Directorate of Distance Education
NALSAR University of Law, Hyderabad

Reading Material

Post-Graduate Diploma in Patents Law

Handbook I – Drafting of Patent Applications

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Contents

Introduction

Patents may be granted to protect inventions that are new, involve an inventive step and are capable of industrial application. The patent has to be for an invention that works, or as it is put in some countries, the invention must be capable of being *"reduced to practice"*. Thus, a clever notion that cannot presently work (e.g. *a time machine*) cannot be patented. Different countries have different ways of expressing the criteria for patents. For example, patents must generally be technical in nature but not all jurisdictions have the same definitions for what is *"technical"* and what is not technical.

The term of a patent is generally twenty years from the filing date of the patent application. A patent gives its owner the right to exclude others from making, using, offering for sale or selling the invention or importing the patented invention into the country where the patent has been granted. In other words, a patent provides a property right that allows the owner to say who cannot use the invention protected by the patent. Anyone who is not the patent owner or who is not licensed by the patent owner and who manufactures, uses, imports, offers for sale or sells the patented invention is called an *"infringer"*. An infringer can be sued in court to force him to stop the infringement and to pay the owner damages. Patents are "territorial;" they have effect only in countries where they have been applied for and granted. Each country has the sovereign right to grant or refuse to grant patent applications.

This Handbook is aimed to help the Patents Law students to familiarise with the approaches of patent drafting and initiate drafting on their own, Large sections of this Module has been carefully compiled from several important publications made by WIPO (World Intellectual Property Organisation), several regional Patent Offices and other Government documents and published articles.

Lastly, a sample *Invention Disclosure Form* a copies of a few granted patents has been added as Annexures for better understanding and in depth study.

CHAPTER I

ANATOMY OF A PATENT

Patents

A patent is a document, issued, upon application, by a government office (or a regional office acting for several countries), which describes an invention and creates a legal situation in which the patented invention can normally only be exploited (manufactured, used, sold, imported) with the authorization of the owner of the patent. "Invention" means a solution to a specific problem in the field of technology. An invention may relate to a product or a process. The protection conferred by the patent is limited in time (generally 20 years).

In a number of countries, inventions are also protectable through registration under the name of *"utility model"* or *"short-term patent".* The requirements are somewhat less strict than for patents, in particular in respect of inventive step, and in comparison, with patents the fees are lower, and the duration of protection is shorter, but otherwise the rights under the utility model or short-term patent are similar. Patents are frequently referred to as *"monopolies"*, but a patent does not give the right to the inventor or the owner of a patented invention to make, use or sell anything. The effects of the grant of a patent are that the patented invention may not be exploited in the country by persons other than the owner of the patent unless the owner agrees to such exploitation. Thus, while the owner is not given a statutory right to practice his invention, he is given a statutory right to prevent others from commercially exploiting his invention, which is frequently referred to as a right to exclude others from making, using or selling the invention. The right to take action against any person exploiting the patented invention in the country without his agreement constitutes the patent owner's most important right, since it permits him to derive the material benefits to which he is entitled as a reward for his intellectual effort and work, and compensation for the expenses which his research and experimentation leading to the invention have entailed.

It should be emphasized, however, that while the State may grant patent rights, it does not automatically enforce them, and it is up to the owner of a patent to bring an action, usually under civil law, for any infringement of his patent rights. The patentee must therefore be his own *"policeman".*

Simply put, a patent is the right granted by the State to an inventor to exclude others from commercially exploiting the invention for a limited period, in return for the disclosure of the invention, so that others may gain the benefit of the invention. The disclosure of the invention is thus an important consideration in any patent granting procedure.

Patent law is designed to encourage inventors to disclose their new technology to the world by offering the incentive of a limited-time monopoly on the technology. This limited-time term of patent is 20 years from the earliest patent application filing date (but this term can be extended via patent term adjustment).

The parts of the patent application typically include the Background, Summary, Detailed Description and Drawings, Claims, and Abstract. The patent agent is unlikely to draft the patent application in this order and should ordinarily draft the claims first. This is because the claims are the heart of a patent. In reading a patent application:

1) the Background section sets the stage for what is to come;
2) the Summary section mirrors the claims;
3) the Detailed Description and Drawings enable the claims by providing a sufficient technical disclosure of the invention;
4) the Claims define the scope of exclusive protection, and
5) the Abstract is primarily an aid for patent searchers and normally receives very little substantive review.

Information Contained Within a Patent

Broadly, the typical patent consists of four main parts:

1) Front page(s)
2) Drawings
3) Specification
4) A background section
5) A list of drawings
6) A detailed description
7) Claims

The specification may also include the following other sections, though these are generally not required:

1) A summary section
2) A cross-reference to related applications
3) A statement of government support

What Patent Won't tell You?

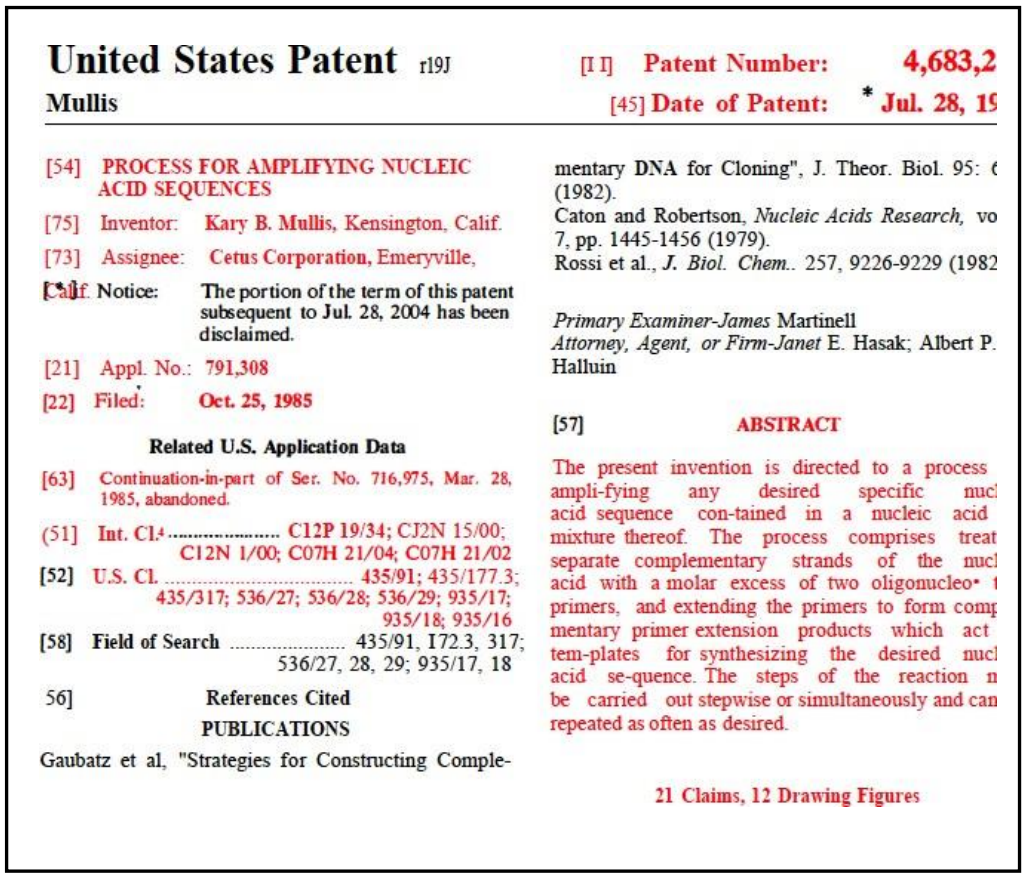
However, keep in mind that there are several important things that the patent will **not** tell you, such as:

1) Have the maintenance fees been paid?
2) Has the patent expired?

3) Who is the current owner of the patent?
4) Has the patent been invalidated, enforced, licensed, or sold?
5) Have any further applications (like continuations or divisional) been filed for this subject matter after this patent was granted?

Sample Portions of a Granted Patent

Front Page (Bibliography)



United States Patent [19]
Mullis

[11] **Patent Number: 4,683,2**
[45] **Date of Patent: * Jul. 28, 19**

[54] **PROCESS FOR AMPLIFYING NUCLEIC ACID SEQUENCES**

[75] Inventor: **Kary B. Mullis, Kensington, Calif.**

[73] Assignee: **Cetus Corporation, Emeryville, Calif.**

[*] Notice: The portion of the term of this patent subsequent to Jul. 28, 2004 has been disclaimed.

[21] Appl. No.: **791,308**

[22] Filed: **Oct. 25, 1985**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 716,975, Mar. 28, 1985, abandoned.

(51) Int. Cl.⁴ C12P 19/34; CJ2N 15/00; C12N 1/00; C07H 21/04; C07H 21/02

[52] U.S. Cl. 435/91; 435/177.3; 435/317; 536/27; 536/28; 536/29; 935/17; 935/18; 935/16

[58] Field of Search 435/91, I72.3, 317; 536/27, 28, 29; 935/17, 18

56] **References Cited**

PUBLICATIONS

Gaubatz et al, "Strategies for Constructing Complementary DNA for Cloning", J. Theor. Biol. 95: (1982).

Caton and Robertson, *Nucleic Acids Research,* vo 7, pp. 1445-1456 (1979).

Rossi et al., *J. Biol. Chem.*. 257, 9226-9229 (1982

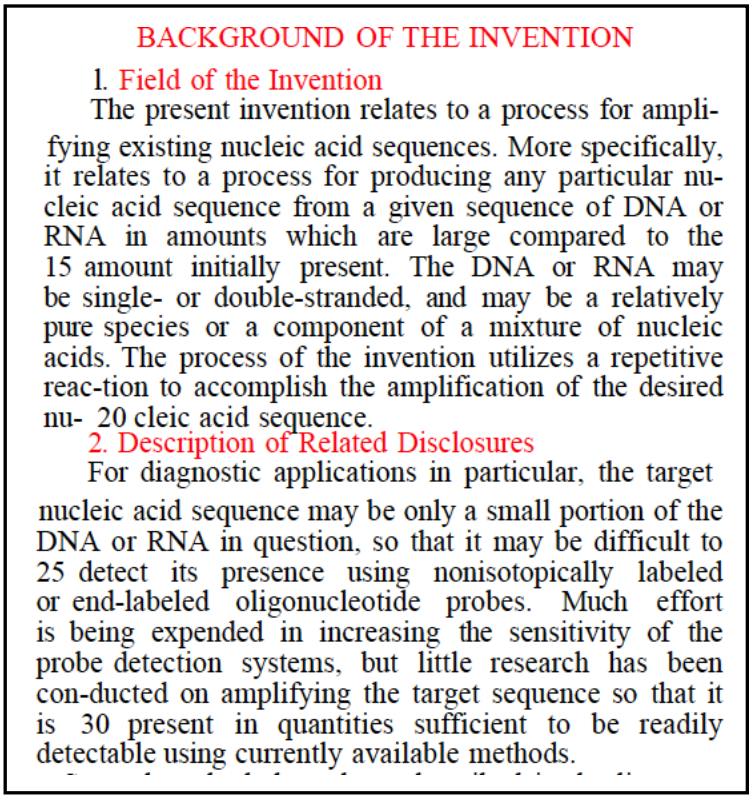
Primary Examiner-James Martinell
Attorney, Agent, or Firm-Janet E. Hasak; Albert P. Halluin

[57] **ABSTRACT**

The present invention is directed to a process ampli-fying any desired specific nucl acid sequence con-tained in a nucleic acid mixture thereof. The process comprises treat separate complementary strands of the nucl acid with a molar excess of two oligonucleo• t primers, and extending the primers to form comp mentary primer extension products which act tem-plates for synthesizing the desired nucl acid se-quence. The steps of the reaction n be carried out stepwise or simultaneously and can repeated as often as desired.

21 Claims, 12 Drawing Figures

Continuity Information & Background



BACKGROUND OF THE INVENTION

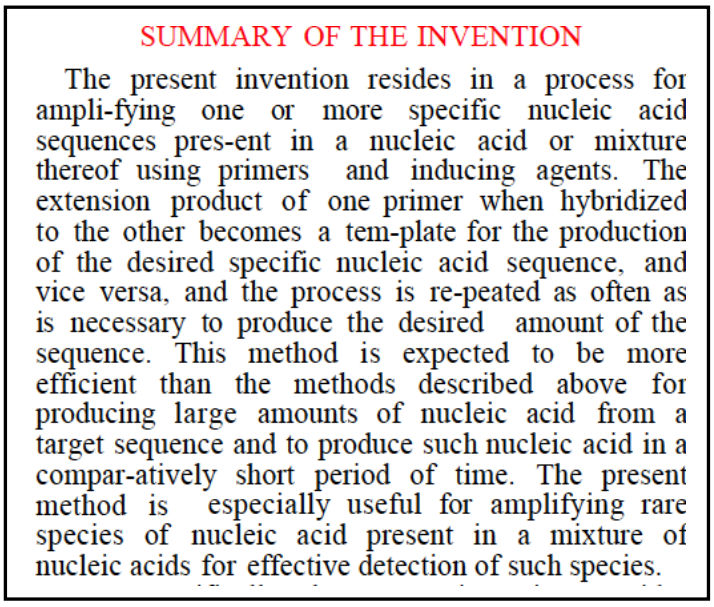
1. Field of the Invention

The present invention relates to a process for amplifying existing nucleic acid sequences. More specifically, it relates to a process for producing any particular nucleic acid sequence from a given sequence of DNA or RNA in amounts which are large compared to the amount initially present. The DNA or RNA may be single- or double-stranded, and may be a relatively pure species or a component of a mixture of nucleic acids. The process of the invention utilizes a repetitive reaction to accomplish the amplification of the desired nucleic acid sequence.

2. Description of Related Disclosures

For diagnostic applications in particular, the target nucleic acid sequence may be only a small portion of the DNA or RNA in question, so that it may be difficult to detect its presence using nonisotopically labeled or end-labeled oligonucleotide probes. Much effort is being expended in increasing the sensitivity of the probe detection systems, but little research has been conducted on amplifying the target sequence so that it is present in quantities sufficient to be readily detectable using currently available methods.

Summary



SUMMARY OF THE INVENTION

The present invention resides in a process for amplifying one or more specific nucleic acid sequences present in a nucleic acid or mixture thereof using primers and inducing agents. The extension product of one primer when hybridized to the other becomes a template for the production of the desired specific nucleic acid sequence, and vice versa, and the process is repeated as often as is necessary to produce the desired amount of the sequence. This method is expected to be more efficient than the methods described above for producing large amounts of nucleic acid from a target sequence and to produce such nucleic acid in a comparatively short period of time. The present method is especially useful for amplifying rare species of nucleic acid present in a mixture of nucleic acids for effective detection of such species.

Figures

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a 94 base pair length sequence of human j3-globin desired to be amplified. The single base pair change which is associated with sickle cell anemia depicted beneath the 94-mer.

FIG. 2 illustrates an autoradiograph of polyacrylamide gel electrophoresis demonstrating amplification of the 94-mer contained in human wild-type DNA and in a plasmid containing a 1.9 kb BamHI fragment of the normal J3-globin gene (pBR328:HbA).

FIG. 3 illustrates an autoradiograph of polyacrylamide gel electrophoresis demonstrating amplification of any of the specific target 94-mer sequence present in pBR328:HbA, a plasmid containing a 1.9 kb BamHI

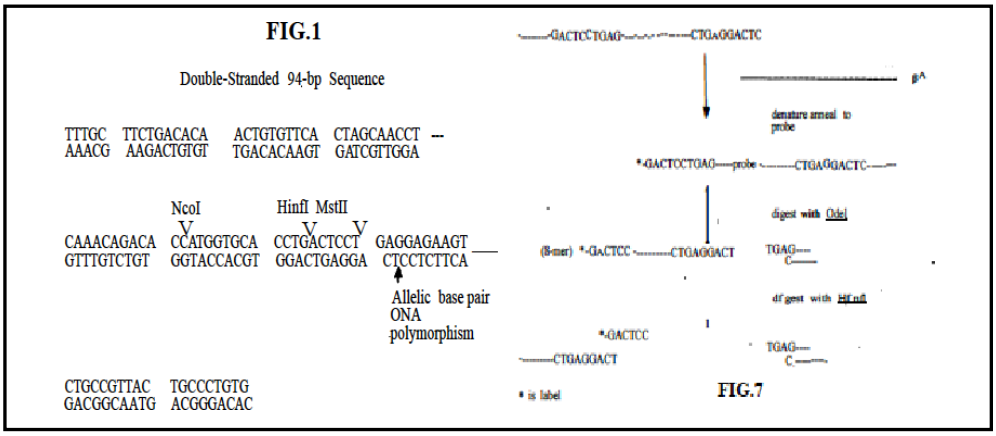
Detailed Description

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "oligonucleotide" as used herein in referring to primers, probes, oligomer fragments to be detected, oligomer controls and unlabeled blocking oligomers is defined as a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a

Figures



Claims

What is claimed is:

1. A process for amplifying at least one specific nucleic acid sequence contained in a nucleic acid or a mixture of nucleic acids wherein each nucleic acid consists of two separate complementary strands, of equal or unequal length, which process comprises:

(a) treating the strands with two oligonucleotide primers, for each different specific sequence being amplified, under conditions such that for each different sequence being amplified an extension product of each primer is synthesized which is complementary to each nucleic acid strand, wherein said primers are selected so as to be sufficiently complementary to different strands of each specific sequence to hybridize therewith such that the extension product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of the extension product of the other primer;

(b) separating the primer extension products from the templates on which they were synthesized to produce single-stranded molecules; and

(c) treating the single-stranded molecules generated from step (b) with the primers of step (a) under conditions that a primer extension product is synthesized using each of the single strands produced in step (b) as a template.

2. The process of claim 1, wherein steps (b) and (c) are repeated at least once.

3. The process of claim 1, wherein said step (b) is accomplished by denaturing.

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CHAPTER II

PATENTABILITY CRITERIA

Patentability

In order to be patentable an invention must fulfill several requirements. These requirements may be broadly classified as novelty, utility (industrial application) and non-obviousness (inventive step). Furthermore, an invention may be patented only if it fits into one of the statutory classes of subject matter protected by the national law. Other legal requirements also exist, such as the necessity for providing an enabling disclosure.

Conditions of Patentability

An invention must meet several criteria if it is to be eligible for patent protection. These include, most significantly, that the invention must consist of patentable subject matter, the invention must be industrially applicable (useful), it must be new (novel), it must exhibit a sufficient *"inventive step"* (be non-obvious), and the disclosure of the invention in the patent application must meet certain standards.

Patentable Subject Matter: In order to be eligible for patent protection, an invention must fall within the scope of patentable subject matter. Patentable subject matter is established by statute and is usually defined in terms of the exceptions to patentability, the general rule being that patent protection shall be available for inventions in all fields of technology (Article 27.1 of the *TRIPS Agreement*[1]).

Subject matter which may be excluded from patentability includes the following (Article 27.3 of the *TRIPS Agreement*[2]). The examples of fields of technology which may be excluded from the scope of patentable subject matter includes the following:

1) discoveries of materials or substances already existing in nature;
2) scientific theories or mathematical methods;
3) plants and animals other than microorganisms, and essentially biological processes for the
4) production of plants and animals, other than non-biological and microbiological processes;
5) schemes, rules or methods, such as those for doing business, performing purely mental
6) acts or playing games;
7) methods of treatment for humans or animals, or diagnostic methods practiced on humans
8) or animals (but not products for use in such methods).

[1] < https://www.wto.org/english/docs_e/legal_e/27-trips_04c_e.htm>
[2] < https://www.wto.org/english/docs_e/legal_e/27-trips_04c_e.htm>

The *TRIPS Agreement* (Article 27.2[3]) further specifies that Members may exclude from patent protection certain kinds of inventions, for instance inventions the commercial exploitation of which would contravene public order or morality.

Industrial Applicability (Utility): An invention, in order to be patentable, must be of a kind which can be applied for practical purposes not be purely theoretical. If the invention is intended to be a product or part of a product, it should be possible to make that product. And if the invention is intended to be a process or part of a process, it should be possible to carry that process out or *"use"* it (the general term) in practice.
"Applicability" and *"industrial applicability"* are expressions reflecting, respectively, the possibility of making and manufacturing in practice, and that of carrying out or using in practice. The term *"industrial"* should be considered in its broadest sense, including any kind of industry. In common language, an *"industrial"* activity means a technical activity on a certain scale, and the *"industrial"* applicability of an invention means the application (making use) of an invention by technical means on a certain scale. National and regional laws and practices concerning the industrial applicability requirement vary significantly. At one end of the spectrum, the requirement of industrial applicability is met as long as the claimed invention can be made in industry without taking into account the use of the invention. At the other end of the spectrum, the *"usefulness"* of the claimed invention is taken into account for the determination of the industrial applicability. On the other hand, some countries do not require industrial applicability, but utility.

Novelty: Novelty is a fundamental requirement in any examination as to substance and is an undisputed condition of patentability. It must be emphasized, however, that novelty is not something which can be proved or established; only its absence can be proved.
An invention is new if it is not anticipated by the prior art. *"Prior art"* is, in general, all the knowledge that existed prior to the relevant filing or priority date of a patent application, whether it existed by way of written or oral disclosure. The question of what should constitute *"prior art"* at a given time is one which has been the subject of debates.

One viewpoint is that the determination of prior art should be made against a background of what is known only in the protecting country. This would exclude knowledge from other countries, if it was not imported into the country before the making of the invention, even if that knowledge was available abroad before the date of the making of the invention.

[3] < https://www.wto.org/english/docs_e/legal_e/27-trips_04c_e.htm>

Another viewpoint is based on the differentiation between printed publications and other disclosures such as oral disclosures and prior use, and where such publications or disclosures occurred.

The disclosure of an invention so that it becomes part of the prior art may take place in three ways, namely:

1) by a description of the invention in a published writing or publication in other form;
2) by a description of the invention in spoken words uttered in public, such a disclosure being called an *"oral disclosure"*;
3) by the use of the invention in public, or by putting the public in a position that enables any member of the public to use it, such a disclosure being a *"disclosure by use".*

Publication in tangible form requires that there be some physical carrier for the information, a document in the broad sense of the term, and that document must have been published, that is to say, made available to the public in any manner such as by offering for sale or deposit in a public collection. Publications include issued patents or published patent applications, writings (whether they be manuscript, typescript, or printed matter), pictures including photographs, drawings or films, and recording, whether they be discs or tapes in either spoken or coded language. Today, publication on the Internet must increasingly be taken into consideration.

Oral disclosure, as the expression suggests, implies that the words or form of the disclosure are not necessarily recorded as such and includes lectures and radio broadcasts.

Disclosure by use is essentially a public, visual disclosure such as by display, sale, demonstration, unrecorded television broadcasts and actual public use.

A document will only destroy the novelty of any invention claimed if the subject matter is explicitly contained in the document. The subject matter set forth in a claim of an application under examination is thus compared element by element with the contents of each individual publication.

Lack of novelty can only be found if the publication by itself contains all the characteristics of that claim, that is, if it anticipates the subject matter of the claim. Lack of novelty may however, be implicit in the publication in the sense that, in carrying out the *"teaching"* of the publication, a person having ordinary skill in the art would inevitably arrive at a result falling within the terms of the claim. Generally speaking, lack of novelty of this kind will only be raised by the Patent

Office where there is no reasonable doubt as to the practical effect of the prior *"teaching".*

It should be noted that in considering novelty, it is not permissible to combine separate items of prior art together.

Inventive Step (Non-Obviousness): In relation to the requirement of inventive step (also referred to as *"non-obviousness"),* the question as to whether or not the invention *"would have been obvious to a person having ordinary skill in the art"* is perhaps the most difficult of the standards to determine in the examination as to substance.

The inclusion of a requirement like this in patent legislation is based on the premise that protection should not be given to what is already known as part of the prior art, or to anything that the person with ordinary skill could deduce as an obvious consequence thereof.

The expression *"ordinary skill"* is intended to exclude the *"best"* expert that can be found. It is intended that the person be limited to one having the average level of skill reached in the field in the country concerned.

It should be noted that novelty and inventive step are different criteria. Novelty exists if there is any difference between the invention and the prior art. The question, *"is there inventive step?"* only arises if there is novelty. The expression *"inventive step"* conveys the idea that it is not enough that the claimed invention is new, that is, different from what exists in the state of the art, but that this difference must have two characteristics:

1) it must be *"inventive"*, that is, the result of a creative idea, and it must be a step, that is, it must be noticeable.

   There must be a clearly identifiable difference between the state of the art and the claimed invention. This is why, in some jurisdictions, there is the concept of an *"advance"* or *"progress"* over the prior art.

2) it is required that this advance or progress be significant and essential to the invention.

   In order to assess the nature of the differences which are relied upon as constituting an inventive step, account has to be taken of the prior art as a whole. Thus, as distinct from the assessment of novelty, the subject matter of the claim under examination is compared not with each publication or other disclosure separately, but with the combinations thereof, insofar as each such combination is obvious to the person having ordinary skill in the art. The combination may be global, whereas the claim may define a set of subject matter known separately. For the inventive step to be denied, it is necessary that not only

the combination, but also the choice of the combined elements, is obvious. It is the sum of the differences that have been discovered which must be compared with the prior art and judged as to obviousness, and not each of the new elements taken individually, except where there is no technical link between them.

In most cases, it is useful to assess inventive step in relation to three aspects, namely:

1) the problem to be solved;
2) the solution to that problem; and
3) the advantageous effects, if any, of the invention with reference to the background art.

If the problem is known or obvious, the examination will bear on the originality of the solution claimed. If no inventive step is found in the solution, the question becomes whether or not the result is obvious or whether it is surprising either by its nature or by its extent. If a person having ordinary skill in the art would have been able to pose the problem, solve it in the manner claimed, and foresee the result, the inventive step is lacking.

Disclosure of the Invention: An additional requirement of patentability is whether or not the invention is sufficiently disclosed in the application. The application must disclose the invention in a manner sufficiently clear for the invention tobe carried out by a person skilled in the art.

The description should set out at least one mode for carrying out the invention claimed. This should be done in terms of examples, where appropriate, and with reference to the drawings, if any. In some countries, the description is required to disclose the best mode for carrying out the invention known to the applicant.

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CHAPTER III

PREPARING FOR A PATENT APPLICATION

I. Predicting Patentability

Prior art searches are a good way to get information on developments in the field of invention. Prior art searches may sometimes reveal what competitors consider worth protecting. Search results may be a critical factor in deciding whether to file a patent application. If a prior art search reveals references that anticipate the claimed invention, the inventor and the patent agent should consider how they can "avoid the prior art" by drafting the claims to overcome it. If this is not possible, they may wish to consider whether filing the patent application is still appropriate. In some cases, a prior art search may reveal patent references that are problematic. Just because one sees a reference that seems similar to the invention does not mean the proposed application should be abandoned. The prior art may instead warrant a *"design around"* effort to see how the claims of the new application can be changed to avoid the prior art.

Prior Art refers to scientific and technical information that exists before the effective date of a given patent application. Prior art may be found in any public documents such as patents, technical publications, conference papers, marketing brochures, products, devices, equipment, processes and materials. The *"effective date"* of a patent application is typically the date of the earliest filed patent application that the pending application can claim.

A prior art search refers to an organized review of prior art contained in public documents. Prior art searches can be of various kinds: patentability searches conducted by an inventor before filing a patent application; invalidity searches in litigation conducted by the accused infringer; patent examination searches conducted by a government examiner in order to determine whether to grant or reject a patent application and state of the art searches for information in a technical field. In all cases, searches are conducted using different kinds of databases, from public databases of issued patents on the Internet to exhaustive databases including technical literature. Searches can be done by legal professionals, by scientists or by researchers. Sometimes, defendants in patent litigation even offer bounties for invalidating prior art.

A patent agent or inventor is not required to search for prior art. However, there may be advantages to performing a search in some cases. A prior art patentability search may be

conducted before the filing of a patent application to gauge the prospects of obtaining broad claim coverage. The purpose of conducting such a search is to find references related to the claimed invention in order to make an assessment of its patentability.

There are varying opinions on how extensive this search should be. Many patent agents and patent lawyers only do a brief search to identify the readily available prior art. These searches are typically fast and inexpensive since the patent agent's clients often do not want to pay for an expensive, thorough search. Also, it is often presumed that the inventor himself will have a good sense of novelty based on his reading of the literature in his field and by communication with his peers. In some cases a more rigorous search may be justified before investing in an expensive foreign patent application.

II. Prior Art Search

The prior art searcher can simply go to the library and conduct research just as he would research any other topic. He can also review existing patents either on-line (via the public databases published on the Internet) or in a public patent library.

An on-line prior art search can be done as either a:

1) Keyword Search

   Before beginning a search based on keywords start by listing those one would use to describe the invention. Think of all possible aspects of the invention and choose keywords that describe each such aspect. The quality of a keyword search will largely depend on the appropriateness of keywords selected.

2) Field Search

   A field search might be used to refine the results of the keyword search. Once the keyword search has been conducted, use the field search to narrow the results down to the field in which the invention at hand operates.

There are several good databases where one can search and expect to obtain comprehensive prior art search results. The databases include national and international databases. The patent searcher does not necessarily have to pay to use these databases as most are free and public and many can be accessed on the Internet.

Patent infringement, rejection of a patent application, or invalidation of an existing patent are all negative outcomes that can result from incomplete prior art searches. Though these conflicts can

often be resolved through negotiation, licensing, designing around, or even costly invalidation of the conflicting patent, as a best practice it is better to mitigate risk and control costs by ensuring all relevant prior art is surfaced before the final IP strategy is set. Patent search experts consider many factors when determining the scope and strategy for a prior art search. Whether one chooses to do one's own prior art searching or outsource it to an expert third-party search firm, here are three fundamental keys to ensuring a complete prior art search so ones clients won't lose sleep:

1) Choosing the right sources
2) Carefully defining the scope
3) Asking the right questions

Arguably, the most important part of a prior search is deciding when one should stop. However, this can be a challenging decision, especially when no relevant references are found. When the goal is to uncover something one can't be sure exists, or verify the absence of something that might exist, how do one know when to stop? At what point can one say that have checked enough sources from enough perspectives to move forward? Convergence is one goal that many search expert use to address this challenge. A good indicator of the search is complete is when one have tried the search in numerous ways - including different sets of keywords, citation searching, a competitor review, a chemical name, text, and chemical structure searching - and the hits (or lack thereof) one gets keep returning the same relevant results. If the results do not converge, and additional relevant answers continue to pop up as one tries different approaches, then one should review the new results to see why they were not found by other methods, and enhance the original search approaches until convergence is achieved, or until it is determined that the body of references retrieved is sufficient to provide an opinion.
The common online resources that may be employed for a prior art search includes:

1) Local Patent Office[1]

   The local patent office will likely have a written or electronic database. Such databases are normally public, and a patent agent or inventor can use them to search issued patents in his or her country. Note that pending patent applications may not be included in the searchable database in many countries.

2) Patent Cooperation Treaty Applications

   WIPO publishes new PCT applications every Thursday. The PCT database (Patentscope[2]) contains published PCT applications dating back to 1978. In many cases the international search report for PCT applications is also available, which may help a prior art search locate further pertinent prior art.

3) United States Patent and Trademark Office

[1] CGPDTM of Indian Patent Office has developed a search tool, INPASS < https://ipindiaservices.gov.in/publicsearch/>, which allows a full-text search to be conducted in all Indian patents as well as patent applications, thereby enabling the stakeholders to conduct an advanced search in the Indian patent database.
[2] <https://patentscope.wipo.int/search/en/search.jsf>

The USPTO has a large and easy-to-use electronic database that anyone with an Internet connection can access free of charge. It covers issued US patents and published patent applications from 1790 with full text searching available for patents issued from 1976 onwards[3]. Note: New US patents issue on Tuesdays and typically appear in the database on the same day.

The USPTO also maintains a database of information on currently pending patent applications provided that the application has been published. The *"Patent Application Information Retrieval"* or *"PAIR"*[4] database provides office actions, responses, related case and other file history information for patents and patent applications. A portion of PAIR is publicly available. Another portion of PAIR is only available to practitioners so that they can review the status of their cases.

The USPTO also makes assignment records available for patents and published patent applications[5].

4) European Patent Office

   One can search the European Patent Office database by going to their home page (Espacenet[6]) - this database contains patents from all over the world.

   The EPO also maintains a database for pending cases much like the USPTO's PAIR system discussed above. This database, known as EPOline[7].

5) Scientific Databases

   There are different scientific and technical databases that are specific to various fields of technology. It is helpful for a patent agent to become familiar with these databases since they contain articles that discuss technological advances in the field. Since prior art encompasses more than just patents, a scan of these scientific databases is important when conducting a patentability search.

Additionally, there are several commercial database providers, who provides information to be searched from multiple sources including both patent and non-patent literature on a subscription basis. It is convenient to use such vendor provided services due to convenience of use and availability of prior art from multiple sources. Often the patent and non-patent literature data such provided are indexed for additional convenience.

[3] <http://patft.uspto.gov/netahtml/PTO/search-bool.html>
[4] <https://portal.uspto.gov/pair/PublicPair>
[5] <https://assignment.uspto.gov/patent/index.html#/patent/search>
[6] <https://worldwide.espacenet.com/>
[7] <https://register.epo.org/regviewer>

There is no perfect prior art search. As one seeks to balance the investment of time and resources with the strategic importance of the outcome to the client, some risk will always remain; however, regardless of the searching budget, ensuring one is using the best available sources, setting an appropriate scope, and focusing on the best ways to ask the question will get one a good way toward avoiding surprises from missed prior art. If the topic is unfamiliar or the outcome critical, it can often be valuable to consult with both technology and IP search experts.

III. Invention Disclosure Form

Invention Disclosure Form (IDF) is the first step in the process of identifying intellectual property (IP) and determining how to proceed with it. This determination process includes decisions about ownership and patenting as well as licensing.

An IDF should be filled up and submitted when something new and useful has been invented to competent and designated internal authorities (if present) or shared with a Patent Agent. Completed IDFs are important legal documents in that they record what has been invented as well as the circumstances under which the invention was made. Submitted IDFs are treated as confidential until a patent application is filed, or the invention is publicly disclosed (i.e. via an inventor's publication). Accordingly, prior to a patent filing or public disclosure, the information in submitted IDFs should not be distributed except:

1) to research sponsors as may be stipulated by contract;
2) to parties that are under attorney-client privilege;
3) to parties that are under confidentiality agreements; or
4) as may be required by law.

A patent agent's clients will likely have different levels of sophistication with respect to their abilities to handle patent documents. Some clients may have fairly sophisticated administrative units that can provide completed invention disclosure packages to patent agents who then conduct a follow-up review as necessary. At the opposite extreme are clients who have no IP infrastructure and require considerable guidance and assistance from the patent agent.

The patent agent will learn over time which approach offers the best results for different types of clients. For some clients, the patent agent may want to provide a blank Invention Disclosure Form and then allow inventor(s) to complete it on their own. For other clients the patent agent may want/need to obtain all his information about the invention via one or more interviews with the inventor(s). In any event, the patent agent should always attempt to have at least one meeting

either in person or by telephone with the inventors. It is highly unlikely that an inventor will be able to supply a patent agent with enough material for the patent agent to have an unambiguous understanding of the invention without some sort of *"live"* meeting with the inventor. Similarly, it is unlikely that the inventor will understand the legal/background information being sought about his invention in the absence of a meeting with the patent agent.

In an ideal situation the inventor will provide the patent agent with an Invention Disclosure Form and supporting documents well before the face-to-face meeting between them. The patent agent will review the disclosure materials and note any places where he has questions or where he believes additional disclosure would be helpful. During the meeting between the patent agent and the inventor, the patent agent verifies that he has a complete understanding of the invention, establishes that there is no additional disclosure information that he should also receive (or that he receives the additional disclosure material), determines the most commercially-significant aspects of the invention and confirms that there are either no pending bar dates or verifies the precise bar dates.

The patent agent should review the invention disclosure well prior to meeting with the inventor. This will ensure that the patent agent will have had sufficient time to identify all the parts of the invention disclosure that raise questions – both technical parts (e.g. *"how does A function with B"?)* and legal parts (e.g. *"Who else could be an inventor?"*). Each patent agent will want to review/modify this form to ensure that it conforms to the legal requirements for his jurisdiction. The patent agent will want to make any necessary changes to this form to provide a full and complete disclosure in the jurisdiction(s) of interest to his clients. He may also wish to provide copies to his clients for them to complete the form and return it to the patent agent well in advance of the disclosure meeting.

In reviewing an invention disclosure and/or in speaking with an inventor the patent agent must keep focused on any/all patentable inventions described. Much of the text in an invention disclosure and/or discussion during the meeting with the inventor will probably not be about a purely patentable novelty but will include other non-patentable technical details. The patent agent should not be surprised to discover that quite often inventors do not know what they have invented, at least in *"patentability"* terms, as they often think in other terms such as *"discoveries"*. Thus, the patent agent will often be the one who articulates what constitutes a patentable invention.

The patent agent should never become the inventor but should strive to have the clearest grasp of

the invention needed to obtain a patent with the broadest claims allowed by law. This means the patent agent must understand the invention well enough to draft claims describing the invention with the fewest possible limitations. In other words, the patent agent must understand the invention well enough to know what elements do not need to be recited in the broadest possible claim for the invention. Understanding the invention also means that the patent agent understands it well enough to prepare a specification for a patent application that discloses all possibly patentable aspects of the invention and enough additional information so that a lay person skilled in the pertinent technical field can understand and make the object invented.

Understanding the invention also means that the patent agent can receive a prior art description such as one used as the basis for a claim rejection by a patent office and be able to explain the differences between the invention and the prior art and/or amend the pending claims to highlight these differences in a manner that minimizes the reduction in the scope of claim coverage The patent agent may discover that the inventor does not know the answer to all his questions. The inventor may be able to speculate about alternatives and in some instances may even have the time to conduct some additional research. The patent agent must make sure, however, that the specification discloses a working embodiment of the invention. Thus, if the inventor is uncertain about the answer to any of the patent agent's questions, the patent agent must use his best professional judgment as to how to deal with the uncertainty. There may be gaps in the technical disclosure that the patent agent can fill but he should always confirm with the inventor that the substitute for any missing material is correct and within the spirit of the invention. The patent agent may assist the inventor in considering possible alternative embodiments for the invention. Often inventors create their inventions for a very specific purpose and have not really considered whether they could be applied to other areas.

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CHAPTER IV

ANATOMY OF A PATENT APPLICATION

Patent Applications

Writing a high-quality patent application is important because it sets out in a clear fashion the terms by which the patent owner and others will be bound. In this sense, drafting a patent application is different from writing a scientific paper. As the patent document contains technical subject matter it will also bear some similarities to a scientific or technical paper, although it does not usually need to rise to the level of a blueprint for making the invention protected by the patent. The issued patent will be reviewed over the years by public officials such as patent examiners and judges and business partners. Thus, the patent application should be drafted with these important audiences in mind.

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The parts of the application are generally:

1) Claims

   One of the first things to do is to prepare the claims for the invention. In fact, the patent agent may even want to sketch out the claims in the disclosure meeting with the inventor. This will often provide confirmation to the patent agent that he has understood the invention. The patent agent may wish to use some sort of *"picture claim"* in the initial meeting with the inventor since inventors are often unfamiliar with patent claim language. For this reason, the patent agent should avoid using highly abstract language to describe the invention in the disclosure meeting with the inventor.

   The majority of patent agents prepare several draft patent claims as their first step in writing a patent application. The claims are the legally-operative part of a patent application; everything revolves around the claims. If the claims are prepared before drafting the specification the patent agent will know which terms need to be described in the specification. While it is generally preferable to draft the claims first, some situations may not provide the patent agent with this luxury.

   The patent agent may consider preparing a *"picture"* claim for an invention. A picture claim is a claim that paints a word picture of the invention. The patent agent will rarely want to file a picture claim although such claims can be useful in helping to understand the invention and may also be helpful in determining all the points of novelty with the inventor. Such claims often contain limitations that do not necessarily provide novelty but

are nevertheless part of a product that includes the invention. Thus, picture claims may be helpful to the drafter of a patent application, but they rarely provide the broadest claims for an invention. Because of the critical importance of claims, the patent agent should carefully revisit them after drafting the specification. This is because after writing the specification, the patent agent will likely come to an even better understanding of the invention. For example, he will now be in a better position to spot extraneous limitations in the claims that could prevent obtaining the broadest possible claim coverage. Similarly, after preparing the specification the patent agent may now see that the claims do not describe the invention as accurately as they could.

Once the claims are completed the patent agent needs to check the drawings and specification to verify that the claim terms have been appropriately described and disclosed.

2) Detailed description (or specification)

The detailed description section, sometimes known as the *"preferred embodiment of invention*" section or the *"disclosed embodiment of the invention"* section breathes life into the claims and provides a sufficient explanation of the invention for an ordinary person skilled in the art to make and understand the invention. In some jurisdictions the term *"specification"* is also used to refer to the description in addition to the summary and background sections of the application; suffice to say that *"detailed description"* and *"specification"* are generally the same for purposes of patent drafting.

The detailed description section must be closely tied to the drawings. This section cannot be substantively amended once the application has been filed. Consequently, the patent agent must make sure that the detailed description section provides an appropriate degree of technical disclosure on the day that the application is filed as he won't have a second chance to alter this part of the application. The patent agent cannot amend his application to include new technical disclosure during prosecution.

If the patent agent uses a highly abstract term in the claims he should consider using the term in the detailed description section, but in a manner that ties the abstract term to a specific embodiment of the invention. Thus, a patent agent should take care that the patent application

i. reflects the disclosure material provided by the inventors,
ii. provides sufficient information to enable an ordinary artisan to reproduce the invention and
iii. provides sufficient depth so that the claims can be narrowed during patent prosecution to avoid close prior art.

In drafting the specification, the patent agent should avoid using phrases such as *"the*

invention is…" The patent agent should instead use phrases like *"in an embodiment of the invention"*. This will ensure that patent claims receive the broadest interpretation possible. Without limiting words to the contrary, the detailed description section is generally presumed to disclose *"an embodiment"* of the invention rather than the invention itself. However, if the patent agent forecloses this broader reading, the scope of the claimed invention may be similarly narrowed. A patent specification filed in the US, for example, must satisfy the three requirements of enablement, written description and best mode. Most of the world's patent laws have requirements identical or very similar to the enablement and written description requirements. In drafting the detailed description section, the patent agent will generally want to err on the side of inclusion for the reasons described above. The patent agent will also want to consider the *"best mode"* requirement that arises in jurisdictions such as the US and India. The patent application must disclose the best mode of carrying out the invention known to the inventors. Basically, the patent application cannot conceal the optimum aspects of the invention from someone who tries to make and use the invention described in the patent.

The *"enablement"* requirement means that a patent application must teach ordinary persons skilled in the art how to make and use the invention. Enablement is usually viewed as of the filing date of the patent application. A patent application that is not enabled as of its filing date cannot become enabled by later technical innovations.

Patent specifications filed with the EPO preferably follow a *"problem-solution"* approach. Thus, the story told by the application is one of outlining a problem first and then describing its solution. Some patent agents find the problem-solution approach easy to follow in crafting their applications and this technique should be suitable for applications filed in most jurisdictions. However, the patent agent should exercise caution as to how the problem is described. The critical aspect of some inventions is a recognition, characterization or re- characterization of a problem, e.g. once the inventor has uniquely formulated the problem, the solution follows fairly easily thereafter. Thus, if the patent agent describes the problem in a manner suggesting that a perfect understanding of the problem was well known in the prior art (when it was not), the patent agent may have inadvertently complicated obtaining patent protection for the client's invention. Aside from this caution, the problem-solution approach may work quite well for many applications, especially those filed with the EPO.

The patent agent must always research and review the law and relevant rules pertaining to the country where he is seeking patent protection for his client. Many patent laws and rules are available online.

3) Drawings

The patent agent must prepare good visual supporting materials that describe the invention. In fact, many patent agents would argue that the drawings are the most important part of the patent application after the claims. Some patent laws require that every claimed element be shown in a drawing. Where possible, the drawings should explain the invention in sufficient detail that reading the detailed description section merely confirms in words the information provided in the drawings. This will not be possible with all inventions. In preparing the drawings the patent agent should think of the story he wants to tell and how he wants to tell it. The patent agent should also think about the level of detail necessary to provide an enabling disclosure.

Conversely, the patent agent should avoid providing too much detail in the drawings – unless the accompanying explanation in the detailed description section explains that the additional detail pertains to but one specific embodiment of the invention. Otherwise, someone may later argue (maybe during litigation) that the additional detail is necessary for the invention. This becomes especially true in many countries if the patent agent also uses a means-plus-function claim, since defendants in a later patent infringement case will argue that all the unnecessary details disclosed in the drawings are necessary structures for performing the recited function. The elements shown in a patent's drawings are typically accompanied by a short description in words and a reference number.

The patent application itself should contain a list of the drawings between the summary of the invention section and the detailed description section. The drawing section should begin with a statement indicating that the drawings are illustrative of one or more embodiments of the invention (and not illustrative of THE invention).

The patent agent should make sure that his drawings are complete and omit no critical details. If the patent agent has a drawing that depicts a process flow, for example, then he should ensure that arrowheads are appropriately depicted. The patent agent may provide sufficient written explanation in the detailed description section to overcome the deficiencies in his drawings – but the patent agent should not rely upon having a more detailed description section that compensates for the short comings of his drawings.

4) Background

The use of background sections varies among the world's patent regimes. In some patent systems the background section serves to disclose to the public the closest prior art applied against the patent application during examination. This is the situation in most European systems.

In some countries such as the US, the prior art submitted by the patent applicant, as well as the prior art found by the examiner, is printed on the cover of the patent itself.

Consequently, patent background sections in many countries suggest caution. Many practitioners attempt to draft the shortest possible background sections out of fear that they will inadvertently and unintentionally deprive their client of the full scope of patent protection by saying too much about the prior art.

The background section is typically considered prior art disclosed by the inventor. Consequently, if the applicant's own inventive disclosure ends up in the background section, the patent examiner may cite this section in the rejection of the applicant's claims. Some patent offices take a fairly hard line about inventive disclosures in background sections, which is one of the reasons why patent agents should draft them carefully. If the patent is ever involved in litigation, they will probably come under even greater scrutiny than they did during initial prosecution.

A final caution about background sections is that sometimes the invention itself is inextricably linked with a *"new understanding"* of the prior art. If this new understanding is described in the background section, possibly the most novel part of the invention has been described there, although it is not the place where the inventor's novelty should ever be described.

Consequently, a good background section should be fairly short and merely set the stage for the technical disclosure to be provided in the detailed description section. The background section could describe the prior art at a very high level. In some jurisdictions it is not generally helpful to mention specific prior art.

The background section may conclude with a short, crisp statement about the shortcomings of the prior art but this must be written in a manner that does not disclose the solution to be described later in the application. Some older patent applications include *"objects of the invention"* paragraphs in either the background or summary sections. Avoid these unless they are required by law in the jurisdictions where you file your applications. The danger is that these statements have a tendency to limit the invention.

Another danger is that these statements have a tendency to provide *"fraud"* arguments. In some countries the findings from various patent cases over the past 10-15 years have caused patent agents to stop drafting such *"objects"* statements for nearly all applications filed in their jurisdictions.

A patent agent should probably not write the background section first. There is a temptation to write this section before anything else. After all, it comes first. However, if the patent agent writes the background section first, there is a danger that he will spend too much time on it and the section will end up being far too long and detailed especially since it is one of the least important parts of the application. One should wait until after

drafting the detailed description section before drafting the background section.

5) Abstract

The patent abstract should describe the invention very clearly in the fewest possible words. The patent agent could use a version of the first paragraph of the summary of the invention section as the abstract. In many of the world's patent systems, abstracts are reviewed only for their adherence to certain maximum length requirements and receive little/no substantive review. For the most part, courts will rarely look to the abstract for an explanation of the invention. Of course, this is not to suggest that the abstract should be misleading or poorly written.

The danger with abstracts is that they may disclose some patentable feature of the invention not also found in the specification. This is a common mistake with new patent agents who tend to draft abstracts early on and not review them again once the patent application has been completed. When reading an abstract that he has drafted, the patent agent should always ask himself: *"Is all this disclosed in the specification?"* If the answer is not strongly *"yes"*, then the patent agent should either add to the specification or modify the abstract.

6) Summary

Not all jurisdictions require a summary of the invention section. However, such sections are customarily prepared in many jurisdictions even when not strictly required by national law. The patent agent may find himself reviewing summary sections drafted by foreign patent agents working on his client's foreign counterpart patent applications. Consequently, the patent agent should understand the precise requirements and customary practice regarding a summary of the invention sections in the jurisdictions of interest to his clients.

Patent agents often make terrible mistakes in drafting summary sections because they are lured into a false sense of the section's importance by virtue of its title. In reality, this is not typically an important section of the application and many errors can be avoided by simply writing the summary section from the claims. In fact, some patent agents prepare the summary of the invention section by taking each of the independent claims in the patent application and turning them into paragraphs. This approach also has as an advantage that the precise words used in the claims will be guaranteed to be in the specification. Many patent agents simply draft the summary of the invention section in a manner that highlights the important aspects of the invention using words drawn from the application's claims.

The summary of the invention section should be one of the last parts of the patent application that the patent agent writes. There is a temptation to draft the summary early on – it comes near the beginning of the application - and the patent agent can test himself

on his knowledge of the invention. But he should avoid these temptations and delay writing the summary section until after he has completed the detailed description.

In preparing the summary of the invention sections, avoid providing some sort of *"big picture"* summary that goes beyond the claims in any manner. The dangers of providing such a summary are many. First, a broad *"meta"* summary will invariably suggest additional prior art that can be applied against the invention. By explicitly linking the invention in writing to a broader subject it will be difficult, if not impossible, to argue later during prosecution that the prior art does not apply.

Second, a broad, big picture summary often includes in some seemingly minor or insignificant way, another concept that is otherwise not well explained in the application. This provides arguments for someone to use against the patent especially during litigation, that the inventor did not provide a complete disclosure because the "summary" mentioned topics not otherwise disclosed in the application. Third, a broad "meta" summary vaguely suggests that the claims are not at the fullest scope of the invention. The client may not be pleased if his patent agent has not sought to protect the full scope of the inventive disclosure.

A patent agent will want to consider the patent application's *Title* fairly early. This title should broadly describe the invention. However, titles are not generally examined. Occasionally a patent examiner will decide that a title is not descriptive of the invention. It is best to avoid being overly narrow in the invention's title, although the title should sufficiently indicate the subject matter of the invention.

The patent application itself should also include all priority information, such as the identification of related applications. In the US, for example, priority information should be provided as the first sentence in the application. The patent agent may have other forms to complete that also provide the inventor's name and priority information but there is more certainty when this information is also included as part of the application itself.

Always remember who the audience will be for the patent application. The key audiences include patent examiners and judges. Of course, the patent agent's client and the inventor are also audiences; the patent agent must make sure the inventor understands his own patent application. Other potential audiences include competitors, infringers and investors. Many investors will often scrutinize a technology company's patent portfolio carefully before making an investment.

A typical patent application has the following sections:

1) Title of invention
2) Cross-reference to related applications (if any);
3) Statement regarding sponsored research or development (if any);

4) Reference to a Sequence Listing or Biological Deposits (if any);
5) Background of the invention;
6) Brief summary of the invention;
7) Brief description of the drawing (if any);
8) Detailed description of the invention;
9) Claims;
10) Drawings; and
11) Abstract.

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CHAPTER V

DRAFTING OF CLAIMS

I. Patent Claims

The claims mark the boundaries of the protection provided by a patent, just as a physical boundary such as a fence, marks the limits of a parcel of real property. Thus, the claims are a written approximation of the abstract inventive concept created by the inventor. The claims define the scope of protection provided by a patent. While jurisdictions around the world may apply differing legal doctrines for claim interpretation, in the most prevalent theory the claims set forth the outer limits of patent protection. The claims clearly and concisely tell the world what the patent applicant claims to be his invention.

The patent agent needs to understand the differences between three legal constructs related to patents:

1) Inventions

   A mental construct inside the mind of the inventor and has no physical substance.

2) Embodiments

   A physical form of the invention in the real world.

3) Claims

   Must protect at least an *"embodiment"* of the invention - but the best patent claims will protect the *"invention"* itself so that no physical embodiments of the invention can be made, used or sold by anyone without infringing the claims.

Early patents did not have claims and the scope of the patented invention was determined in court proceedings during patent infringement litigation by reviewing the specification filed by the inventor. Not surprisingly, this process eventually became unworkable and the process of patent claiming was born as a means for providing greater notice of the boundaries of the patent. Additionally, in a substantive examination patent system the claims are reviewed by a patent examiner, which provides the courts and the public with some assurance that a typical patent claim does not exceed the maximum scope of protection the inventor should receive. Thus, the claims were originally based on the concept that they were to serve as a guideline to explain what the inventor perceived as his invention at the time he made his invention and filed his patent application. Today, the claims define the protection given by a patent and lie at the heart of any invention. In fact, claims are typically the first portion of the patent application examined and scrutinized by a patent examiner or anyone studying the patent.

The patent agent will generally strive for a broad set of claims that cover various aspects of the invention at various levels of detail. The patent agent will probably not want all the claims to meet the apparent theoretical maximum of protection since subsequent litigation will likely raise invalidity arguments not contemplated by the patent examiner. Thus, the patent agent will want to draft some narrower claims in the event that the broadest claims are invalidated. A narrower set of claims will often be upheld as valid during litigation but will still be *"broad enough"* to prove infringement against the patent infringer.

Once the claims have been drafted and the specification has been written, the patent agent must re-read the specification and claims to ensure that every single claim has adequate support in the specification. The choice of words and terminology used in claims should be traced back to the specification to ensure that the specification and claims are consistent, and that the same terminology is used throughout. A claim that is not supported by the specification can easily be thrown out for lack of support.

Patent claims may be amended during patent prosecution. Some jurisdictions place limits on the degree to which claims may be amended and/or canceled and replaced with new claims. Nevertheless, the patent agent will typically have some flexibility in adjusting the pending claims to avoid newly- discovered prior art or to satisfy other legal requirements. Similarly, hindsight may sometimes suggest to a client and/or the patent agent that the initially-filed claims could have been more broadly recited. Accordingly, the patent agent may also amend the claims to give them greater breadth.

While jurisdictions may differ over format and interpretation issues regarding patent claims, the theory of what a good patent claim should accomplish is essentially the same worldwide. For instance, the following advice originated largely from claims construction guidelines provided by the EPO: The application must contain *"one or more claims".* These claims must:

1) *"define the matter for which protection is sought"*;
2) *"be clear and concise"*; and
3) *"be supported by the description"*.

Since the extent of the protection conferred by a patent is determined by the terms recited in the claims (interpreted with the help of the description and the drawings), clarity of the claims is of the utmost importance. The EPO recommends that claims be drafted in terms of the *"technical features of the invention".* This advice means that claims should not contain statements relating, for example, to commercial advantages or other non-technical matters although statements of

purpose are allowed when they assist in defining the invention. This is sound advice for claims drafters in anyjurisdiction.

II. Unity of Invention

A patent application must typically relate to one invention only or to a group of inventions so linked as to form a single general inventive concept. The second of these alternatives, i.e. the single-concept linked group may give rise to a plurality of independent claims in the same category although the more usual case is a plurality of independent claims in different categories.

If a patent examiner determines that the claims in a patent application lack unity of invention, the patent agent will typically be required to elect certain claims and cancel or remove the non-elected claims. However, the patent agent will typically be allowed to file another patent application with the unelected claims from the first application. The rule for unity of invention is essentially a fee-regulation mechanism that prevents patent offices from having to examine a plethora of separate inventions for an applicant who has only paid for examination of a single invention. As such, the finding of a lack of unity of invention is not typically a fatal flaw for a patent application, although it may incur additional fees for the client as well as additional delay.
In some jurisdictions such as the EPO, a lack of unity may be directly evident a priori, i.e., before considering the claims in relation to the prior art, or may only become apparent *posteriori*, i.e. after taking the prior art into consideration - e.g. a document within the state of the art shows that there is lack of novelty or inventive step in an independent claim, thus leaving two or more dependent claims without a common inventive concept.

The phrase *"restriction requirement",* which is US terminology related to a finding of lack of unity. The patent agent should bear in mind that the absence of unity of invention is not a fatal flaw; it is simply a mechanism that allows the government to collect additional fees. The patent agent will typically file a divisional application with the claims that have been restricted out of the application due to lack of unity of invention.

III. Claim Format

A patent claim is traditionally written as a single sentence in most jurisdictions. Each of these *"sentences"* is preceded by a number that becomes the claim's identifier, e.g. *"Claim 1"*. Although a patent claim is a single sentence, it is a heavily punctuated single sentence. The patent claims typically appear in a separate section towards the end of the application and the issued patent.

A patent claim has three parts:

1) The Preamble

   A preamble is an introductory phrase that identifies the category of the invention protected by that claim. For example, the invention may be an apparatus, article, composition, method or process. It is a good idea to keep the preamble consistent with the title of the invention. The claim can also recite an object of the invention in the preamble, but for the same reasons noted in the specification writing section the patent agent must use caution to avoid accidentally limiting the scope of the invention.

2) The Transitional Phrase

   There are two types of transitional phrases:

   i. open-ended and
   ii. closed phrases.

   Open-ended phrases do not exclude any additional, unrecited elements or method steps. In other words, open-ended phrases are inclusive, not exclusive. In the US for example, open- ended phrases include the terms *"comprising"*, *"including"*, *"containing",* and *"characterized by"*. These terms have been construed or interpreted to mean *"including the following elements but not excluding others".* The words *"comprising"* and *"including"* are the most commonly used transitional phrases in most jurisdictions.

3) The Body

   The body of the claim recites the elements and limitations of the claim. The body also explains how the different elements exist in relationship to one another. Basically, the body of the claim recites and inter-relates all the elements of the claim.

I claim:

1. [An apparatus], [consisting of]:
   [a pencil;
   an eraser attached to the pencil; and
   a light attached to the pencil].

[Preamble**]** **[**Transition Phrase**]** **[**Body**]**

IV. Claim Punctuations

To the novice, it might seem illogical, confusing or perhaps even insulting to discuss something as basic as how a patent claim is to be punctuated. Many topics related to patent claims are certainly much more exciting than punctuation. However, nearly every patent office sets forth strict requirements for how patent claims are punctuated and will not grant a patent application unless/until these seemingly arbitrary rules are followed to the letter. Thus, if the patent agent solely focuses on matters such as attuning his patent claims to the client's business needs, while not understanding proper patent claim format, he will find that his otherwise well-crafted patent claims will never be issued by any patent office anywhere in the world.

A comma typically separates the preamble from the transitional phrase and a colon typically separates the transition from the body. The body itself is typically broken into small paragraphs that define the logical elements of the claim. Many jurisdictions do not have specific laws requiring such punctuation but the patent agent should strive to make sure that the claim will be interpreted as he intends. Similarly, in many jurisdictions a claim *"element"* might not have a precise and/or legal meaning, with all the words of a claim simply being *"limitations"* to the claim. That said, the patent agent must write the claim in a manner that does not complicate claim interpretation by the patent examiner and later by courts and potential licensees. Thus, the *"elements"* of a claim are typically separated by semi-colons and the penultimate element ends with *"; and"*.

I claim: 1. An apparatus, comprising: a plurality of printed pages; a binding configured to hold the printed pages together; and a cover attached to the binding.	1.Preamble, Transition Phrase: Element (#1); Element (#2); and Element (#3).

V. Claim Sets

A set of claims in a patent specification will normally include one or more independent (or main) claims and a number of dependent or subsidiary claims (or sub claims) which depend on one or more preceding independent claim(s). All patent applications must contain at least one *"independent"* claim directed to the essential features of the invention, i.e. those features necessary to satisfy the legal requirements of novelty and inventive step. Each independent claim may be followed by one or more dependent claims concerning more specific embodiments of the invention recited in the independent claim. Any claim relating to a particular embodiment

includes also the essential features of the invention recited in the corresponding independent claim.

Any claim which refers to another claim will include all features of the other claim, even if they are not explicitly recited. Claims containing references to other claims are called "dependent claims." Since a dependent claim does not by itself define all the characterizing features of the subject-matter which it claims, expressions such as *"characterized in that"* or *"characterized by"* are not necessary in such a claim but are nevertheless permissible. A claim defining further particulars of an invention may include all the features of another dependent claim and should then refer back to that claim. Also, in some cases a dependent claim may define a particular feature or features which may appropriately be added to more than one previous claim (independent or dependent). It follows that there are several possibilities: a dependent claim may refer back to one or more independent claims, to one or more dependent claims or to both independent and dependent claims.

Independent Claims

The independent claims in a patent represent the broadest claims. Some independent claims are broader than other independent claims but a given independent claim is always broader than any claim that depends on it. An independent claim is a claim that stands alone and does not need a limitation from another claim in order to be complete. Each claim set begins with an independent claim. A patent application may have more than one independent claim. For instance, sometimes a single invention might encompass several different inventive concepts, in which case it may not be possible to have one broad claim that covers all the different inventive concepts. In general, it is wise to have several independent claims, each of which separately covers a different inventive concept. Some jurisdictions such as the EPO may prefer that the number of independent claims be limited to one independent claim in each category.

Many patent agents find that claim limiting rules are not particularly well enforced and/or that exceptions are easy to find. As a general rule, among the world's three large patent offices, US patents tend to have the most claims; Japanese patents tend to have the fewest claims and the EPO tends to be in the middle. As with all patent matters, the patent agent should strive to make sure that his client has been accorded the appropriate number of claims for his invention. Experience will teach one the moment when adding more claims reaches the point of diminishing returns in terms of the additional cost in excess claim fees, annuity fees, etc.

An independent claim should typically specify the essential features needed to define the

invention except insofar as such features are implied by the generic terms used. If a claim is to a process for producing the product of the invention, the process as claimed should be one which, when carried out in a manner which would seem reasonable to a person skilled in the art, necessarily has as its end result that particular product.

Dependent Claims

A dependent claim is one that depends from another claim – either an independent claim or another dependent claim. Such dependencies are signaled by the identification of parent claim. For example: *"2. The apparatus of Claim 1, further comprising…"* indicates that Claim 2 is dependent from Claim

1. The format of a dependent claim really provides little more than a time and money-saving mechanism. By reciting another claim, the dependent claim is saying that it includes everything from the parent claim plus whatever is newly-recited in the dependent claim itself. Dependent claims tend to be considerably shorter than independent claims and patent novices sometimes mistakenly believe that dependent claims are broader than independent claims when the complete opposite is correct. Dependent claims are always narrower than the claim from which they depend. A dependent claim can only add elements or limitations to the claim to which it refers. It cannot subtract any elements or limitations from the same.

Dependent claims should be grouped together in the most appropriate way possible. The arrangement must, therefore, be one which enables the association of related claims to be readily determined and their meaning in association to be readily construed. In no way can a dependent claim extend the scope of protection of the invention defined in the corresponding independent claim.

A patent examiner will sometimes allow a dependent claim over the prior art and merely object that the claim depends from a rejected independent claim. This means that the patent applicant can obtain a patent by simply canceling the rejected independent claim (and any other intervening dependent claims) and add the cancelled limitations to the allowable dependent claim. The patent agent can also amend the other claims in the application to depend from the newly independent (formerly dependent) claim. Of course, a patent agent and his client may decide not to accept the allowance of the dependent claim and continue to fight for the patentability of the parent claim.

Multiple Dependent Claims

Multiple dependent claims provide another format for dependent claims. The preamble of a multiple dependent claim refers to more than one claim in the alternative. For example, a

preamble of a multiple dependent claim might read *"the apparatus of Claim 1 or Claim 2"* or *"the apparatus of one of Claims 1 and 2"*. Here, Claims 1 and 2 are referred to in the alternative – meaning the claim depends on Claim 1 or Claim 2 but not both. Like dependent claims, the body of a multiple dependent claim must narrow the claim from which it depends. In some jurisdictions multiple dependent claims may not depend on another multiple dependent claim. Like many aspects of patent practice, different jurisdictions may have different formatting requirements for multiple dependent claims and the patent agent must make his claims conform to the precise requirements for the jurisdictions of interest to his client.

Omnibus Claims

An omnibus claim is a claim which refers to the description and/or drawings as the subject matter of the claim (Omnibus is a Latin word meaning *'For all, for everyone'*). Omnibus claims limit the claim scope to only what the applicant actually disclosed. Despite of this however, there have been cases where the omnibus claim was the only claim left at the end of validity challenges and sometimes the sole remaining claim upon which to base an infringement action. Therefore for patent applications that are not very strong, omnibus claims can play an integral role to serve the purpose of filing the patent application and forms the sole basis of protection of claimed novelty. A key difference between omnibus claims and independent/dependent claims is that while non-omnibus claims draw boundaries of what is being claimed as the novelty and dependent features of novelty, omnibus claims expand those boundaries to include the parts of the invention mentioned in the specification and drawings but not expressly mentioned in the claims. This is why an omnibus claim is usually added as the last claim in order to include the drawings and description within the scope of the claims and ensuring that no aspect of the invention has been missed out. Hence, inclusion of omnibus claims is recommended in jurisdictions where applicable, for the sake of protecting whatever is disclosed in the drawings and specification apart from the claim.

Omnibus claims can be narrowly or broadly drafted based on the patent application and scope of coverage, and can also include both narrow and broad omnibus claims. The narrow omnibus claims are intentionally limited in scope.

I claim:

1. An apparatus, comprising: a pencil and a light attached to the pencil.
2. The apparatus of Claim 1, wherein the light is detachably attached to the pencil.
3. A pencil as recited in Claims 1 or 2, further comprising an eraser.
4. An apparatus, substantially as herein described.

Claim1: Independent

Claim 2: Dependent (on Claim 1)

Claim 3: Multiple Dependent (on Claim 1 & 2)

Claim 4: Omnibus

VI. Two-part claim or Improvement Claims

In a two-part claim (also known as an *Improvement Claim* or a *Jepson Claim*), the preamble of the claim sets out the most relevant known prior art, and the body characterizes the improvement of the invention. The preamble and body are connected by a specific transitional phrase that signals the claim is a *two- part claim* or *Jepson claim*. Thus, two-part claims still have a preamble, a transition and a body, but with a two-part claim, the preamble is the statement of the prior art, the transition is a phrase such as *"characterized by"*, and the body provides the novelty.

In Europe, for example, the preamble is followed by the transition *"characterized in that"* or *"characterized by"*. In the US the preamble is typically followed by the transition *"wherein the improvement comprises…"* The preamble should typically reference only a single piece of prior art since the preamble is considered an implied admission that it is prior art.

Some jurisdictions such as the EPO have a preference for two-part claims. The EPO advises that applicants should follow the two-part formulation in claims where, for example, it is clear that the invention resides in a distinct improvement in an old combination of parts or steps. As with many rules created for bureaucratic efficiency this *"preference"* is somewhat flexible in actual practice. Thus, patent agents need to consider whether conformity with the two-part preference is in their client's best interest given that it requires an explicit admission that certain parts of the claims are definitely in the prior art. Some patent agents may wish to recite their claims initially in a conventional form and then see if (and/or how determined) the examiner is in requiring the

two-part form. On other occasions, the client may be best served by drafting claims in the two-part format from the beginning, given the nature of the invention and the prior art.

While expressing a preference for two-part claims the EPO concedes that such claims are inappropriate in some circumstances. Thus, the nature of an invention may be such that this form of claim is unsuitable, e.g. because it would give a distorted or misleading picture of the invention or the prior art.

I claim:

1. A pencil having an eraser, wherein the improvement comprises a light attached to the pencil.

In this claim, a pencil having an eraser is the relevant known prior art and the claimed improvement is the attached light.

Examples of the kind of invention which may require a different presentation are:

1) the combination of known integers of equal status, the inventive step lying solely in the combination;
2) the modification of, as distinct from addition to, a known chemical process e.g. by omitting one substance or substituting one substance for another; and
3) a complex system of functionally inter-related parts, the inventive step concerning changes in several of these or in their inter-relationships.

VII. Means-plus-function Claims

Means-plus-function claims recite elements that do not have specifically-defined structures but instead recite functions performed by structures disclosed in the specification. The interpretation of means- plus-function claims varies from jurisdiction to jurisdiction and even varies within jurisdictions over time. For example, a given jurisdiction may interpret a means-plus-function claim as the means disclosed in the patent's specification for performing the recited function plus the reasonable equivalents of those means. Means-plus-function claims could receive either a broad or narrow interpretation in a given jurisdiction since the claims do not specifically define the structure. Litigants in patent infringement cases sometimes expend considerable energy arguing over whether or not an asserted claim even is a means-plus-function claim. The format of a classic means-plus-function claim is the word *"means"* followed by a function.

Not all the elements in a means-plus-function claim need to be means elements. In other words, each element of a claim can receive different treatment. Assume, for example, that a claim recites three elements, two in means-plus-function format and one that recites a structural element. The structural element will typically be construed according to its ordinary meaning in the art. Each of the two means- plus-function elements will be construed by first determining the recited function and then respectively determining the structure disclosed in the specification for performing the function.

I claim:

1. An apparatus for cooking rice,
    comprising a means for
    holding rice, and
    a heater configured to heat the rice holding means.

Instead of restricting a rice holding structure by name (like a bowl) – a device is referenced that performs the function of holding rice. Thus specific name is avoided but only function it performs is recited; although the invention is for a rice cooker.

Means-plus-function claims are helpful in jurisdictions where such claims receive broader interpretation than claims that specifically recite a structural element. Means-plus-function claims are even helpful in jurisdictions that do not necessarily afford a broad interpretation to means-plus-function claims but nevertheless interpret such claims differently from claims where the structural limitations are affirmatively recited. The *"difference"*, whatever it might be, allows for a more complete range of claim coverage – assuming the patentee includes both types of claims in his application. Also, claims interpretation by courts has a tendency to change over time.

VIII. Markush Claims

A Markush claim is a claim that defines multiple *"functionally equivalent"* alternative entities for one or more of the features of the invention. This type of claim is mainly encountered in the chemistry field. Generally, there will be a consistent core structure that provides the activity of a compound. However even relatively straightforward Markush structures might comprise several thousand compounds, while more complex structures have been estimated to include in the order of 10^{61} compounds (*The number of actual known compounds number in the order of* 10^{7}).

Where a single claim defines (chemical or non-chemical) alternatives, i.e. a so-called *"Markush*

grouping", unity of invention should be considered to be present if the alternatives are of a similar nature. When the Markush grouping is for alternatives of chemical compounds, they should be regarded as being of a similar nature where:

1) all alternatives have a common property or activity, and
2) a common structure is present, i.e. a significant structural element is shared by all of the alternatives,
3) or all alternatives belong to a recognized class of chemical compounds in the art to which the invention pertains.

A *"significant structural element is shared by all the alternatives"* where the compounds share a common chemical structure which occupies a large portion of their structures or, in case the compounds only have in common a small portion of their structures, the commonly-shared structure constitutes a structurally distinctive portion in view of existing prior art. The structural element may be a single component or a combination of individual components linked together. The alternatives belong to a *"recognized class of chemical compounds"* if there is an expectation from the knowledge in the art that members of the class will behave in the same way in the context of the claimed invention, i.e. that each member could be substituted one for the other with the expectation that the same intended result would be achieved. If it can be shown that at least one Markush alternative is not novel, unity of invention should be reconsidered. However, it should be noted that the mere existence of compound(s) falling within the scope of a claim is not unusual and will rarely result in an objection of lack of unity. This may be an over-technical approach which at its most extreme would result in an objection of lack of unity. When in such cases, a novelty objection will be taken that will generally result in the applicant amending the claim to remove the prior art compound(s). The Examiner should take a broad consideration of the relationship between the alternatives. In these situations the issue may be closely linked to inventive step.

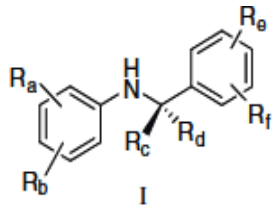
The key consideration for unity is whether there is a common or corresponding special technical feature between alternatives. In the case of a Markush structure this requirement is met when the alternatives (that is the compounds defined by the claim) are of a *similar nature***.**

In chemical cases, a claim directed to a genus expressed as a group consisting of certain specified materials is allowable, provided it is clear from the known nature of the alternative materials or from the prior art that the materials in the group possess at least one property in common which is mainly responsible for their function in the claimed relationship. Therefore, a Markush claim will generally be construed with a generic expression covering a group of two or

more different materials (elements, radicals, compounds).

I claim:

1. A compound of structural formula I



I

where: R_a, R_b, R_e, and R_f are each independently selected from cholorine, methyl, ethyl, propyl, or methoxy; and Rc and Rd are each independently selected from hydrogen or C_{1-3} alkyl.

IX. Swiss-type Claims

In Europe, a Swiss-type claim or *"Swiss type of use claim*" is a formerly used claim format intended to cover the first, second or subsequent medical use (or indication of efficacy) of a known substance or composition, as it was a decision of the *Swiss Federal Intellectual Property Office* in 1984 that first allowed patents with this type of claims.. A Swiss claim is directed at the manufacture of the known substance, ensuring that the invention is not excluded on the basis that it is a method of medical treatment. The novelty of a Swiss claim arises from the new therapeutic application (the drug and first medical use always being known), with the result that the focus of the patent shifts so that novelty of the invention is not in the way the substance is used, nor in relation to the substance itself. In certain countries, including New Zealand,[46] the Philippines,[47] and Canada, methods of medical treatment are not patentable either. however *"Swiss-type claims"* are allowed.

Swiss-type claims usually define a second medical use with the wording *"use of substance X for the preparation of a medicament (or pharmaceutical composition) for treating indication Y"* – have since been superseded by revised legislation in the EPC 2000 which permits second medical uses to be protected by the wording *"product X for treating indication Y"*. The biggest difference is that a Swiss- type claim is a process, and the other is a product claim, the infringement of which are covered by different subsections of the UK Patents Act 1977. However, many patents containing Swiss-type claims are still in force, and as the scope of these claims differs from the new EPC 2000 type second medical use claims, the recent judgements could have a far reaching impact for years to come.

I claim:

1. The use of compound mefloquine in the manufacture of a treatment for malaria.

X. Proper Antecedent Basis

The elements in a patent claim must have the correct antecedent basis. This means that the first time an element is introduced, the indefinite article *"a"* or *"an"* should be used. Later when referring back to previously introduced elements, the definite article *"the"* or *"said"* should be used. Proper antecedent basis is not just a good idea - it is the law. In each new claim set the antecedent basis must be re- established. In essence, each independent claim needs to be drafted independently and with proper antecedent basis.

I claim:

1. A device, comprising:
   a pencil; and
   a light attached to the pencil.
2. The device recited in claim 1 wherein the light is detachably attached to the pencil.
3. The device recited in claim 2 wherein the pencil is red in color.

In Claim 1, "pencil" is introduced for the first time by referring to it as "a pencil". In the same claim, light is also introduced for the first time as "a light". However, when one wants to specify that the light was attached to the pencil, pencil is referred as "the pencil". The use of the word "the" signaled that the pencil was the one that had previously defined in the claim. Otherwise, there would be ambiguity as to whether it was the same pencil or another pencil. The words "the" and "said" are interchangeable in claims drafting.

Similarly, for Claims 2 & 3.

XI. Reference Numerals

In some jurisdictions, claims are encouraged and/or required to recite the reference numerals associated with particular elements in the patent application's drawings. Thus, if Figure 1 of the patent shows a computer memory and this computer memory is labeled *"123"*, for example, if

the claims recite this particular computer memory, the computer memory element will be followed by the reference number *"123"*. Thus, if the application contains drawings and the comprehension of the claims would be improved by establishing the connection between the features mentioned in the claims and the corresponding reference signs in the drawings, appropriate reference signs should be placed in parentheses after the features mentioned in the claims. If there are a large number of different embodiments, only the reference signs of the most important embodiments typically need be incorporated in the independent claim(s). Where claims are drafted in the two-part form, the reference signs should be inserted not only in the characterizing part but also in the preamble of the claims according to recommendations from the EPO. This advice may not apply to all jurisdictions.

I claim:

1. An apparatus, comprising:

a plurality of printed pages (11);

a binding (14) configured to hold the printed pages (11) together; and

a cover (21) attached to the binding (14).

The numbers in parenthesis are the reference numbers from the patent application's drawings.

****

I claim:

1. The crystalline Form II according to any preceding claim that is characterized by a powder X-ray diffraction pattern substantially as shown in Figure 1.

The crystalline Form is tagged to Figure 1 (pXRD).

XII. Bracketed Expressions

If text is added to reference signs in parentheses in the claims, lack of clarity can arise. Expressions such as *"securing means (screw 13, nail 14)"* or *"valve assembly (valve seat 23, valve element 27, & valve seat 28)"* may not be considered as mere reference signs but as *"special features"*. Consequently, it is unclear whether the features added to the reference signs are limiting or not. Accordingly, such bracketed features are not generally permissible or recommended.

In some jurisdictions a lack of clarity can also arise with bracketed expressions that do not include reference signs, e.g. *"(concrete) molded brick"*. In contrast, bracketed expressions with a

generally accepted meaning are allowable, e.g. *"(meth)acrylate"* which is known as an abbreviation for *"acrylate and methacrylate"*. Thus, the use of brackets in chemical or mathematical formulae is typically unobjectionable.

XIII. Claim Phrases

Words like *"comprising"* have a special meaning when applied to claims. Similarly, other words can have special meanings when applied to patent claims. Some words are used to further define a structure or provide a function associated with a given structure. Some of these words are *"wherein"*, *"whereby"*, and *"such that"*, and *"so as to"*. The patent agent must know how the courts in the jurisdictions of interest have opted to interpret these words and then he must use them in a manner appropriate to

their legal interpretation. For example, a *"wherein clause"* is generally used to describe either a function, operation or result that flows from the previously-recited structure or function of the claim. Thus, *"wherein clauses"* should be used where the result necessarily follows the recited structure or function.

I claim:

1. A folder for keeping files, wherein the folder is configured to receive the files…

A claim for a folder for keeping files, the wherein format is effectively employed.

XIV. Multiple Elements

Many Patent Offices require claims to recite at least two elements. A patent claim without many limitations can be impossibly broad. However, in many cases the specification will define an element and we can also assume that element in discussion exists in the prior art.

I claim:

1. A computer, comprising:
   a processor.
2. A computer, comprising:
   a processor;
   a memory; and
   a bus configured to transmit data between the memory and the processor.

Claim 1 does not tell much about a computer other than that it is something containing a processor.

Thus, the applicant appears to be claiming anything that contains a processor especially if the preamble is not considered to be limiting. Such a claim is impossibly broad.

XV. Specific Types of Claims

For many inventions, claims in more than one category are needed for full protection. One could argue that there are only two basic kinds of claim: claims to a physical entity (*product, apparatus*) and claims to an activity (*process, use*).

The first basic kind of claim (product claim) includes a substance or compositions (e.g. a chemical compound or a mixture of compounds) as well as any physical entity (e.g. object, article, apparatus, machine or system of co-operating apparatus) which is produced by a person's technical skill. The second basic kind of claim (process claim) is applicable to all kinds of activities in which the use of some material product for effecting the process is implied; the activity may be exercised upon material products, upon energy, upon other processes (as in control processes) or upon living things.

Listed below are the major specific type claims:

1) Apparatus or Device Claims

   An apparatus or device claim protects embodiments of an invention in the form of a physical apparatus, system or device. When drafting an apparatus claim, the patent agent can begin by reciting in a preamble what the apparatus is and what it does. Next, the

patent agent can list the essential elements of the invention. Essential elements are ones required for the functioning of the inventive device in its most basic form, e.g. the essence of the invention. The novelty of the invention lies in the essential components.

I claim:

1. An apparatus for supporting a camera, comprising:
    a pivotal mounting configured to hold the camera; and
    a plurality of legs arranged to support the pivotal mounting.

The preamble recites that it is an apparatus for supporting a camera. The body of the claim recites that the essential elements of this apparatus are a pivotal mounting for the camera and legs arranged to support the pivotal mounting.

2) Method Claims or Process Claims

Method claims are claims that recite a sequence of steps which together complete a task such as making an article of some sort. However, note that in many jurisdictions the steps performed in a method claim are presumed to occur in any order, unless otherwise stated, for both prior art and infringement purposes. The patent agent will always want to look for words and limitations that can be removed from claims.

I claim:

1. A method for making tea, the method comprising:
    boiling water;
    adding sugar to the boiling water;
    adding tea leaves to the boiling water to form a mixture;
    adding milk to the mixture; and
    filtering the mixture.

This claims a method or process involving the series of steps performed in the process of making tea are stated sequentially in the order they are performed.

3) Product-by-Process Claims

Claims for products defined in terms of a process of manufacture are allowable in some jurisdictions provided that the products as such fulfill the requirements for patentability, i.e. they are new and inventive. A product is not typically rendered novel merely by the fact that it is produced by means of a new process. A claim defining a product in terms of a process will be construed as a claim to the product as such in many jurisdictions. The claim may for instance take the form *"Product X obtainable by process Y"*. Irrespective of whether the term *"obtainable", "obtained", "directly obtained"* or an equivalent wording is used in the product- by-process claim, it is still directed to the product per se and confers absolute protection upon the product.

In some jurisdictions such as the EPO, if the subject-matter of a patent is a process, the protection conferred by the patent extends to the products directly obtained by such process. Many jurisdictions apply similar tests for product-by-process claims as described above. Other jurisdictions treat product-by-process claims as method claims. Consequently, the patent agent should verify that a product-by-process claim is the best approach for protecting his client's invention before employing this type of claim. Such claims may be employed as part of a mix of claim formats.

I claim:

1. A wafer for production of an LED prepared by a process comprising the steps of:

(a) providing an n-GaAs single crystalline substrate;

(b) contacting the substrate with a melt comprising GaAs and Si in an amount of from 0.1 to 0.5 wt% at a temperature of from 900 to 950EC;

(c) cooling the melt while maintaining contact with the substrate at a rate of 5E/min to form a Si-doped GaAs epitaxial layer on the substrate comprising a pn junction; and

(d) separating the substrate from the melt.

The claim relates to an LED wafer prepared by a liquid phase epitaxy (LPE) process.

4) Result to be Achieved & parameter Claims

As a general rule, claims which attempt to define the invention by a result to be achieved will not be allowed, particularly if they only amount to claiming the underlying technical problem. In fact, many jurisdictions will never allow such claims under any circumstances. Additionally, the patent agent must strive to capture the essence of his client's invention and characterizing a product by its parameters may easily lead to a

claim that is significantly narrower than the client's invention.

Some jurisdictions such as the EPO, may allow such claims if the invention can either only be defined in such terms or cannot otherwise be defined more precisely without unduly restricting the scope of the claims and if the result is one which can be directly and positively verified by tests or procedures adequately specified in the description or known to the person skilled in the art and which do not require undue experimentation.

Similarly, where the invention relates to a product, it may be defined in a claim in various ways, such as a chemical product by its chemical formula, as a product of a process (if no clearer definition is possible) or, exceptionally, by its parameters. However, the patent agent is advised to use caution when drafting such claims as they may not be accepted and/or may be subject to later misinterpretation.

Parameters are characteristic values which may be values of directly measurable properties (e.g. the melting point of a substance, the flexural strength of steel, the resistance of an electrical conductor) or may be defined as more or less complicated mathematical combinations of several variables in the form of formulae.

I claim:

A method for treating pain in a human subject in need of acute or chronic pain relief, comprising the steps of:

providing a solid oral dosage form comprising about 5 mg to about 80 mg oxymorphone or a pharmaceutically acceptable salt thereof in a controlled release delivery system with a release rate profile designed to provide an adequate blood plasma level over at least 12 hours to provide sustained pain relief over this same period, the system comprising a filler and a hydrophilic material, wherein oxymorphone is the sole active ingredient; and,

administering the dosage form to the subject, wherein the oxymorphone C_{max} is at least about 50% higher when the dosage form is administered to the subject under fed versus fasted conditions.

This claims method for treating pain for acute or chronic pain relief using a solid oral dosage of oxymorphone.

5) Composition Claims

Claims related to compositions are used where the invention to be claimed has to do with the chemical nature of the materials or components used. While drafting claims it is up to

the patent agent to claim each of the elements as narrowly or as broadly as necessary in view of the prior art, the scope of the invention and other relevant factors.

> I claim:
>
> 1. A copper electroplating solution, comprising:
>
> an alkaline solution of copper sulfate, from 30-50 grams per liter;
>
> sulfuric acid, from 2-4 times the copper acetate solution; and
>
> an aqueous solution of a pH-modifying substrate in an amount sufficient to adjust the pH to a value of from 3.5-5.0.

6) Use Claims

Some jurisdictions permit claims to new uses of known substances, particularly second or subsequent medical uses or indications of known substances and compositions. These use claims are also known as Swiss-type claims since Switzerland was the first country to allow them.

Use claims are not permitted in all jurisdictions. Such claims are permitted in the EPO, even though the EPO generally, does not allow claims directly to a method of treating the human body. Such claims, however, are not permitted in the US or India, for example.

> I claim:
>
> 1. The use of recombinant insulin in the manufacture of a treatment for diabetes..

7) Biotechnology Claims

Biotechnology in general relates to all practical uses of living organisms. Uses for biological/life science inventions may be either commercial or therapeutic. Thus, biotechnology inventions may include cDNA, recombinant DNA, DNA fragments, protein, monoclonal antibodies, anti-sense DNA and RNA, recombinant vectors and expression vectors. Where an invention involves a biological material and words alone cannot sufficiently describe how to make and use the invention in a reproducible manner, access to the biological material may be necessary for the satisfaction of the statutory requirements for patentability

The Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure was established in 1977 to facilitate the recognition

of deposited biological material in patent applications throughout the world.

I claim:

1. An isolated polynucleotide comprising a member selected from the group consisting of:
   (a) a polynucleotide encoding a polypeptide comprising amino acid 1 to amino acid 255 as set forth in SEQ ID NO:2; and
   (b) a polynucleotide which hybridizes to and which is at least 95% complementary to the polynucleotide of (a).
2. The polynucleotide of claim 1 comprising nucleotide 1 to nucleotide 1080 of SEQ ID NO: 1.

****

I claim:

1. *Aspergillus niger* ATCC 1015.

8) Software Clams

Patent applications related to computer software and/or hardware devices that execute specialized algorithms typically include apparatus and method claims. Such applications also often contain some specialized claim formats for software inventions. The acceptable claim formats for computer software inventions may vary from country to country. Some acceptable formats may include computer-readable medium claims, data structure claims and propagated signal claims. The software arts have some other specialized claim types, but these are typically modifications of more basic claim types and best left for an advanced treatise.

I claim:

1. A computer readable device storing print control instructions to be executed by a computer communicatively connected to at least one printer via a network, the print control instructions comprising at least a monitoring program, wherein:

the monitoring program, when executed by the computer, causes the computer to function as:

a monitoring module configured to monitor setting information in which location information of the printer on the network is written to detect writing of the location information, and

a port setting module configured to, upon detection of the writing of the location information, set a logical print port having the written location information set therein as a print port to be used.

****

I claim:

1. A method for preventing hacker attacks and virus infection in a computer system comprising:

prompting a user to perform a switching allowing temporarily writing to a write- protectable storage area, wherein a respective physical write-protection mechanism comprises a switching means which is exclusively operable from outside of an operating system of said computer system;

storing security-relevant information into said write-protectable storage area;

prompting a user to perform a manual switching to restrict writing to said write- protectable storage area, thus resulting in generating a write-protected storage area; restricting any write access to said write protected storage area in response to said manual switching during further runtime of said computer system.

A computer-readable medium claim, also known in the US as a *Beauregard claim*, attempts to protect an invention when it is embodied in a particular medium, e.g. a CD ROM. Such claims, which can have several different formats, would allow a patent holder to seek damages not only against persons who made infringing software and persons who used infringing software but also to seek damages against sellers of such software, including retail sellers and wholesalers. One of the more common formats is to take the body of a method claim for the invention and add a "*computer-readable medium"* preamble.

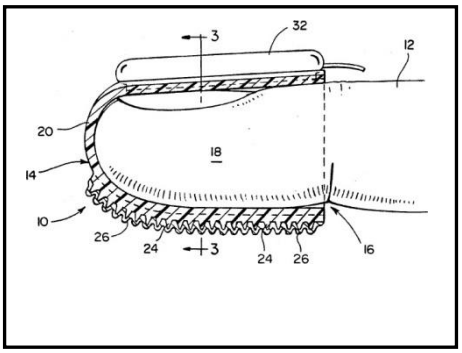
A data structure claim, also known in the US as a *Lowry claim*, attempts to provide protection for novel computer data structures. Of course, not every computerized invention includes a unique data structure, but some inventions do include new data structures.

Following points should be considered while drafting patent claims:

1) Each claim should be a single sentence and should be clearly worded.
2) Each claim should be precise and without unnecessary repetition.
3) Rights are given to claims only, not for any matter described in the complete specification.
4) Claims define the boundaries of legal protection and form a protective fence around the invention.
5) Each claim is evaluated on its own merit and, therefore, if one of the claims is objected, it does not mean that the rest of the claims are invalid.

XVI. Sample Claims

BIODEGRADABLE TOOTHBRUSH



I claim:
1. A disposable toothbrush comprising:
a cap including an opening for receipt of a fingertip, a flat surface located on one side of said cap having bristles projecting therefrom for brushing of teeth, a layer of dehydrated toothpaste being located on said bristles, and
at least one dental hygiene accessory located on a side of said cap opposite to said one side,
said at least one dental hygiene accessory being located within a capsule slidably mounted on said cap.

ADJUSTABLE PEDAL ASSEMBLY WITH ELECTRONIC THROTTLE CONTROL

What is claimed is:

1. A vehicle control pedal apparatus **(12)** comprising:

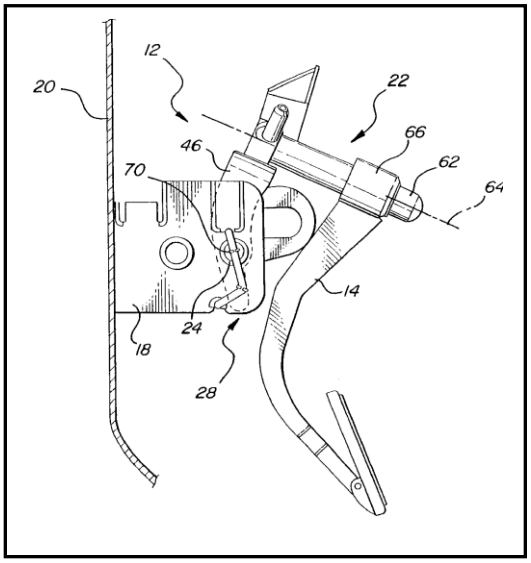
a support **(18)** adapted to be mounted to a vehicle structure **(20);**

an adjustable pedal assembly **(22)** having a pedal arm **(14)** moveable in fore and aft directions with respect to said support **(18);**

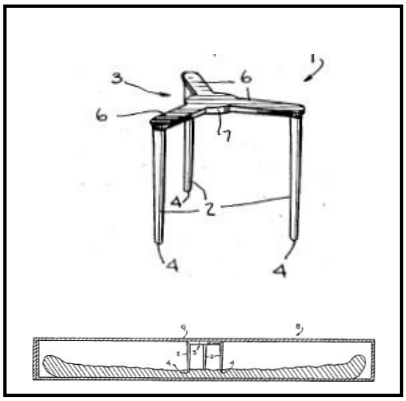
a pivot **(24)** for pivotally supporting said adjustable pedal assembly **(22)** with respect to said support **(18)** and defining a pivot axis **(26);** and

an electronic throttle control **(28)** attached to said support **(18)** for controlling an engine throttle **(30);**

said apparatus **(12)** characterized by said electronic throttle control **(28)** being fixed relative to said vehicle structure **(20)** such that said pedal arm **(14)** moves in fore and aft directions with respect to said electronic throttle control **(28),** said electronic throttle control **(28)** being responsive to said pivot **(24)** for providing a signal **(32)** that corresponds to pedal arm position as said pedal arm **(14)** pivots about said pivot axis **(26)** between rest and applied positions.



PACKAGE SAVER



Having thus described my invention, I claim:

1. in combination a package having a flexible cover, food article packaged therein and spaced downwardly from the cover, a unitary molded plastic package saving device for positioning between the cover and the article for supporting the package cover thereby preventing damage to the packaged food article by the cover, said device comprising the combination of three or more spaced legs, each leg having one relatively flat end adapted for engaging the packaged article and having its opposite end attached to a device cover portion.

2. The combination as claimed in claim **1** in which said device cover portion comprises a number of flat cover sections radiating from a common flat central portion.

3. The combination as claimed in claim **1** wherein said device is formed of heat resistant plastic.

4. The combination as claimed in claim **1** wherein said device is formed of thermo-setting plastic.

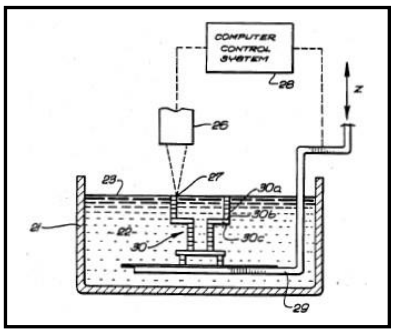
APPARATUS FOR PRODUCTION OF THREE-DIMENSIONAL OBJECTS BY STEREOLITHOGRAPHY

I claim:

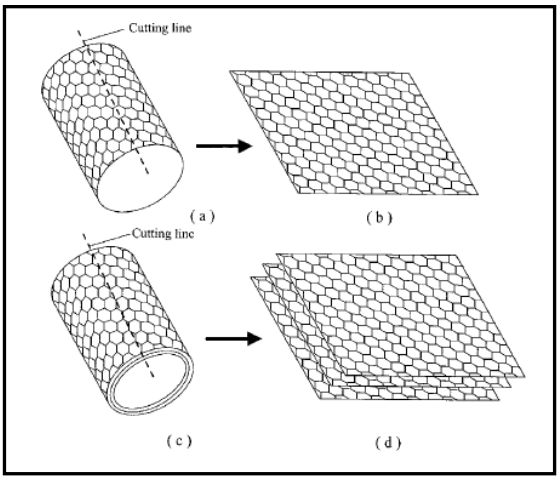
1. A system for producing a three-dimensional object from a flnid medium capable of solidification when subjected to prescribed synergistic stimulation, said system comprising:

means for drawing upon and forming successive cross-sectional laminae of said object at a two-di-mensional interface; and

means for moving said cross-sections as they are formed and building up said object in step wise fashion, whereby a three-dimensional object is extracted from a substantially two-dimensional surface.



NANO-SCALED GRAPHENE PLATES



What is claimed is:

1. A nano-scaled graphene plate material comprising at least a nanometer-scaled plate with said plate comprising a sheet of graphite plane or a multiplicity of sheets of graphite plane; said graphite plane comprising a two-dimensional hexagonal lattice of carbon atoms and said plate having a length and a width parallel to said graphite plane and a thickness orthogonal to said graphite plane characterized in that the length, width, and thickness values are all smaller than 10 nanometers.

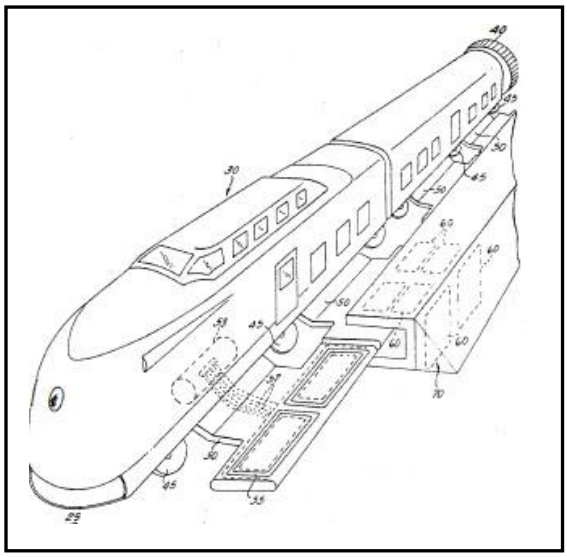
2. A composite material containing a matrix material and a predetermined proportion of nano-scaled graphene plate material as a reinforcement material, said nano-scaled graphene plate material comprising at least a nanometer-scaled plate with said plate comprising a sheet of graphite plane or a multiplicity of sheets of graphite plane; said graphite plane comprising a two-dimensional hexagonal lattice of carbon atoms and said plate having a length and a width parallel to said graphite plane and a thickness orthogo-nal to said graphite plane characterized in that the length, width, and thickness values are all 100 nanometers or smaller.

ELECTROMAGNETIC INDUCTIVE SUSPENSION AND STABILIZATION SYSTEM FOR A GROUND VEHICLE

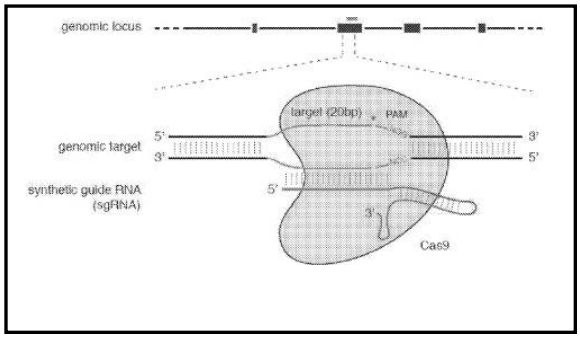
What is claimed is:

1. An electromagnetic inductive suspension and stabilization system for a ground vehicle, comprising in combination, a plurality of superconducting magnets carried by the vehicle and a track bed formed by a longitudinally 25 extending series of oriented shorted loops composed of metal conductors, said magnets and said shorted loops being arranged in electromagnetic inductive relationship to each other, whereby, when the vehicle is propelled at speeds above a certain transitional sreed, there is generated 30 a suspension force for floating the vehicle above the ground and for maintaining the vehicle at an equilibrium position above the track bed, stabilized against vertical displacements.

2. A svstem in accordance with claim l, in which the 35 said track bed comprises additional longitudinally extend- ing series of oriented ·shorted loops, whereby, when the vehicle is propelled at speeds above a certain transitionai speed, there are generated, in addition to said suspension force, restoring and damping forces to stabilize the w- 40 hicle against lateral, rotational and oscillatory displace-ments.



CRISPR-CAS SYSTEMS AND METHODS FOR ALTERING EXPRESSION OF GENE PRODUCTS



1. A method of altering expression of at least one gene product comprising introducing into a eukaryotic cell containing and expressing a DNA molecule having a target sequence and encoding the gene product an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)—CRISPR associated (Cas) (CRISPR-Cas) system comprising one or more vectors comprising:

a) a first regulatory element operable in a eukaryotic cell operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA that hybridizes with the target sequence, and

b) a second regulatory element operable in a eukaryotic cell operably linked to a nucleotide sequence encoding a Type-II Cas9 protein,

wherein components (a) and (b) are located on same or different vectors of the system, whereby the guide RNA targets the target sequence and the Cas9 protein cleaves the DNA molecule, whereby expression of the at least one gene product is altered; and, wherein the Cas9 protein and the guide RNA do not naturally occur together.

VIRTUAL REALITY GENARATOR FOR DISPLAYING ABSTRACT INFORMATION

What is claimed is:

1. A virtual reality generator to display abstract information as a multi-dimensional information terrain, the virtual reality generator comprising:

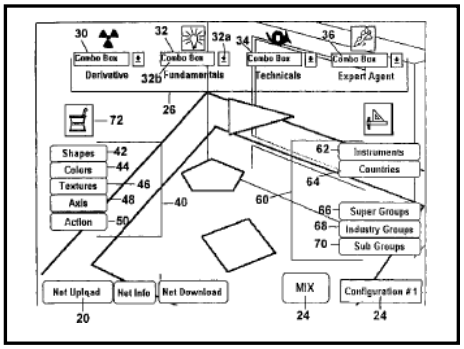
an input module receiving the abstract information from an information source, the information source generating the information as a function of a predetermined analysis of real-time and pre-stored data;

a user interface module including a first input selecting a categorical dimension for each of a first dimension of

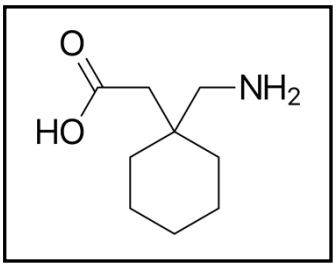
a multi-dimensional information terrain and a second dimension of the multi-dimensional information terrain and a second input for selecting a numerical dimension 50 for a third dimension of the multi-dimensional information terrain, the user interface module selecting a portion of the abstract information as a function of the categorical dimensions and the numerical dimension; and

a virtual reality generator module coupled to the input module and the user interface module, the virtual reality generator generating, continuously modifying and displaying on a display device a multi-dimensional information terrain that enables a user to simulate movement through and interact with the abstract information, the information terrain representing selected portions of the information,

wherein when the user simulates movement through and interacts with the abstract information, the user viewing the display device has a sensation of traveling through and within the information terrain.



PROCESS FOR THE PREPARATION OF GABAPENTIN



Claims

1) A process for the preparation of gabapentin which comprises:

a. the Hofi:nann rearrangement of 1,1-cyclohexanediacetic acid monoamide;

b. the precipitation of gabapentin by acidification of the reaction mixture obtained by said rearrangement to a pH comprised between 4 and 6.3 with an organic or inorganic acid.

2) A process according to claim 1 wherein the reaction mixture is acidified to a pH comprised between 5.5 and 6.3.

3) A process according to claim 1 wherein the reaction mixture is acidified to a pH value around 6.2-6.3.

4) A process according to claim 1 wherein the acidification of the reaction mixture is carried out with an organic acid.

5) A process according to claim 1 wherein the acidification of the reaction mixture is carried out with an acid selected among acetic, citric, hydrochloric, formic, maleic, methanesulphonic, oxalic and tartaric acid or mix1nres thereof.

WIRELESS INTERNET SYSTEMAND METHOD

The invention claimed is:

1. A computing device comprising:

a communication module adapted to:

(1) wirelessly connect said computing device to an IP based network via a first wireless access point (AP) having a first AP Identification (APID); and

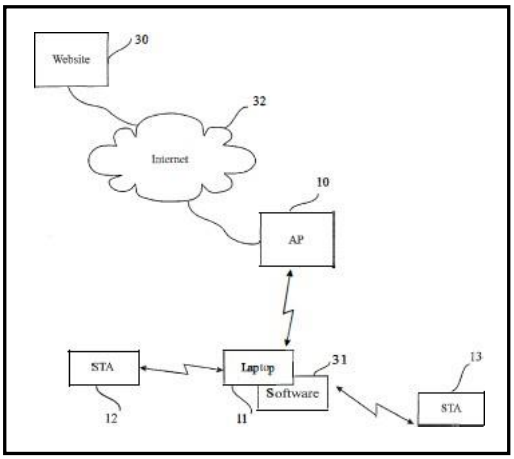
(2) wirelessly communicate with other wireless enabled computing devices ;

a user interface and display adapted to allow a user of said computing device to interact with destinations over the IP based network, through the first wireless AP, using a first public IP address; and

an AP module adapted to:

(1) provide a given device of the other wireless enabled computing devices with access to the IP based network by causing said computing device to serve the given device as a second AP having a second APID, distinct from the first APID, and provide the given device access to the network via the first AP; and

(2) tunnel data traffic from the given device, through said computing device, through the first AP, through the IP network, to a proxy server, such that the proxy server acts as a proxy of the given device and the data traffic is secure from said computing device and first AP and the given device operates on the network with a public IP address distinct from the first public IP address.



Tips on Drafting Claims:

9) Start claims on a new page and number each claim using Arabic numbers starting with 1.

10) Precede your claims with a short statement such as *"I/We claim: ..."*

11) Each claim should consist of a preamble, transition phrase and the body.

12) Figure out the all essential features or elements of your invention that you want to claim rights to.

13) Start with broadest claims of your invention and then progress to narrower claims.

14) The first claim would be the Independent claim and subsequent claim would be dependent claims.

15) All claims should be linked so as to form a single inventive concept.

16) There is no restriction to the number of claims to be incorporated in the specification. But the applicant

17) has to pay additional fee, if there are more than ten claims.

18) Claims must be supported by the description and should be based on the description. This means that all the characteristics of the invention that form the part of the claims must be fully explained in the description.

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CHAPTER VI

CLAIM CONSTRUCTION BY COURTS

Claim Construction by Courts

The greatest test of a patent agent's claims will likely come not before the patent examiner but before the courts if the patent is ever litigated. In patent litigation, the interpretation of the claims is typically the most critical factor in determining whether the patent has been infringed or is even valid over the prior art. The process of interpreting the claims is known as "claims construction." The scope of protection provided by a given patent is often determined by the meaning of just a few specific terms used in a claim.

While construing claims, particularly in the US, courts have increasingly used dictionaries. The US, however, begins by reading the claims, giving them their ordinary meaning and reviewing the specification and prosecution history to see if the claims have some different or special meaning. For ordinary words with no specialized meanings, courts often use a standard dictionary to define the words. In contrast, technical dictionaries, encyclopedias and treatises may be used for establishing specialized meanings of terms in a particular field of the invention.

A court will generally give a claim term the full range of its ordinary meaning as understood by a person with ordinary skills in the field of the invention. For instance, if the invention is a chemical invention and the term *"amorphous"* needs to be construed, the court will likely be persuaded to take into account the ordinary meaning of the term as understood by an average chemist. Likewise, if an invention relates to software and the claim term to be construed by the courts is for *"cache"*, for instance, then the court may be persuaded to take into account the ordinary meaning of the term as understood by an average software programmer.

It is common to find that words used in claims have multiple dictionary meanings. Some of the meanings have no relation to the claimed invention. If a particular term has more than one possible meaning, in the absence of any other factors, courts will more likely be persuaded to interpret the term by its customary meaning in a specific art rather than its ordinary meaning. In construing claims, the court may be persuaded to examine the intrinsic evidence carefully and ascertain the meaning of the term that is most consistent with the selection of words made by the patent agent in preparing the specification and in prosecuting the patent application. Intrinsic

evidence means evidence that is specific to the patent. Examples of intrinsic evidence are the patent itself (*claims, description, drawings etc.*) and the prosecution file or history of the patent. Thus, the patent agent must always be extremely careful about what he writes in the patent application and in responses to office received during patent prosecution.

The laws of some jurisdictions provide legal protection beyond the literal scope of the words used in a patent claim. This additional protection is known as the *"doctrine of equivalents"*. The doctrine of equivalents does not necessarily provide the same scope of protection from one jurisdiction to the next.

A patent claim recites that a *"nail"* holds Widget A to Widget B. An accused infringer literally infringes the patent claim except that the accused infringer uses a *"screw"* to hold Widget A to Widget B instead of a nail.
Under the doctrine of equivalents, the patentee could argue that a screw was equivalent to a nail for purposes of the patented invention. If the court accepted the patentee's arguments, then infringement would be found.

The patent agent had written in an office action response that "only" nails are used in the invention, it would be difficult for the patentee to argue later that either "glue" or "screws" were equivalent to nails.

Some countries believe that it is entirely up to the inventor to set forth in his claims what he considers to be his invention and maintain no doctrine of equivalents. In reviewing the example above, judges in such countries would find that the patentee could have simply drafted his patent claims using a term that encompassed both nails and screws, such as *"metal fastener"*. Other countries believe that it can be nearly impossible to find words that adequately describe the full scope of a complicated invention and maintain a doctrine of equivalents. In a broad doctrine of equivalents regime, the patentee might even be able to argue that *"glue"* was equivalent to a nail for purposes of the invention.

The doctrine of equivalents is a complicated legal topic whose requirements vary significantly from country to country. The patent agent should be aware of the doctrine, however, and should know what the courts require in the jurisdictions where he prosecutes patent applications. For example, in many jurisdictions, the patent agent's communications during patent prosecution can be used to foreclose application of the doctrine of equivalents.

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CHAPTER VII

PATENT STRATEGY

Patent Strategy

The common reasons for the failure of a patent are often poor claims and close prior art. In fact, some *"famous"* patents were not nearly as successful at cornering a market as is commonly believed. As an aside, a common misconception about patents is that the patent office considers infringement issues when awarding patents. In reality, Patent Offices only look at prior art pertinent to the pending patent application. One would likely have to remind your clients of this fact from time to time. Patent strategy also becomes more complicated and typically more lucrative as the number of patents in a portfolio increase. Holding a single patent rarely provides the same power and flexibility that holding a dozen, or a hundred patents provides.

Patent valuation is a complicated topic that relates to patent strategy. A thorough discussion of patent valuation is outside the scope of this manual. However, the student may appreciate what is known as the *"real property"* metaphor. Intellectual property bears many similarities to real property. Prior art is analogous to public lands (*non-patented prior art*) and/or lands already claimed by others (*issued patents that are still in force*). The real estate catch phrase *"location, location, location"* applies equally to patents - a patent whose claims fall squarely on top of a valuable invention is worth much more than a patent whose claims map to a less lucrative space. A patent as a legal instrument is analogous to the quality of a home's construction - a patent located on top of a valuable invention can still be worthless if the patent has not been properly constructed. The legal remedies associated with patent infringement are similar to the legal remedies associated with encroachment on another's real property.

Patenting of inventions can be notoriously expensive; however, the monopoly attracted because of it has the potential to exceed, by far, any initial patenting costs. The problem remains that a decision on whether to patent an invention often has to be made long before the commercial potential of an invention can readily be adjudged. How then should one decide what to patent and what not to patent?

A tension has always existed between the monopoly that attracts by reason of patents, and competition law, which seeks to even the commercial playing field, thereby preventing monopolies. But patenting is about more than a monopoly - so it has been advocated for many

years. It is about a *quid pro quo* (or *"something for something"*) - a temporary monopoly in exchange for knowledge transfer and the advancement of the state of the art. The monopoly is the proverbial carrot that entices innovators and investors to continue pursuing improvement and sharing same with the public at large. Perhaps then,this fundamental reasoning behind patenting can be used to inform business decisions on whether or not to seek patent protection for new inventions.

In reality, this has always been a multi-pronged approach:

1) Offensive Blocking Patenting to Mount Attacks on Competitors

   A patentee may employ his patents directly against any and all infringers. A patent typically does not give its owner any rights to make, use or sell the invention covered by the patent. In fact, it is quite possible to obtain a patent for an invention that could not be made, used or sold due to the infringement of someone else's patent or without approval from a government regulatory agency. Fortunately, a patent cannot infringe another patent.

   Selling a product is often, although not always, more lucrative than licensing the intellectual property necessary to manufacture the product. Consequently, many patent owners who also manufacture products use their patents to force competitors either to design around their patents (and produce, hopefully, an inferior product) or to license their patents.

   Some companies apply their patent royalties to their research departments as a matter of policy. This makes some sense in that the company's research and development department probably created the invention that resulted in the patent being used to generate royalties – and by giving the R&D department *"extra"* money from the patent licensing, the company may end up better enabled to create new products and services.

   In developing an offensive patent strategy, the patent owner should continually consider the nature of the licensing target's infringement. The infringer could be guilty of direct infringement, contributory infringement and/or inducement of infringement. The nature of the damages may also vary based on the use of the infringing technology. Direct infringers do not necessarily incur greater damages than contributory infringers. Some country's patent laws also recognize infringement under the doctrine of equivalents. Thus, a defendant who is not directly infringing a patent claim might still be considered an infringer by virtue of his use of a substantially similar component used in a substantially similar manner. Analysis under the doctrine of equivalents is quite complicated; however, one key factor is whether the patent's prosecution history

includes statements that would indicate that the patentee surrendered claim coverage for the substantially similar component during patent prosecution. This is another reason why responses to office actions need to be carefully worded and preferably short.

2) Defensive Patenting to Defend Oneself from Infringement Actions

Patents are *"swords"* and not *"shields"* in the sense that a patent does not give its owner the right to manufacture a product protected by the patent. A patent provides a negative right that allows the owner to say who cannot practice the invention protected by the patent. Holding a patent will provide its owner with little assurance that his manufacture of a product covered by the patent will not infringe another patent owned by someone else. However, patents can sometimes effectively operate as shields with respect to patent-holding competitors who will refrain from suing you for infringement out of fear that you will counter-sue them or patent infringement. In defending oneself against claims of patent infringement, it is frequently of little help for the defendant to say that he has a patent and that his own products fall within the scope of protection accorded by the patent – unless the defendant's patent is so different from the plaintiff's patent that a legal fact finder (e.g. a judge or jury) could readily see the differences between the two inventions. However, even in such situations it is often easier for the defendant simply to explain why he doesn't infringe the claims in suit. In certain circumstances a patent or group of patents may provide a defensive shield for a patentee against his own competitors. Certainly, whether a given company will benefit from having more patents depends somewhat on the company's industry segment and the company's particular technical characteristics and business strategy. There is typically little reason for a company to acquire patents without a specific business purpose.

In many industries where the major players each hold substantial numbers of patents, it is quite common for these competitors to cross-license their patent portfolios to each other. Such cross- licenses may include some royalty formula, or they may be completely free. Additionally, the cross-licenses may include a major limitation such as a field of use limitation which would still permit infringement litigation outside the field of use.

A patent owner can employ many tools in his efforts to determine how best to use his patents. One should probably model various economic scenarios before deciding how to exploit his patents. A simple matrix may be helpful in some situations. For example, the company can list its products and decide on a per-product-basis how to exploit the intellectual property related to the product. For some products, the company may decide to use the related patents in a purely defensive manner to block out all competitors = while for other products, the company may decide to follow a licensing strategy. At a

high level, the company can provide estimates of its likelihood of success following each path and/or the likelihood of senior management agreeing to follow a particular approach. The options that receive the highest ratings for a given product can then be analyzed further to arrive at the company's ultimate intellectual property strategy for the product. This analysis will also likely require analysis of the strengths/weaknesses of the patents involved as well as the values of the relative markets. In the end a well-developed patent portfolio focuses on the company's core businesses and protects particular features and functions that transcend the company's specific product offerings. A well-developed patent portfolio will also likely create barriers to market entry and/or success for actual and potential competitors.

3) Design-around Techniques

Designing around one or more patents involves determining the scope of claim coverage provided by the patent. Designing around also typically involves detailed review of the patent specification, review of the prior art cited and applied during the prosecution and close analysis of the prosecution history of the patent application to see if the applicant made any damaging admissions about the invention during prosecution (e.g. *"This invention pertains to improved buggy whips and absolutely nothing else!"*).

The attorney performing the design around analysis may wish to determine the precise meanings for the terms used in the patent's claims by applying the laws regarding patent claim construction or claim interpretation in the forum jurisdiction. It is essential always to bear in mind that the claims define the scope of the protection. The rules for determining the scope of claim coverage vary from jurisdiction to jurisdiction. In many jurisdictions claim limitations will initially receive the *"plain meaning"* (or *ordinary meaning*) of the terms recited, but may be further interpreted in light of their use in the patent's specification and/or in the prosecution history for the patent. If *"means-plus-function"* claim language is used, the attorney will need to consider how such claims are interpreted in the forum jurisdiction.

The attorney will likely prepare his analysis in the form of an *"opinion"*. In some cases, the opinion may be fairly short while in other cases it may be extremely detailed. "Opinion letters" may be quite helpful in jurisdictions that recognize some form of *"willful infringement"*. Willful infringement arises when an infringer knows of another party's patent and deliberately infringes it and/or when the infringer makes no effort to determine if he infringes the patent. Obtaining a non-infringement or invalidity opinion from a neutral attorney may provide a defense to *"willful"* infringement in many of the jurisdictions that recognize willful infringement. The damages associated with willful

infringement are typically a multiple of the actual or direct damages for patent infringement.

Obtaining a non-infringement or invalidity opinion may be helpful even in a jurisdiction that does not recognize willful infringement - since such opinions can provide valuable guidance to a company on the basic question of whether they have an infringement problem with respect to a particular competitor's patent.

Patent agents are not allowed to prepare opinions in many jurisdictions such as the US Patent opinions are typically written by an attorney, usually by a patent attorney. Many law firms and attorneys will not prepare opinions due to the possibility of a high malpractice claim should the opinion turn out to be inadequately prepared. Of course, an attorney will not typically write an opinion for a client if, in the attorney's opinion, the client really is infringing a valid patent. In such situations, the attorney typically expresses his concerns in a non-permanent medium(e.g. *verbally*) and not on paper. Because a patent agent typically has in-depth knowledge of a particular technology, the patent agent may assist the attorney in preparation of an opinion.

Opinions of counsel in many countries are typically protected by the attorney-client privilege and do not need to be disclosed to adverse parties. A plaintiff typically must seek special permission from the court in order to compel a defendant to produce an opinion. Accordingly, whenever a company has an opinion prepared byits counsel, the appropriate persons in the company should make sure that the opinion is retained by the company in strictest confidence and not shared with anyone other than the company's key executives on a need-to-know basis. Additionally, the opinion should not be provided to the company's customers. Note that reliance on an opinion of counsel may result in a waiver of attorney-client privilege for all opinions relating to the subject matter of the opinion. In some circumstances, the company may share its opinions with other parties using a vehicle known as either a *"common interest agreement"* or a *"joint defense agreement".*

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ANNEXURE I

Source: WIPO Manual

Confidential
Disclosure No.:
Status:

INVENTION DISCLOSURE FORM

Name:

Work phone number:

Fax number:

1. PROPOSED TITLE:

2. FIELD OF INVENTION

This invention relates primarily to:

3. BACKGROUND AND RELATED ART

A. The technical problem addressed by the invention is as follows:

B. The closest related art is described as follows:

C. Advantages presented by the invention are as follows:

4. DRAWING(S)

Drawings for this invention are available/not available. If available, please attach.

Comments about drawings provided:

5. WRITTEN DESCRIPTION

The invention is described as follows:

NOTE 1: Please attach additional pages as necessary.
NOTE 2: If you have other documents and/or drawings related to the invention, please attach copies to this form.

6. CONCEPTION OF INVENTION

Date of conception:

Date of first written description:

7. REDUCTION TO PRACTICE

Has the invention been reduced to practice (does it work)?

COMMENTS, if any, on conception of invention and/or first written description:

8. INVENTOR(S) (this section must be completed)

INVENTOR 1:

Name:

Residence Address:

Citizenship:

INVENTOR 2:

Name:

Residence Address:

Citizenship:

COMMENTS on inventors or inventorship (please note if any of the inventors resides out of the country).

9. DATES OR PRODUCT TESTING AND RELEASE

Alpha Testing:

Beta Testing:

General release or sale:

Offers for sale:

COMMENTS on product testing and release:

10. DISCLOSURE OF INVENTION

Has there been any disclosure or use of the invention by the public? When and to whom? Under a non-disclosure agreement?

Please attach a copy of the disclosure.

11. INTERNAL DISCLOSURE(S)

First internal disclosure date:

Name of first person to whom invention was disclosed:

COMMENTS about first internal disclosure:

12. ARTICLE(S)

Have any articles been published?

DETAILS about publication of articles(s):

Please attach a copy of any published article(s).

13. ADVERTISEMENTS, PRESS RELEASES AND PRODUCT ANNOUNCEMENTS

Any advertisements, press releases or product announcements?

DETAILS about any advertisements, press releases and product announcements:

Please attach copies of any advertisements, press releases and/or product announcements.

14. OUTSIDE DISCLOSURE(S)

Have there been any disclosures outside the company?

Were all outside disclosures under a non-disclosure agreement?

DETAILS about any disclosures outside the company:

Please attach copies of any information disclosed.

15. TRADE SHOWS AND CONFERENCES

Are there any upcoming trade shows or conferences?

DETAILS about upcoming trade shows and/or conferences:

ADDITIONAL COMMENTS BY INVENTOR:

Signed: Witnessed and Understood by:

Date: Date:

ANNEXURE II(i)

United States Patent [19]

Mullis

[11] **Patent Number: 4,683,202**

[45] **Date of Patent: * Jul. 28, 1987**

[54] **PROCESS FOR AMPLIFYING NUCLEIC ACID SEQUENCES**

[75] Inventor: **Kary B. Mullis,** Kensington, Calif.

[73] Assignee: **Cetus Corporation,** Emeryville, Calif.

[*] Notice: The portion of the term of this patent subsequent to Jul. 28, 2004 has been disclaimed.

[21] Appl. No.: **791,308**

[22] Filed: **Oct. 25, 1985**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 716,975, Mar. 28, 1985, abandoned.

[51] **Int. Cl.**[4] **C12P 19/34;** C12N 15/00; C12N 1/00; C07H 21/04; C07H 21/02

[52] **U.S. Cl.** **435/91;** 435/177.3; 435/317; 536/27; 536/28; 536/29; 935/17; 935/18; 935/16

[58] **Field of Search** 435/91, 172.3, 317; 536/27, 28, 29; 935/17, 18

[56] **References Cited**

PUBLICATIONS

Gaubatz et al, "Strategies for Constructing Complementary DNA for Cloning", J. Theor. Biol. 95: 679 (1982).

Caton and Robertson, *Nucleic Acids Research,* vol. 7, pp. 1445–1456 (1979).

Rossi et al., *J. Biol. Chem.,* 257, 9226–9229 (1982).

Primary Examiner—James Martinell

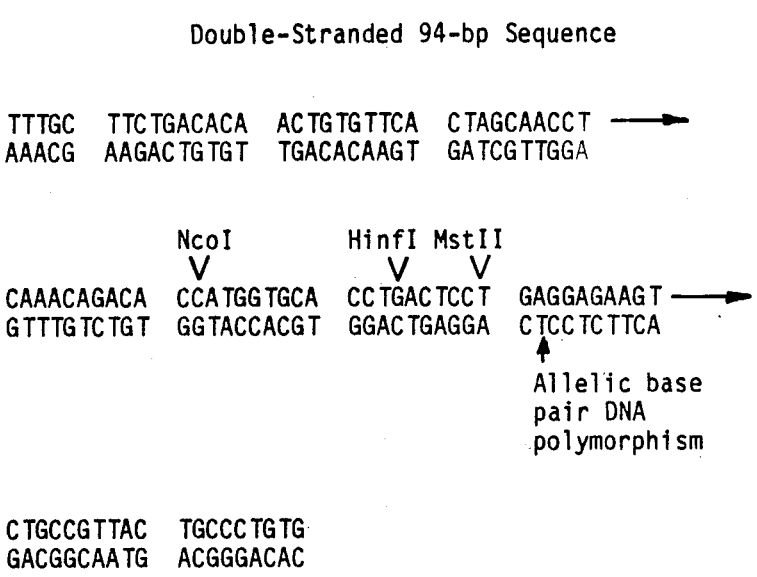
Attorney, Agent, or Firm—Janet E. Hasak; Albert P. Halluin

[57] **ABSTRACT**

The present invention is directed to a process for amplifying any desired specific nucleic acid sequence contained in a nucleic acid or mixture thereof. The process comprises treating separate complementary strands of the nucleic acid with a molar excess of two oligonucleotide primers, and extending the primers to form complementary primer extension products which act as templates for synthesizing the desired nucleic acid sequence. The steps of the reaction may be carried out stepwise or simultaneously and can be repeated as often as desired.

21 Claims, 12 Drawing Figures

FIG.1


Double-Stranded 94-bp Sequence
TTTGC TTCTGACACA ACTGTGTTCA CTAGCAACCT
AAACG AAGACTGTGT TGACACAAGT GATCGTTGGA
NcoI
HinfI MstII
CAAACAGACA CCATGGTGCA CCTGACTCCT GAGGAGAAGT
GTTTGTCTGT GGTACCACGT GGACTGAGGA CTCCTCTTCA
Allelic base pair DNA polymorphism
CTGCCGTTAC TGCCCTGTG
GACGGCAATG ACGGGACAC

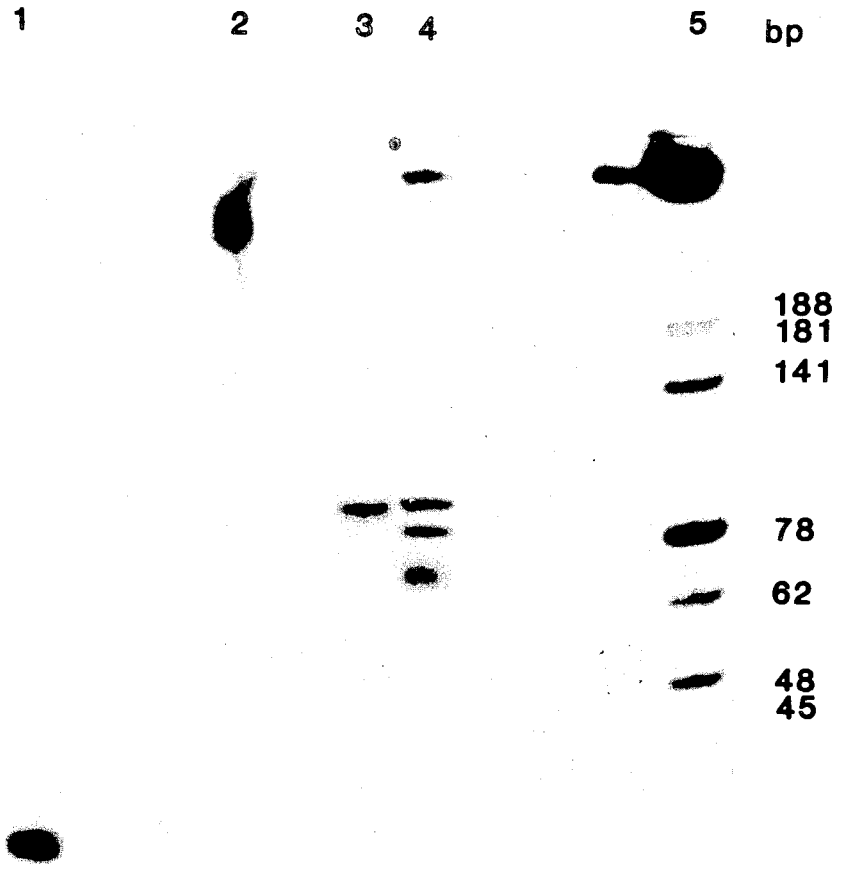


FIG.2

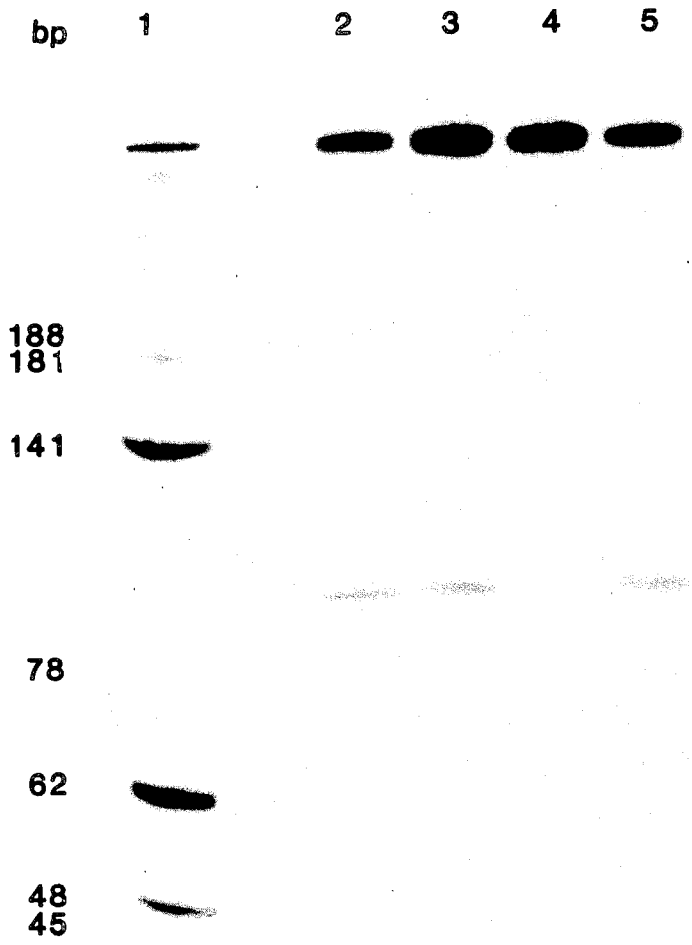


FIG.3

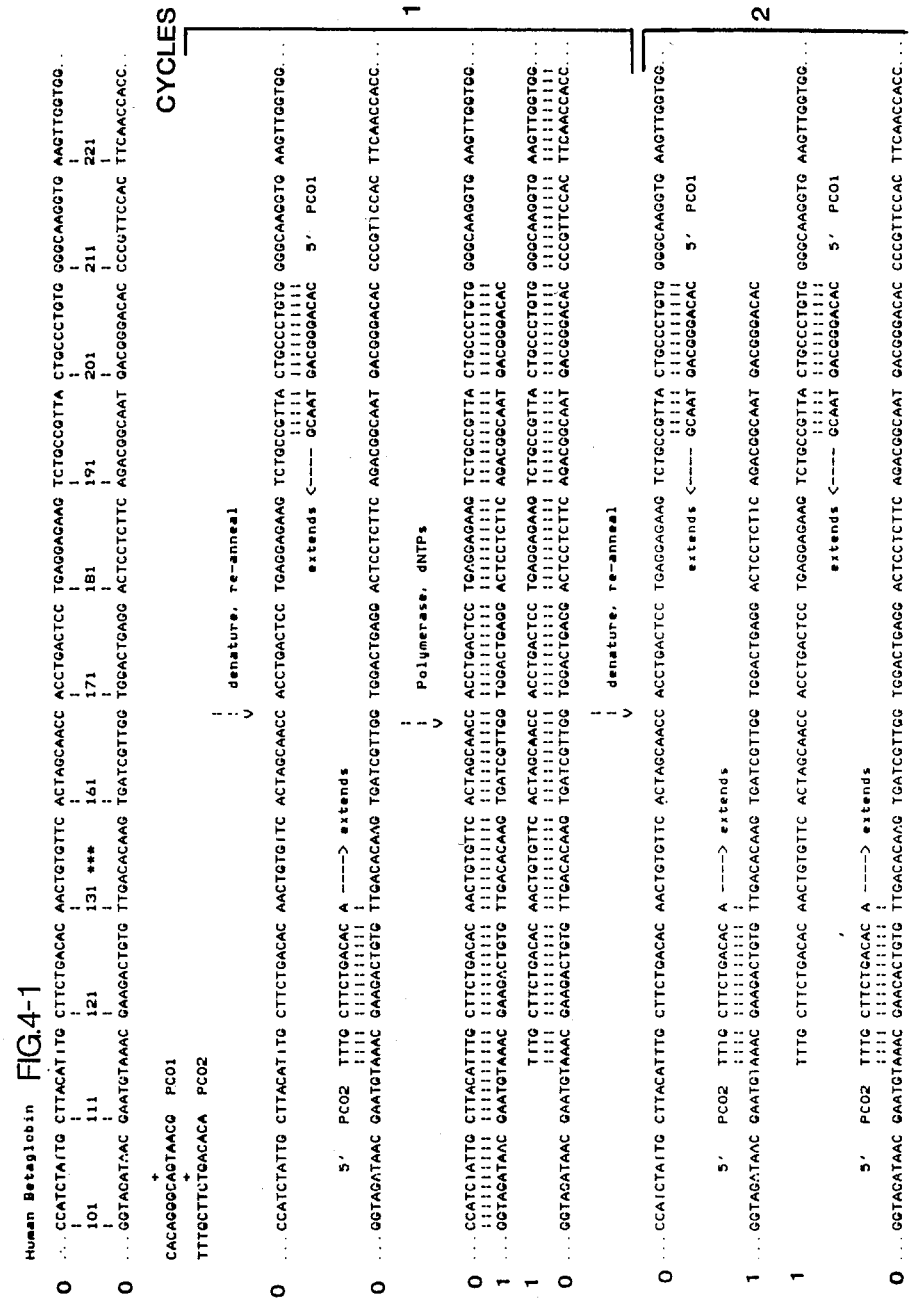

Human Beteglobin
CYCLES
denature, re-anneal
Polymerase, dNTPs
extends
PCO1
PCO2

FIG.4-1

FIG.4-2

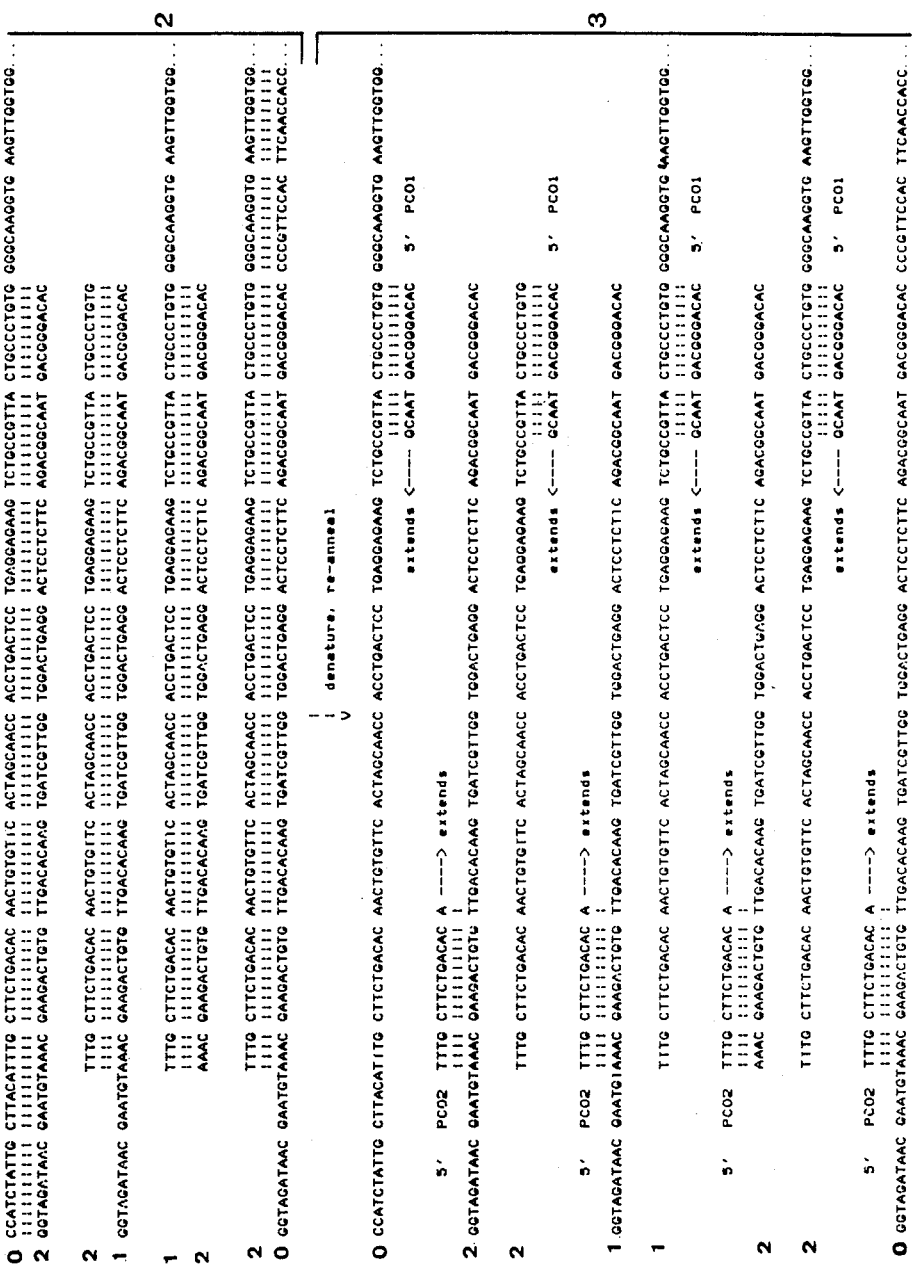
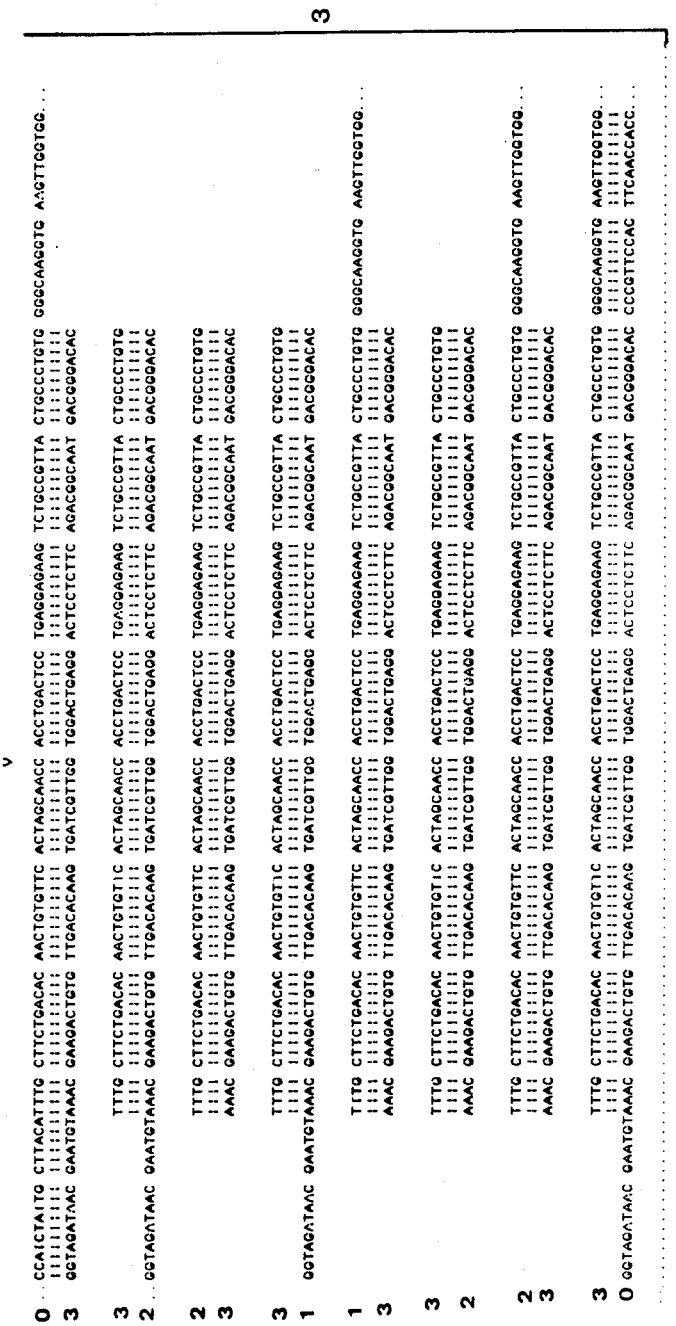

Polymerase, dNTPs
denature, re-anneal
extends
PCO2
PCO1
5'
3'
2
3

FIG.4-3


Polymerase, dNTPs
3
COPIES OF DNA SEQUENCE AFTER N CYCLES
N
0
1
5
10
15
20
template
long product
short product
=2(exp N)-N-1
1
0
0
1
1
5
26
1
10
1013
1
15
32,752
1
20
1,048,555

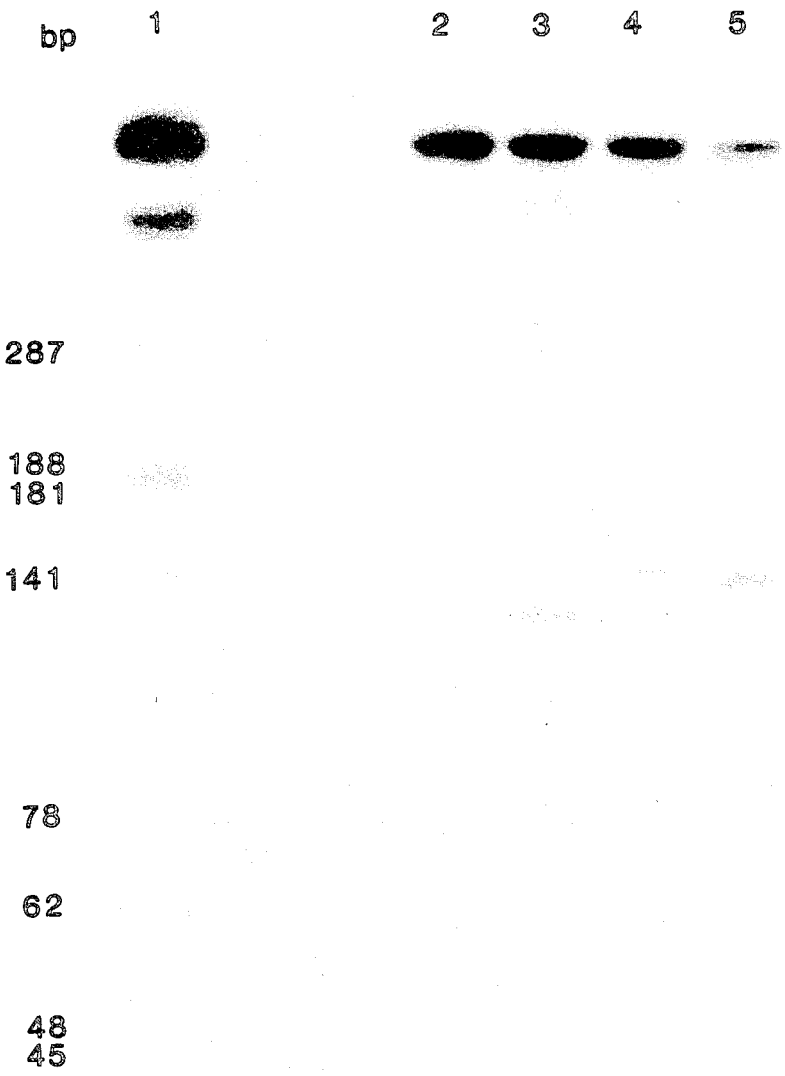


FIG.5

FIG.6

```
                    ==========
β^A   CATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA
      GTACCACGTGGACTGAGGACTCCTCTTCAGACGGCAATGACGGGACACCCCGTTCCACTT
                    ==========

                    =====  *
β^S   CATGGTGCACCTGACTCCTGTGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA
      GTACCACGTGGACTGAGGACACCTCTTCAGACGGCAATGACGGGACACCCCGTTCCACTT
                    =====

* Marks the mutation (A to T) in the sickle cell gene which disrupts
  the DdeI site
```

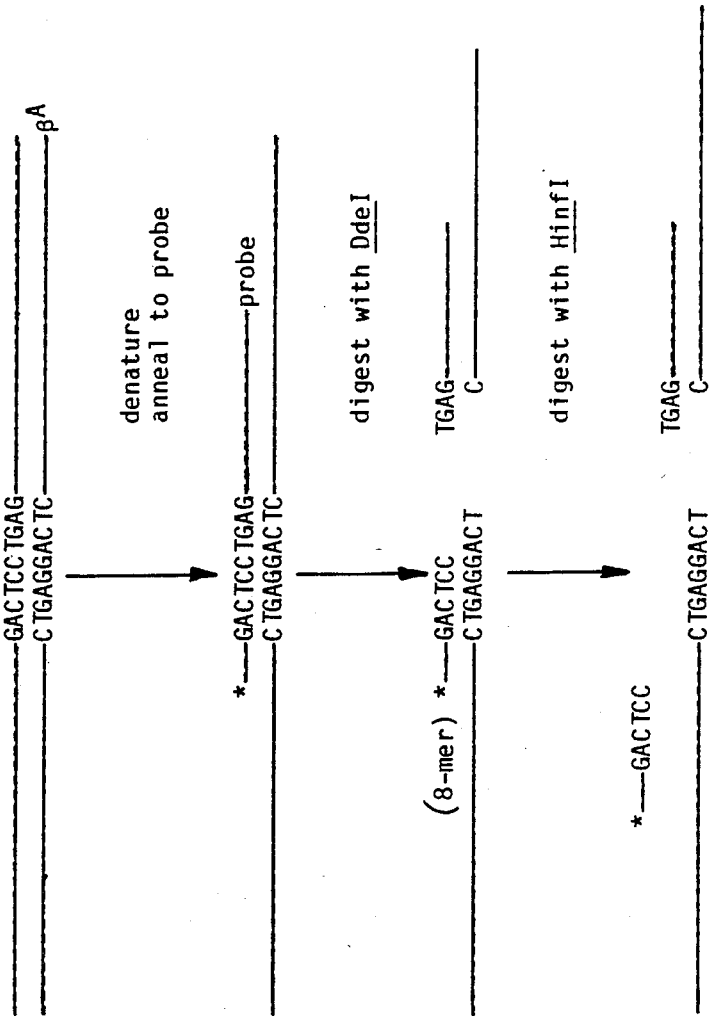

GACTCCTGAG
CTGAGGACTC
pA
denature
anneal to probe
*—GACTCCTGAG
probe
CTGAGGACTC
digest with DdeI
(8-mer) *—GACTCC
CTGAGGACT
TGAG
C
digest with HinfI
*—GACTCC
CTGAGGACT
TGAG
C
* is label

FIG.7

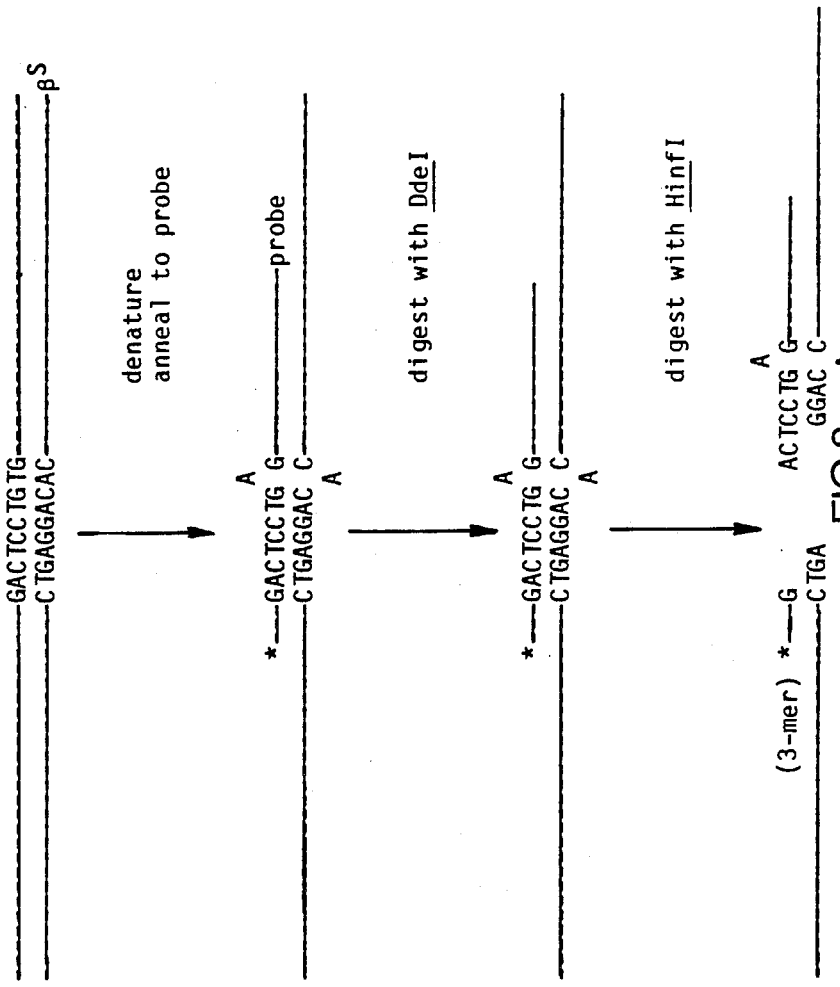

GACTCCTGTG
CTGAGGACAC
βS
denature
anneal to probe
probe
digest with DdeI
digest with HinfI
(3-mer)
ACTCCTG G
GGAC C
* is label

FIG.8

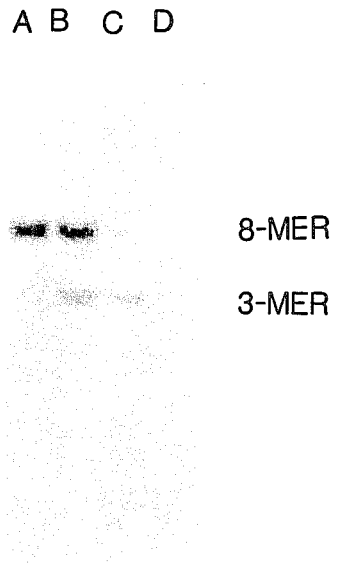
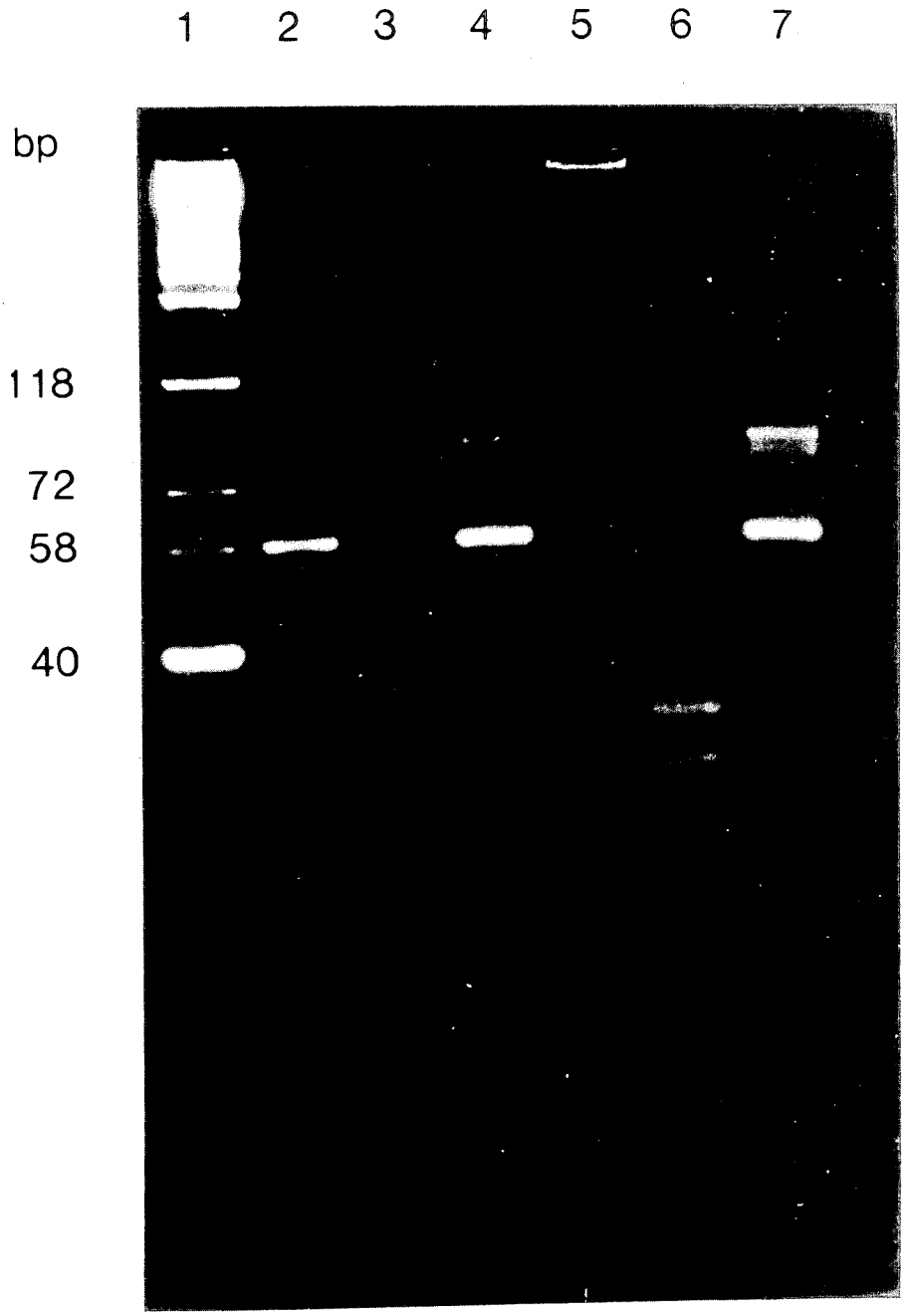


FIG.9

FIG. 10



PROCESS FOR AMPLIFYING NUCLEIC ACID SEQUENCES

This application is a continuation-in-part of copending U.S. application Ser. No. 716,975 filed Mar. 28, 1985, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a process for amplifying existing nucleic acid sequences. More specifically, it relates to a process for producing any particular nucleic acid sequence from a given sequence of DNA or RNA in amounts which are large compared to the amount initially present. The DNA or RNA may be single- or double-stranded, and may be a relatively pure species or a component of a mixture of nucleic acids. The process of the invention utilizes a repetitive reaction to accomplish the amplification of the desired nucleic acid sequence.

2. Description of Related Disclosures

For diagnostic applications in particular, the target nucleic acid sequence may be only a small portion of the DNA or RNA in question, so that it may be difficult to detect its presence using nonisotopically labeled or end-labeled oligonucleotide probes. Much effort is being expended in increasing the sensitivity of the probe detection systems, but little research has been conducted on amplifying the target sequence so that it is present in quantities sufficient to be readily detectable using currently available methods.

Several methods have been described in the literature for the synthesis of nucleic acids de novo or from an existing sequence. These methods are capable of producing large amounts of a given nucleic acid of completely specified sequence.

One known method for synthesizing nucleic acids de novo involves the organic synthesis of a nucleic acid from nucleoside derivatives. This synthesis may be performed in solution or on a solid support. One type of organic synthesis is the phosphotriester method, which has been utilized to prepare gene fragments or short genes. In the phosphotriester method, oligonucleotides are prepared which can then be joined together to form longer nucleic acids. For a description of this method, see Narang, S. A., et al., *Meth. Enzymol.*, 68, 90 (1979) and U.S. Pat. No. 4,356,270. The patent describes the synthesis and cloning of the somatostatin gene.

A second type of organic synthesis is the phosphodiester method, which has been utilized to prepare a tRNA gene. See Brown, E. L., et al., *Meth. Enzymol.*, 68, 109 (1979) for a description of this method. As in the phosphotriester method, the phosphodiester method involves synthesis of oligonucleotides which are subsequently joined together to form the desired nucleic acid.

Although the above processes for de novo synthesis may be utilized to synthesize long strands of nucleic acid, they are not very practical to use for the synthesis of large amounts of a nucleic acid. Both processes are laborious and time-consuming, require expensive equipment and reagents, and have a low overall efficiency. The low overall efficiency may be caused by the inefficiencies of the synthesis of the oligonucleotides and of the joining reactions. In the synthesis of a long nucleic acid, or even in the synthesis of a large amount of a shorter nucleic acid, many oligonucleotides would need to be synthesized and many joining reactions would be required. Consequently, these methods would not be practical for synthesizing large amounts of any desired nucleic acid.

Methods also exist for producing nucleic acids in large amounts from small amounts of the initial existing nucleic acid. These methods involve the cloning of a nucleic acid in the appropriate host system, where the desired nucleic acid is inserted into an appropriate vector which is used to transform the host. When the host is cultured the vector is replicated, and hence more copies of the desired nucleic acid are produced. For a brief description of subcloning nucleic acid fragments, see Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, pp. 390–401 (1982). See also the techniques described in U.S. Pat. Nos. 4,416,988 and 4,403,036.

A third method for synthesizing nucleic acids, described in U.S. Pat. No. 4,293,652, is a hybrid of the above-described organic synthesis and molecular cloning methods. In this process, the appropriate number of oligonucleotides to make up the desired nucleic acid sequence is organically synthesized and inserted sequentially into a vector which is amplified by growth prior to each succeeding insertion.

The present invention bears some similarity to the molecular cloning method; however, it does not involve the propagation of any organism and thereby avoids the possible hazards or inconvenience which this entails. The present invention also does not require synthesis of nucleic acid sequences unrelated to the desired sequence, and thereby the present invention obviates the need for extensive purification of the product from a complicated biological mixture.

SUMMARY OF THE INVENTION

The present invention resides in a process for amplifying one or more specific nucleic acid sequences present in a nucleic acid or mixture thereof using primers and inducing agents. The extension product of one primer when hybridized to the other becomes a template for the production of the desired specific nucleic acid sequence, and vice versa, and the process is repeated as often as is necessary to produce the desired amount of the sequence. This method is expected to be more efficient than the methods described above for producing large amounts of nucleic acid from a target sequence and to produce such nucleic acid in a comparatively short period of time. The present method is especially useful for amplifying rare species of nucleic acid present in a mixture of nucleic acids for effective detection of such species.

More specifically, the present invention provides a process for amplifying at least one specific nucleic acid sequence contained in a nucleic acid or a mixture of nucleic acids wherein each nucleic acid consists of two separate complementary strands, of equal or unequal length, which process comprises:

(a) treating the strands with two primers, for each different specific sequence being amplified, under conditions such that for each different sequence being amplified an extension product of each primer is synthesized which is complementary to each nucleic acid strand, wherein said primers are selected so as to be substantially complementary to different strands of each specific sequence such that the extension product synthesized from one primer, when it is separated from its complement,

can serve as a template for synthesis of the extension product of the other primer;

(b) separating the primer extension products from the templates on which they were synthesized to produce single-stranded molecules; and

(c) treating the single-stranded molecules generated from step (b) with the primers of step (a) under conditions such that a primer extension product is synthesized using each of the single strands produced in step (b) as a template.

The steps may be conducted sequentially or simultaneously. In addition, steps (b) and (c) may be repeated until the desired level of sequence amplification is obtained.

In other embodiments the invention relates to methods for diagnosing the presence of specific nucleic acid sequences suspected of being in a sample and diagnostic kits applicable thereto.

The present invention may be useful not only for producing large amounts of an existing nucleic acid of completely specified sequence, but also for producing nucleic acid sequences which are known to exist but are not completely specified. In either case an initial copy of the sequence to be amplified must be available, although it need not be pure or a discrete molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates a 94 base pair length sequence of human β-globin desired to be amplified. The single base pair change which is associated with sickle cell anemia is depicted beneath the 94-mer.

FIG. **2** illustrates an autoradiograph of polyacrylamide gel electrophoresis demonstrating amplification of the 94-mer contained in human wild-type DNA and in a plasmid containing a 1.9 kb BamHI fragment of the normal β-globin gene (pBR328:HbA).

FIG. **3** illustrates an autoradiograph of polyacrylamide gel electrophoresis demonstrating amplification of any of the specific target 94-mer sequence present in pBR328:HbA, a plasmid containing a 1.9 kb BamHI fragment of the sickle cell allele of β-globin (pBR328:HbS), pBR328:HbA where the sequence to be amplified is cleaved with MstII, and pBR328:HbS where the sequence to the amplified has been treated but not cleaved with MstII.

FIGS. **4-1-4-3** illustrate in detail the steps and products of the polymerase chain reaction for amplification of the desired 94-mer sequence of human β-globin for three cycles using two oligonucleotide primers.

FIG. **5** represents an autoradiograph of polyacrylamide gel electrophoresis demonstrating amplification after four cycles of a 240-mer sequence in pBR328:HbA, where the aliquots are digested with NcoI (Lane 3), MstII (Lane 4) or HinfI (Lane 5). Lane 1 is the molecular weight standard and Lane 2 contains the intact 240-bp product.

FIG. **6** illustrates the sequence of the normal (β^A) and sickle cell (β^S) β-globin genes in the region of the DdeI and HinfI restriction sites, where the single lines for β^A mark the position of the DdeI site (CTGAG) and the double bars for β^A and β^S mark the position of the HinfI site (GACTC).

FIG. **7** illustrates the results of sequential digestion of normal β-globin using a 40-mer probe and DdeI followed by HinfI restriction enzymes.

FIG. **8** illustrates the results of sequential digestion of sickle β-globin using the same 40-mer probe as in FIG. 7 and DdeI followed by HinfI restriction enzymes.

FIG. **9** illustrates an autoradiograph of polyacrylamide gel electrophoresis demonstrating the use of the same 40-mer probe as in FIG. 7 to specifically characterize the beta-globin alleles present in samples of whole human DNA which have been subjected to amplification by the present method.

FIG. **10** illustrates a photograph of a 6% NuSieve agarose gel visualized using ethidium bromide and UV light. This photograph demonstrates amplification of a sub-fragment of a 110-bp amplification product which sub-fragment is an inner nested set within the 110-bp fragment.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "oligonucleotide" as used herein in referring to primers, probes, oligomer fragments to be detected, oligomer controls and unlabeled blocking oligomers is defined as a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, i.e., in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH. The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is firt treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and use of the method. For example, for diagnostics applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15–25 or more nucleotides, although it may contain fewer nucleotides. For other applications, the oligonucleotide primer is typically shorter, e.g., 7–15 nucleotides. Such short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with template.

The primers herein are selected to be "substantially" complementary to the different strands of each specific sequence to be amplified. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand to be amplified to hybridize therewith and thereby form a template for synthesis of the extension product of the other primer.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes

each of which cut double-stranded DNA at or near a specific nucleotide sequence.

As used herein, the term "DNA polymorphism" refers to the condition in which two or more different nucleotide sequences coexists in the same interbreeding population in a DNA sequence.

The term "restriction fragment length polymorphism" ("RFLP") refers to the differences in DNA nucleotide sequences that are randomly distributed throughout the entire human genome and that produce different restriction endonuclease patterns.

The present invention is directed to a process for amplifying any one or more desired specific nucleic acid sequences found in a nucleic acid. Because large amounts of a specific sequence may be produced by this process, the present invention may be used for improving the efficiency of cloning DNA or messenger RNA and for amplifying a target sequence to facilitate detection thereof. The present invention is also useful for obtaining large amounts of the desired sequence from a mixture of nucleic acids resulting from an imperfect chemical synthesis.

In general, the present process involves a chain reaction for producing, in exponential quantities relative to the number of reaction steps involved, at least one specific nucleic acid sequence given (a) that the ends of the required sequence are known in sufficient detail that oligonucleotides can be synthesized which will hybridize to them, and (b) that a small amount of the sequence is available to initiate the chain reaction. The product of the chain reaction will be a discrete nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

Any source of nucleic acid, in purified or nonpurified form, can be utilized as the starting nucleic acid or acids, provided it contains or is suspected of containing the specific nucleic acid sequence desired. Thus, the process may employ, for example, DNA or RNA, including messenger RNA, which DNA or RNA may be single stranded or double stranded. In addition, a DNA-RNA hybrid which contains one strand of each may be utilized. A mixture of any of these nucleic acids may also be employed, or the nucleic acid produced from a previous amplification reaction herein using the same or different primers may be so utilized. The specific nucleic acid sequence to be amplified may be only a fraction of a larger molecule or can be present initially as a discrete molecule, so that the specific sequence constitutes the entire nucleic acid. It is not necessary that the sequence to be amplified be present initially in a pure form; it may be a minor fraction of a complex mixture, such as a portion of the β-globin gene contained in whole human DNA or a portion of nucleic acid sequence due to a particular microorganism which organism might constitute only a very minor fraction of a particular biological sample. The starting nucleic acid may contain more than one desired specific nucleic acid sequence which may be the same or different. Therefore, the present process is useful not only for producing large amounts of one specific nucleic acid sequence, but also for amplifying simultaneously more than one different specific nucleic acid sequence located on the same or different nucleic acid molecules.

The nucleic acid or acids may be obtained from any source, for example, from plasmids such as pBR322, from cloned DNA or RNA, or from natural DNA or RNA from any source, including bacteria, yeast, viruses, and higher organisms such as plants or animals. DNA or RNA may be extracted from blood, tissue material such as chorionic villi or amniotic cells by a variety of techniques such as that described by Maniatis et al., *Molecular Cloning A Laboratory Manual* (New York: Cold Spring Harbor Laboratory, 1982), pp. 280–281.

Any specific nucleic acid sequence can be produced by the present process. It is only necessary that a sufficient number of bases at both ends of the sequence be known in sufficient detail so that two oligonucleotide primers can be prepared which will hybridize to different strands of the desired sequence and at relative positions along the sequence such that an extension product synthesized from one primer, when it is separated from its template (complement), can serve as a template for extension of the other primer into a nucleic acid of defined length. The greater the knowledge about the bases at both ends of the sequence, the greater can be the specificity of the primers for the target nucleic acid sequence, and thus the greater the efficiency of the process. It will be understood that the word primer as used hereinafter may refer to more than one primer, particularly in the case where there is some ambiguity in the information regarding the terminal sequence(s) of the fragment to be amplified. For instance, in the case where a nucleic acid sequence is inferred from protein sequence information a collection of primers containing sequences representing all possible codon variations based on degeneracy of the genetic code will be used for each strand. One primer from this collection will be 100% homologous with the end of the desired sequence to be amplified.

The oligonucleotide primers may be prepared using any suitable method, such as, for example, the phosphotriester and phosphodiester methods described above, or automated embodiments thereof. In one such automated embodiment diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage et al., *Tetrahedron Letters* (1981), 22: 1859–1962. One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Pat. No. 4,458,066. It is also possible to use a primer which has been isolated from a biological source (such as a restriction endonuclease digest).

The specific nucleic acid sequence is produced by using the nucleic acid containing that sequence as a template. If the nucleic acid contains two strands, it is necessary to separate the strands of the nucleic acid before it can be used as the template, either as a separate step or simultaneously with the synthesis of the primer extension products. This strand separation can be accomplished by any suitable method including physical, chemical or enzymatic means. One physical method of separating the strands of the nucleic acid involves heating the nucleic acid until it is completely (>99%) denatured. Typical heat denaturation may involve temperatures ranging from about 80° to 105° C. for times ranging from about 1 to 10 minutes. Strand separation may also be induced by an enzyme from the class of enzymes known as helicases or the enzyme RecA, which has helicase activity and in the presence of riboATP is known to denature DNA. The reaction conditions suitable for separating the strands of nucleic acids with helicases are described by Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLIII "DNA: Replication and Recombination" (New York: Cold Spring Harbor Laboratory, 1978), B. Kuhn et al., "DNA Helicases", pp. 63–67, and techniques for using RecA are

reviewed in C. Radding, *Ann. Rev. Genetics*, 16: 405–37 (1982).

If the original nucleic acid containing the sequence to be amplified is single stranded, its complement is synthesized by adding one or two oligonucleotide primers thereto. If an appropriate single primer is added, a primer extension product is synthesized in the presence of the primer, an inducer or catalyst of the synthesis and the four nucleotides described below. The product will be partially complementary to the single-stranded nucleic acid and will hybridize with the nucleic acid strand to form a duplex of unequal length strands that may then be separated into single strands as described above to produce two single separated complementary strands. Alternatively, two appropriate primers may be added to the single-stranded nucleic acid and the reaction carried out.

If the original nucleic acid constitutes the sequence to be amplified, the primer extension product(s) produced will be completely complementary to the strands of the original nucleic acid and will hybridize therewith to form a duplex of equal length strands to be separated into single-stranded molecules.

When the complementary strands of the nucleic acid or acids are separated, whether the nucleic acid was originally double or single stranded, the strands are ready to be used as a template for the synthesis of additional nucleic acid strands. This synthesis can be performed using any suitable method. Generally it occurs in a buffered aqueous solution, preferably at a pH of 7–9, most preferably about 8. Preferably, a molar excess (for cloned nucleic acid, usually about 1000:1 primer:template, and for genomic nucleic acid, usually about 10^6:1 primer:template) of the two oligonucleotide primers is added to the buffer containing the separated template strands. It is understood, however, that the amount of complementary strand may not be known if the process herein is used for diagnostic applications, so that the amount of primer relative to the amount of complementary strand cannot be determined with certainty. As a practical matter, however, the amount of primer added will generally be in molar excess over the amount of complementary strand (template) when the sequence to be amplified is contained in a mixture of complicated long-chain nucleic acid strands. A large molar excess is preferred to improve the efficiency of the process.

The deoxyribonucleoside triphosphates dATP, dCTP, dGTP and TTP are also added to the synthesis mixture in adequate amounts and the resulting solution is heated to about 90°–100° C. for from about 1 to 10 minutes, preferably from 1 to 4 minutes. After this heating period the solution is allowed to cool to room temperature, which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for inducing or catalyzing the primer extension reaction, and the reaction is allowed to occur under conditions known in the art. This synthesis reaction may occur at from room temperature up to a temperature above which the inducing agent no longer functions efficiently. Thus, for example, if DNA polymerase is used as inducing agent, the temperature is generally no greater than about 40° C. Most conveniently the reaction occurs at room temperature.

The inducing agent may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, reverse transcriptase, and other enzymes, including heat-stable enzymes, which will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each nucleic acid strand. Generally, the synthesis will be initiated at the 3′ end of each primer and proceed in the 5′ direction along the template strand, until synthesis terminates, producing molecules of different lengths. There may be inducing agents, however, which initiate synthesis at the 5′ end and proceed in the other direction, using the same process as described above.

The newly synthesized strand and its complementary nucleic acid strand form a double-stranded molecule which is used in the succeeding steps of the process. In the next step, the strands of the double-stranded molecule are separated using any of the procedures described above to provide single-stranded molecules.

New nucleic acid is synthesized on the single-stranded molecules. Additional inducing agent, nucleotides and primers may be added if necessary for the reaction to proceed under the conditions prescribed above. Again, the synthesis will be initiated at one end of the oligonucleotide primers and will proceed along the single strands of the template to produce additional nucleic acid. After this step, half of the extension product will consist of the specific nucleic acid sequence bounded by the two primers.

The steps of strand separation and extension product synthesis can be repeated as often as needed to produce the desired quantity of the specific nucleic acid sequence. As will be described in further detail below, the amount of the specific nucleic acid sequence produced will accumulate in an exponential fashion.

When it is desired to produce more than one specific nucleic acid sequence from the first nucleic acid or mixture of nucleic acids, the appropriate number of different oligonucleotide primers are utilized. For example, if two different specific nucleic acid sequences are to be produced, four primers are utilized. Two of the primers are specific for one of the specific nucleic acid sequences and the other two primers are specific for the second specific nucleic acid sequence. In this manner, each of the two different specific sequences can be produced exponentially by the present process.

The present invention can be performed in a step-wise fashion where after each step new reagents are added, or simultaneously, where all reagents are added at the initial step, or partially step-wise and partially simultaneous, where fresh reagent is added after a given number of steps. If a method of strand separation, such as heat, is employed which will inactivate the inducing agent, as in the case of a heat-labile enzyme, then it is necessary to replenish the inducing agent after every strand separation step. The simultaneous method may be utilized when an enzymatic means is used for the strand separation step. In the simultaneous procedure, the reaction mixture may contain, in addition to the nucleic acid strand(s) containing the desired sequence, the strand-separating enzyme (e.g., helicase), an appropriate energy source for the strand-separating enzyme, such as rATP, the four nucleotides, the oligonucleotide primers in molar excess, and the inducing agent, e.g., Klenow fragment of *E. coli* DNA polymerase I. If heat is used for denaturation in a simultaneous process, a heat-stable inducing agent such as a thermostable poly-

merase may be employed which will operate at an elevated temperature, preferably 65°–90° C. depending on the inducing agent, at which temperature the nucleic acid will consist of single and double strands in equilibrium. For smaller lengths of nucleic acid, lower temperatures of about 50° C. may be employed. The upper temperature will depend on the temperature at which the enzyme will degrade or the temperature above which an insufficient level of primer hybridization will occur. Such a heat-stable enzyme is described, e.g., by A. S. Kaledin et al., *Biokhimiya,* 45, 644–651 (1980). Each step of the process will occur sequentially notwithstanding the initial presence of all the reagents. Additional materials may be added as necessary. After the appropriate length of time has passed to produce the desired amount of the specific nucleic acid sequence, the reaction may be halted by inactivating the enzymes in any known manner or separating the components of the reaction.

The process of the present invention may be conducted continuously. In one embodiment of an automated process, the reaction may be cycled through a denaturing region, a reagent addition region, and a reaction region. In another embodiment, the enzyme used for the synthesis of primer extension products can be immobilized in a column. The other reaction components can be continuously circulated by a pump through the column and a heating coil in series; thus the nucleic acids produced can be repeatedly denatured without inactivating the enzyme.

desired sequence [S] comprised of complementary strands [S+] and [S−] is utilized as the nucleic acid. During the first and each subsequent reaction cycle extension of each oligonucleotide primer on the original template will produce one new ssDNA molecule product of indefinite length which terminates with only one of the primers. These products, hereafter referred to as "long products," will accumulate in a linear fashion; that is, the amount present after any number of cycles will be proportional to the number of cycles.

The long products thus produced will act as templates for one or the other of the oligonucleotide primers during subsequent cycles and will produce molecules of the desired sequence [S+] or [S−]. These molecules will also function as templates for one or the other of the oligonucleotide primers, producing further [S+] and [S−], and thus a chain reaction can be sustained which will result in the accumulation of [S] at an exponential rate relative to the number of cycles.

By-products formed by oligonucleotide hybridizations other than those intended are not self-catalytic (except in rare instances) and thus accumulate at a linear rate.

The specific sequence to be amplified, [S], can be depicted diagrammatically as:

[S+] 5′ AAAAAAAAAAXXXXXXXXXXCCCCCCCCCC 3′
[S−] 3′ TTTTTTTTTTYYYYYYYYYYGGGGGGGGGG 5′

The appropriate oligonucleotide primers would be:

Primer 1: GGGGGGGGGG
Primer 2: AAAAAAAAAA

so that if DNA containing [S]

. . . zzzzzzzzzzzzzzzzAAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzzzz . . .
. . . zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGGzzzzzzzzzzzzzzzz . . .

is separated into single strands and its single strands are hybridized to Primers 1 and 2, the following extension reactions can be catalyzed by DNA polymerase in the presence of the four deoxyribonucleoside triphosphates:

3′ 5′
extends ← GGGGGGGGGG Primer 1
. . . zzzzzzzzzzzzzzzzAAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzzzz . . .
original template strand+

original template strand−
. . . zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGGzzzzzzzzzzzzzzzz . . .
Primer 2 AAAAAAAAAA → extends
5′ 3′

The present invention is demonstrated diagrammatically below were double-stranded DNA containing the

On denaturation of the two duplexes formed, the products are:

3′ 5′
. . .zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGG
newly synthesized long product 1

5′ 3′
. . .zzzzzzzzzzzzzzzzAAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzzzz . . .
original template strand+

-continued

3′ 5′
. . .zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGGzzzzzzzzzzzzzzzz . . .
original template strand

5′ 3′
AAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzzzz . . .
newly synthesized long product 3

If these four strands are allowed to rehybridize with Primers 1 and 2 in the next cycle, inducing agent will catalyze the following reactions:

Primer 2 5′ AAAAAAAAAA ——————→ extends to here
3′ . . . zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGG 5′
newly synthesized long product 1

extends ←—————— GGGGGGGGGG 5′ Primer 1
5′ . . .zzzzzzzzzzzzzzAAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzz . . . 3′
original template strand+

Primer 2 5′ AAAAAAAAAA ——————→ extends
3′ . . . zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGGzzzzzzzzzz . . . 5′
original template strand−

extends to here ←—————— GGGGGGGGGG 5′ Primer 1
5′ AAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzzz . . . 3′
newly synthesized long product 2

If the strands of the above four duplexes are separated, the following strands are found:

5′ AAAAAAAAAAXXXXXXXXXXCCCCCCCCCC 3′
newly synthesized [S+]
3′ . . . zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGG 5′
first cycle synthesized long product 1
3′ . . . zzzzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGG 5′
newly synthesized long product 1
5′ . . . zzzzzzzzzzzzzzzzAAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzz . . . 3′
original template strand+
5′ AAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzzz . . . 3′
newly synthesized long product 2
3′ . . . zzzzzzzzzzzzzzTTTTTTTTTTYYYYYYYYYYGGGGGGGGGGzzzzzzzzzzzzzzz . . . 5′
original template strand−
3′ TTTTTTTTTTYYYYYYYYYYGGGGGGGGGG 5′
newly synthesized [S−]
5′ AAAAAAAAAAXXXXXXXXXXCCCCCCCCCCzzzzzzzzzzzzzzz . . . 3′
first cycle synthesized long product 2

Cycle Number	Template	Long Products	Specific Sequence [S]
0	1	—	—
1	1	1	0
2	1	2	1
3	1	3	4
5	1	5	26
10	1	10	1013
15	1	15	32,752
20	1	20	1,048,555
n	1	n	$(2^n$-n-1)

Number of Double Strands After 0 to n Cycles

It is seen that each strand which terminates with the oligonucleotide sequence of one primer and the complementary sequence of the other is the specific nucleic acid sequence [S] that is desired to be produced.

The steps of this process can be repeated indefinitely, being limited only by the amount of Primers 1 and 2, inducing agent and nucleotides present. The amount of original nucleic acid remains constant in the entire process, because it is not replicated. The amount of the long products increases linearly because they are produced only from the original nucleic acid. The amount of the specific sequence increases exponentially. Thus, the specific sequence will become the predominant species. This is illustrated in the following table, which indicates the relative amounts of the species theoretically present after n cycles, assuming 100% efficiency at each cycle:

When a single-stranded nucleic acid is utilized as the template, only one long product is formed per cycle.

The method herein may be utilized to clone a particular nucleic acid sequence for insertion into a suitable expression vector. The vector may then be used to transform an appropriate host organism to produce the gene product of the sequence by standard methods of recombinant DNA technology.

In addition, the process herein can be used for in vitro mutagenesis. The oligodeoxyribonucleotide primers need not be exactly complementary to the DNA se-

quence which is being amplified. It is only necessary that they be able to hydridize to the sequence sufficiently well to be extended by the polymerase enzyme or by whatever other inducing agent is employed. The product of a polymerase chain reaction wherein the primers employed are not exactly complementary to the original template will contain the sequence of the primer rather than the template, thereby introducing an in vitro mutation. In further cycles this mutation will be amplified with an undiminished efficiency because no further mispaired primings are required. The mutant thus produced may be inserted into an appropriate vector by standard molecular biological techniques and might confer mutant properties on this vector such as the potential for production of an altered protein.

The process of making an altered DNA sequence as described above could be repeated on the altered DNA using different primers so as to induce further sequence changes. In this way a series of mutated sequences could gradually be produced wherein each new addition to the series could differ from the last in a minor way, but from the original DNA source sequence in an increasingly major way. In this manner changes could be made ultimately which were not feasible in a single step due to the inability of a very seriously mismatched primer to function.

In addition, the primer can contain as part of its sequence a non-complementary sequence provided that a sufficient amount of the primer contains a sequence which is complementary to the strand to be amplified. For example, a nucleotide sequence which is not complementary to the template sequence (such as, e.g., a promoter, linker, coding sequence, etc.) may be attached at the 5′ end of one or both of the primers, and thereby appended to the product of the amplification process. After the extension primer is added, sufficient cycles are run to achieve the desired amount of new template containing the non-complementary nucleotide insert. This allows production of large quantities of the combined fragments in a relatively short period of time (e.g., two hours or less) using a simple technique.

The method herein may also be used to enable detection and/or characterization of specific nucleic acid sequences associated with infectious diseases, genetic disorders or cellular disorders such as cancer. Amplification is useful when the amount of nucleic acid available for analysis is very small, as, for example, in the prenatal diagnosis of sickle cell anemia using DNA obtained from fetal cells. Amplification is particularly useful if such an analysis is to be done on a small sample using non-radioactive detection techniques which may be inherently insensitive, or where radioactive techniques are being employed but where rapid detection is desirable.

For purposes of this invention genetic diseases may include specific deletions and/or mutations in genomic DNA from any organism, such as, e.g., sickle cell anemia, cystic fibrosis, α-thalassemia, β-thalassemia, and the like. Sickle cell anemia can be readily detected via oligomer restriction analysis or a RFLP-like analysis following amplification of the appropriate DNA sequence by the present method. α-Thalassemia can be detected by the absence of a sequence, and β-thalassemia can be detected by the presence of a polymorphic restriction site closely linked to a mutation which causes the disease.

All of these genetic diseases may be detected by amplifying the appropriate sequence and analyzing it by Southern blots without using radioactive probes. In such a process, for example, a small sample of DNA from, e.g., amniotic fluid containing a very low level of the desired sequence is amplified, cut with a restriction enzyme, and analyzed via a Southern blotting technique. The use of non-radioactive probes is facilitated by the high level of the amplified signal.

In another embodiment a small sample of DNA may be amplified to a convenient level and then a further cycle of extension reactions performed wherein nucleotide derivatives which are readily detectable (such as ^{32}P-labeled or biotin labeled nucleoside triphosphates) are incorporated directly into the final DNA product, which may be analyzed by restriction and electrophoretic separation or any other appropriate method. An example of this technique in a model system is demonstrated in FIG. **5**.

In a further embodiment, demonstrated in a model system in FIG. **3**, the nucleic acid may be exposed to a particular restriction endonuclease prior to amplification. Since a sequence which has been cut cannot be amplified, the appearance of an amplified fragment, despite prior restriction of the DNA sample, implies the absence of a site for the endonuclease within the amplified sequence. The presence or absence of an amplified sequence can be detected by an appropriate method.

A practical application of this technique can be illustrated by its use in facilitating the detection of sickle cell anemia via the oligomer restriction technique described herein and in copending U.S. application Ser. No. 716,982 to Erlich et al. entitled "Method For Detection of Polymorphic Restriction Sites and Nucleic Acid Sequences" filed Mar. 28, 1985. Sickle cell anemia is a hemoglobin disease which is caused by a single base pair change in the sixth codon of the β-globin gene. FIG. **6** illustrates the sequences of normal and sickle cell β-globin genes in the region of their polymorphism, where the single bars mark the location of a DdeI site present only in the normal gene and where the double bars mark the location of a HinfI site which is non-polymorphic and thus present in both the normal and sickle cell alleles. FIG. **7** illustrates the process of oligomer restriction of normal β-globin DNA using a probe spanning both restriction sites and labeled where the asterisk appears. The DNA, amplified as provided herein, is denatured and annealed to the labeled probe. The enzyme DdeI cleaves the DNA at the reformed DdeI site and generates a labeled octamer. Under the conditions used in the test the octamer is short enough to dissociate from the duplex. The subsequent addition of the enzyme HinfI has no effect on the now single-stranded octamer. FIG. **8** illustrates the same process applied to the sickle cell allele of β-globin DNA. The enzyme DdeI cannot cleave the duplex formed by the amplified DNA and the labeled probe because of the A—A base pair mismatch. The enzyme HinfI, however, does restrict the hybrid and a labeled trimer is produced. In practice the method can diagnose the DNA of an individual as being either homozygous for the wild type, homozygous for the sickle type or a heterozygous carrier of the sickle cell trait, since a specific signal is associated with the presence of either allele. Use of this above-described method to amplify the pertinent sequence allows for a rapid analysis of a single copy gene using a probe with only a single ^{32}P label.

Various infectious diseases can be diagnosed by the presence in clinical samples of specific DNA sequences characteristic of the causative microorganism. These

include bacteria, such as Salmonella, Chlamydia, and Neisseria; viruses, such as the hepatitis viruses; and protozoan parasites, such as the Plasmodium responsible for malaria. U.S. Pat. No. 4,358,535 issued to Falkow describes the use of specific DNA hybridization probes for the diagnosis of infectious diseases. A problem inherent in the Falkow procedure is that a relatively small number of pathogenic organisms may be present in a clinical sample from an infected patient and the DNA extracted from these may constitute only a very small fraction of the total DNA in the sample. Specific amplification of suspected sequences prior to immobilization and hybridization detection of the DNA samples could greatly improve the sensitivity and specificity of these procedures.

Routine clinical use of DNA probes for the diagnosis of infectious diseases would be simplified considerably if non-radioactively labeled probes could be employed as described in EP 63,879 to Ward. In this procedure biotin-containing DNA probes are detected by chromogenic enzymes linked to avidin or biotin-specific antibodies. This type of detection is convenient, but relatively insensitive. The combination of specific DNA amplification by the present method and the use of stably labeled probes could provide the convenience and sensitivity required to make the Falkow and Ward procedures useful in a routine clinical setting.

The amplification process can also be utilized to produce sufficient quantities of DNA from a single copy human gene such that detection by a simple non-specific DNA stain such as ethidium bromide can be employed so as to make a DNA diagnosis directly.

In addition to detecting infectious diseases and pathological abnormalities in the genome of organisms, the process herein can also be used to detect DNA polymorphism which may not be associated with any pathological state.

The following examples are offered by way of illustration and are not intended in limit the invention in any manner. In these examples all percentages are by weight if for solids and by volume if for liquids, and all temperatures are in degrees Celsius unless otherwise noted.

EXAMPLE 1

A 25 base pair sequence having the nucleotide sequence

5′ CCTCGGCACCGTCACCCTGGATGCT 3′

3′ GGAGCCGTGGCAGTGGGACCTACGA 5′

contained on a 47 base pair FokI restriction fragment of pBR322 obtainable from ATCC was prepared as follows. A FokI digest of pBR322 containing the 47-bp fragment was produced by digesting pBR322 with FokI in accordance with the conditions suggested by the supplier, New England Biolabs Inc. The primers which were utilized were 5′ d(CCTCGGCACCG) 3′ and 5′ d(AGCATCCAGGGTG) 3′, and were prepared using conventional techniques. The following ingredients were added to 33 μl of buffer which consisted of 25 mM potassium phosphate, 10 mM magnesium chloride and 100 mM sodium chloride at pH 7.5: 2433 pmoles of each of the primers described above, 2.4 pmoles of the FokI digest of pBR322, 12 nmoles of dATP, 22 nmoles of dCTP, 19 nmoles of dGTP and 10 nmoles of TTP.

The mixture was heated to 85° C. for five minutes and allowed to cool to ambient temperature. Five units of the Klenow fragment of *E. coli* DNA polymerase I were added and the temperature was maintained for 15 minutes. After that time, the mixture was again heated to 85° C. for five minutes and allowed to cool. Five units of the Klenow fragment were again added and the reaction was carried out for 15 minutes. The heating, cooling and synthesis steps were repeated eleven more times.

After the final repetition, a 5μl aliquot was removed from the reaction mixture. This was heated to 85° C. for three minutes and allowed to cool to ambient temperature. 12.5 pmoles of α-P^{32}-deoxycytidine triphosphate and 5 units of Klenow fragment were added and the reaction was allowed to proceed for 15 minutes. The labeled products were examined by polyacrylamide gel electrophoresis. The FokI digest was labeled in a similar fashion and served as a control and molecular weight markers. The only heavily labeled band visible after the 13 cycles was the intended 25 base pair sequence.

EXAMPLE 2

The desired sequence to be amplified was a 94 base pair sequence contained within the human beta-globin gene and spanning the MstII site involved in sickle cell anemia. The sequence has the nucleotide sequence shown in FIG. 1.

I. Synthesis of Primers

The following two oligodeoxyribonucleotide primers were prepared by the method described below:

5′ CACAGGGCAGTAACG 3′ Primer A

and

5′ TTTGCTTCTGACACA 3′ Primer B

Automated Synthesis Procedures: The diethylphosphoramidites, synthesized according to Beaucage and Caruthers (*Tetrahedron Letters* (1981) 22:1859–1862), were sequentially condensed to a nucleoside derivatized controlled pore glass support using a Biosearch SAM-1. The procedure included detritylation with trichloroacetic acid in dichloromethane, condensation using benzotriazole as activating proton donor, and capping with acetic anhydride and dimethylaminopyridine in tetrahydrofuran and pyridine. Cycle time was approximately 30 minutes. Yields at each step were essentially quantitative and were determined by collection and spectroscopic examination of the dimethoxytrityl alcohol released during detritylation.

Oligodeoxyribonucleotide Deprotection and Purification Procedures: The solid support was removed from the column and exposed to 1 ml concentrated ammonium hydroxide at room temperature for four hours in a closed tube. The support was then removed by filtration and the solution containing the partially protected oligodeoxyribonucleotide was brought to 55° C. for five hours. Ammonia was removed and the residue was applied to a preparative polyacrylamide gel. Electrophoresis was carried out at 30 volts/cm for 90 minutes after which the band containing the product was identified by UV shadowing of a fluorescent plate. The band was excised and eluted with 1 ml distilled water overnight at 4° C. This solution was applied to an Altech RP18 column and eluted with a 7–13% gradient of acetonitrile in 1% ammonium acetate buffer at pH 6.0. The elution was monitored by UV absorbance at 260 nm and the appropriate fraction collected, quantitated by UV absorbance in a fixed volume and evaporated to dryness at room temperature in a vacuum centrifuge.

Characterization of Oligodeoxyribonucleotides: Test aliquots of the purified oligonucleotides were ^{32}P la-

beled with polynucleotide kinase and γ-^{32}P-ATP. The labeled compounds were examined by autoradiography of 14–20% polyacrylamide gels after electrophoresis for 45 minutes at 50 volts/cm. The procedure verifies the molecular weight. Base composition was determined by digestion of the oligodeoxyribonucleotide to nucleosides by use of venom diesterase and bacterial alkaline phosphatase and subsequent separation and quantitation of the derived nucleosides using a reverse phase HPLC column and a 10% acetonitrile, 1% ammonium acetate mobile phase.

II. Source of DNA

A. Extraction of Whole Human Wild-Type DNA

Human genomic DNA homozygous for normal β-globin was extracted from the cell line Molt4 (obtained from Human Genetic Mutant Cell Repository and identified as GM2219c) using the technique described by Stetler et al., *Proc. Nat. Acad. Sci. USA* (1982), 79:5966–5970.

B. Construction of Cloned Globin Genes

A 1.9 kb BamHI fragment of the normal β-globin gene was isolated from the cosmid pFC11 and inserted into the BamHI site of pBR328 (Soberon, et al., Gene (1980) 9:287–305). This fragment, which encompasses the region that hybridizes to the synthetic 40-mer probe, includes the first and second exons, first intron, and 5' flanking sequences of the gene (Lawn et al., Cell (1978), 15:1157–1174). This clone was designated pBR328:HbAand deposited under ATCC No. 39,698 on May 25, 1984.

The corresponding 1.9 kb BamHI fragment of the sickle cell allele of β-globin was isolated from the cosmid pFC12 and cloned as described above. This clone was designated pBR328:HbS and deposited under ATCC No. 39,699 on May 25, 1984.

Each recombinant plasmid was transformed into and propagated in *E. coli* MM294 (ATCC No. 39,607).

C. Digestion of Cloned Globin Genes with MstII

A total of 100 μg each of pBR328:HbA and pBR328:HbS were individually digested with 20 units of MstII (New England Biolabs) for 16 hours at 37° C. in 200 μl of 150 mM NaCl, 12 mM Tris HCl (pH 7.5), 12 mM $MgCl_2$, 1 mM dithiothreitol (DTT), and 100 μg/ml bovine serum albumin (BSA). The products are designated pBR328:HbA/MstII and pBR328:HbS/MstII, respectively.

III. Polymerase Chain Reaction

To 100 μl of buffer consisting of 60 mM sodium acetate, 30 mM Tris acetate and 10 mM magnesium acetate at pH 8.0 was added 2 μl of a solution containing 100 picomoles of Primer A (of the sequence d(CACAGGGCACTAACG)), 100 picomoles of Primer B (of the sequence d(TTTBCTTCTGACACA)) and 1000 picomoles each of dATP, dCTP, dGTP and TTP. In addition, one of the following sources of DNA described above was added:

10 μg whole human wild-type DNA (Reaction I)
0.1 picomole pBR328:HbA (Reaction II)
0.1 picomole pBR328:HbS (Reaction III)
0.1 picomole pBR328:HbA/MstII (Reaction IV)
0.1 picomole pBR328:HbS/MstII (Reaction V)
No target DNA (Reaction VI)

Each resulting solution was heated to 100° C. for four minutes and allowed to cool to room temperature for two minutes, whereupon 1 μl containing four units of Klenow fragment of *E. coli* DNA polymerase was added. Each reaction was allowed to proceed for 10 minutes, after which the cycle of adding the primers, nucleotides and DNA, heating, cooling, adding polymerase, and reacting was repeated nineteen times for Reaction I and four times for Reactions II–VI.

Four microliter aliquots of Reactions I and II removed before the first cycle and after the last cycle of each reaction were applied to a 12% polyacrylamide gel 0.089M in Tris-borate buffer at pH 8.3 and 2.5 mM in EDTA. The gel was electrophoresed at 25 volts/cm for four hours, transferred to a nylon membrane serving as solid phase support and probed with a 5'-^{32}P-labeled 40 bp synthetic fragment, prepared by standard techniques, of the sequence

5'd(TCCTGAGGAGAAGTCTGCCGT-TACTGCCCTGTGGGGCAAG)3'

in 30% formamide, 3×SSPE, 5×Denhardt's, 5% sodium dodecyl sulfate at pH 7.4. FIG. 2 is an autoradiograph of the probed nylon membrane for Reactions I and II. Lane 1 is 0.1 picomole of a 58-bp synthetic fragment control one strand of which is complementary to the above probe. Lane 2 is 4 μl of Reaction I prior to the first amplification cycle. Lane 3 is 4 μl of Reaction I after the 20th amplification cycle. Lane 4 is 4 μl of Reaction II after five amplification cycles. Lane 5 is a molecular weight standard consisting of a FokI (New England Biolabs) digest of pBR322 (New England Biolabs) labeled with alpha-^{32}P-dNTPs and polymerase. Lane 3 shows that after twenty cycles the reaction mixture I contained a large amount of the specific sequence of the proper molecular weight and no other detectable products. Reaction mixture II after five cycles also contained this product, as well as the starting nucleic acid and other products, as shown by Lane 4.

To 5.0 μl aliquots of Reactions II–VI after the fourth cycle were added 5 pmoles of each primer described above. The solutions were heated to 100° C. for four minutes and allowed to equilibrate to room temperature. Three pmoles each of alpha-^{32}P-dATP, alpha-^{32}P-dCTP, alpha-^{32}P-dGTP and alpha-^{32}P-TTP and four units of Klenow fragment were added. The reaction, in a final volume of 10 μl and at the salt concentrations given above, was allowed to proceed for 10 minutes. The polymerase activity was terminated by heating for 20 minutes at 60° C. Four μl aliquots of Reactions II–VI were loaded onto a 12% polyacrylamide gel 0.089M in Tris-borate buffer at pH 8.3 and 2.5 mM in EDTA. The gel was electrophoresed at 25 volts/cm for four hours after which autoradiography was performed.

FIG. 3 is an autoradiograph of the electrophoresis. Lane 1 is a molecular weight standard, Lane 2 is Reaction II, Lane 3 is Reaction III, Lane 4 is Reaction IV and Lane 5 is Reaction V. Another lane for Reaction VI with no DNA as control had no images in any of the lanes. It can be seen from the figure that the 94--bp fragment predicted from the target DNA was present only where intact β-globin DNA sequences were available for amplification, i.e., pBR328: HbA (Lane 2), pBR328: HbS (Lane 3) and pBR328: HbS/MstII (Lane 5). MstII digestion cuts pBR328: HbA in the 94-mer sequence rendering it incapable of being amplified, and the 94-mer band does not appear in Lane 4. In contrast, the 94-mer sequence in pBR328: HbS does not cut when the plasmid is digested with MstII and thus is available for amplification as shown in Lane 5.

FIG. 4 illustrates the chain reaction for three cycles in amplifying the 94-bp sequence. PC01 and PC02 are

Primers A and B. The numbers on the right indicate the cycles, whereas the numbers on the left indiciates the cycle number in which a particular molecule was produced.

EXAMPLE 3

This example illustrates amplification of a 110 bp sequence spanning the allelic MstII site in the human hemoglobin gene.

A total of 1.0 microgram whole human DNA, 100 picomoles D(ACACAACTGTGTTCACTAGC) and 100 picomoles (dCAACTTCATCCACGTTCACC), the primers having been prepared by the technique of Example 2, were dissolved in 100 μl of a solution which was:

1.5 mM in each of the four deoxyribonucleoside triphosphates
30 mM in Tris acetate buffer at pH 7.9
60 mM in sodium acetate
10 mM in magnesium acetate
0.25 mM in dithiothreitol

The solution was heated to 100° C. for one minute and brought rapidly to 25° C. for one minute, after which was added 2.5 units Klenow fragment of DNA polymerase. The polymerase reaction was allowed to proceed for two minutes at 25° C., after which the cycle of heating, cooling, adding Klenow, and reacting was repeated as often as desired.

With a 70% efficiency at each cycle, 15 cycles resulted in the synthesis of 1.4 femtomoles of the desired 110 bp fragment of the β-globin gene.

EXAMPLE 4

This example illustrates amplification of a 240 bp sequence spanning the allelic MstII site in the human hemoglobin gene. This sequence contains NcoI, HinfI and MstII restriction sites.

To 100 μl of a mixture of 60 mM sodium acetate, 30 mM Tris acetate and 10 mM magnesium acetate at pH 8.0 containing 0.1 pmole pBR328: HbA was added 2 μof Solution A containing:

100 pmoles d(GGTTGGCCAATCTACTCCAGG) primer
100 pmoles d(TAACCTTGATACCAACCTGCCC) primer
1000 pmoles each of dATP, dCTP, dGTP and TTP

The two primers were prepared by the technique described in Example 2. The solution was heated to 100° C. for four minutes and allowed to cool in ambient air for two minutes, after which was added 1 μl containing four units Klenow fragment of *E. coli* DNA polymerase. The reaction was allowed to proceed for 10 minutes after which the cycle of solution A addition, heating, cooling, adding polymerase, and reacting was repeated three times. To a 5.0 μl aliquot of the reactions was added 5 picomoles of each oligonucleotide primer described above. The solution was heated to 100° C. for four minutes and allowed to come to ambient temperature, after which 3 picomoles each of the alpha-^{32}P-labeled deoxyribonucleoside triphosphates and 4 units Klenow fragment were added. The reaction, in a final volume of 10 μl and at the salt concentrations given above, was allowed to proceed for 10 minutes. The polymerase activity was terminated by heating for 20 minutes at 60° C. Two μl aliquots were digested with NcoI, MstII, or HinfI and loaded onto a 12% polyacrylamide gel 0.089M in Tris-borate buffer at pH 8.3 and 2.5 mM in EDTA. The gel was electrophoresed at 25 volts/cm for four hours and autoradiography was performed. FIG. 5 illustrates the autoradiograph of the electrophoresis, were Lane 1 is the molecular weight standard, Lane 2 is without digestion with enzyme (240 bp intact), Lane 3 is digestion with NcoI (131 and 109 bp), Lane 4 is digestion with MstII (149 and 91 bp), and Lane 5 is digestion with HinfI (144 and 96 bp). The autoradiograph is consistent with the amplification of the 240 bp sequence.

EXAMPLE 5

This example illustrates use of the process herein to detect sickle cell anemia by sequential digestion.

Synthesis and Phosphorylation of Oligodeoxyribonucleotides

A labeled DNA probe, RS06, of the sequence

5′ *CTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGG 3′

where * indicates the label, and an unlabeled blocking oligomer, RS10, of the sequence:

3′ GACAGAGGTCACCTCTTCAGACGGCAATGACGGGACACCC 5′

which has three pair mismatches with RS06 were synthesized according to the procedures provided in Example 2(I). The probe RS06 was labeled by contacting five pmole thereof with 4 units of T4 polynucleotide kinase (New England Biolabs) and 50 pmole γ-^{32}P-ATP (New England Nuclear, about 7200 Ci/mmole) in a 40 μl reaction volume containing 70 mM Tris buffer (pH 7.6), 10 mM $MgCl_2$, 1.5 mM spermine, and 2.5 mM dithiothreitol for 90 minutes at 37° C. The total volume was then adjusted to 100 μl with 25 mM EDTA and purified according to the procedure of Maniatis et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory, 1982), pp. 464–465 over a 1 ml Bio Gel P-4 spin dialysis column from Bio-Rad equilibrated with Tris-EDTA (TE) buffer (10 mM Tris buffer, 0.1 mM EDTA, pH 8.0). The labeled probe was further purified by electrophoresis on a 18% polyacrylamide gel (19:1 acrylamide:BIS, Bio-Rad) in Tris-boric acid-EDTA (TBE) buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) for 500 vhr. After localization by autoradiography, the portion of the gel containing the labeled probe was excised, crushed and eluted into 0.2 ml TE buffer overnight at 4° C. TCA precipitation of the reaction product indicated that the specific activity was 4.9 Ci/mmole and the final concentration was 20 pmole/ml.

The unlabeled RS10 blocking oligomer was used at a concentration of 200 pmole/ml.

Isolation of Human Genomic DNA from Cell Lines

High molecular weight genomic DNA was isolated from the lymphoid cell lines Molt4, SC-1 and GM2064 using essentially the method of Stetler et al., Proc. Natl. Acad. SC1. USA (1982), 79, 5966–5970 (for Molt4) and Maniatis et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory, 1982), pp. 280–281

Molt4 (Human Mutant Cell Repository, GM2219C) is a T cell line homozygous for normal β-globin, and SC-1, deposited with ATCC on Mar. 19, 1985, is an EBV-transformed B cell line homozygous for the sickle cell allele. GM2064 (Human Mutant Cell Repository, GM2064) was originally isolated from an individual homozygous for hereditary persistance of fetal hemoglobin (HPFH) and contains no beta- or delta- globin

gene sequences. All cell lines were maintained in RPMI-1640 with 10% fetal calf serum.

Isolation of Human Genomic DNA from Clinical Blood Samples

A clinical blood sample designated CH12 from a known sickle cell carrier (AS) was obtained from Dr. Bertram Lubin of Children's Hospital in Oakland, Calif. Genomic DNA was prepared from the buffy coat fraction, which is composed primarily of peripheral blood lymphocytes, using a modification of the procedure described by Nunberg et al., Proc. Nat. Acad. Sci. U.S.A., 75, 5553-5556 (1978).

The cells were resuspended in 5 ml Tris-EDTA-NaCl (TEN) buffer (10 mM Tris buffer pH 8, 1 mM EDTA, 10 mM NaCl) and adjusted to 0.2 mg/ml proteinase K, 0.5% SDS, and incubated overnight at 37° C. Sodium perchlorate was then added to 0.7 M and the lysate gently shaken for 1-2 hours at room temperature. The lysate was extracted with 30 ml phenol/chloroform (1:1), then with 30 ml chloroform, and followed by ethanol precipitation of the nucleic acids. The pellet was resuspended in 2 ml of TE buffer and RNase A added to 0.005 mg/ml. After digestion for one hour at 37° C., the DNA was extracted once each with equal volumes of phenol, phenol/chloroform, and chloroform, and ethanol precipitated. The DNA was resuspended in 0.5 ml TE buffer and the concentration was determined by absorbance at 260 nm.

Polymerase Chain Reaction to Amplify Selectively β-Globin Sequences

Two micrograms of genomic DNA was amplified in an initial 100 μl reaction volume containing 10 mM Tris buffer (ph 7.5), 50 mM NaCl, 10 mM $MgCl_2$, 150 pmole of Primer A of the sequence d(CACAGGGCACTAACG), and 150 pmole of Primer B of the sequence d(CTTTGCTTCTGACACA) and overlayed with about 100 μl mineral oil to prevent evaporation.

Each DNA sample underwent 15 cycles of amplification where one cycle is composed of three steps:

(1) Denature in a heat block set at 95° C. for two minutes.

(2) Transfer immediately to a heat block set at 30° C. for two minutes to allow primers and genomic DNA to anneal.

(3) Add 2 μl of a solution containing 5 units of the Klenow fragment of E. coli DNA polymerase I (New England Biolabs), 1 nmole each of dATP, dCTP, dGTP and TTP, in a buffer composed of 10 mM Tris (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$, and 4 mM dithiothreitol. This extension reaction was allowed to proceed for 10 minutes at 30° C.

After the final cycle, the reaction was terminated by heating at 95° C. for two minutes. The mineral oil was extracted with 0.2 ml of chloroform and discarded. The final reaction volume was 130 μl.

Hybridization/Digestion of Amplified Genomic DNA with Probes and DdeI/HinfI

Forty-five microliters of the amplified genomic DNA was ethanol precipitated and resuspended in an equal volume of TE buffer. Ten microliters (containing the pre-amplification equivalent of 154 ng of genomic DNA) was dispensed into a 1.5 ml Microfuge tube and 20 μl of TE buffer to a final volume of 30 μl. The sample was overlayed with mineral oil and denatured at 95° C. for 10 minutes. Ten microliters of 0.6 M NaCl containing 0.02 pmole of labeled RS06 probe was added to the tube, mixed gently, and immediately transferred to a 56° C. heat block for one hour. Four microliters of unlabeled RS10 blocking oligomer (0.8 pmole) was added and the hybridization continued for an additional 10 minutes at the same temperature. Five microliters of 60 mM $MgCl_2$/0.1% BSA and 1 μl of DdeI (10 units, New England Biolabs) were added and the reannealed DNA was digested for 30 minutes at 56° C. One microliter of HinfI (10 units, New England Biolabs) was then added and incubated for another 30 minutes. The reaction was stopped by the addition of 4 μl 75 mM EDTA and 6 μl tracking dye to a final volume of 61 μl.

The mineral oil was extracted with 0.2 ml chloroform, and 18 μl of the reaction mixture (45 ng genomic DNA) was loaded onto a 30% polyacrylamide mini-gel (19:1, Bio-Rad) in a Hoeffer SE200 apparatus. The gel was electrophoresed at approximately 300 volts for one hour until the bromphenol blue dye front migrated to 3.0 cm offorigin. The top of 1.5 cm of the gel was removed and the remaining gel was exposed for four days with one intensification screen at −70° C.

Discussion of Autoradiograph (FIG. 9)

Each lane contains 45 ng of amplified genomic DNA. Lane A contains Molt4 DNA; Lane B, CH12; Lane C, SC-1; and Lane D, GM2064. Molt4 represents the genotype of a normal individual with two copies of the β^A gene per cell (AA), CH12 is a clinical sample from a sickle cell carrier with one β^A and one β^S gene per cell (AS), and SC-1 represents the genotype of a sickle cell individual with two copies of the β^S gene per cell (SS). GM2064, which contains no beta- or delta-globin sequences, is present as a negative control.

As seen in the autoradiogram, the DdeI-cleaved, β^A-specific octamer is present only in those DNA's containing the β^A gene (Lanes A and B), and the HinfI-cleaved, β^S-specific trimer is present only in those DNA's containing the β^S gene (Lanes B and C). The presence of both trimer and octamer (Lane B) is diagnostic for a sickle cell carrier and is distinguishable from a normal individual (Lane A) with only octamer and a sickle cell afflicted individual (Lane C) with only trimer.

As a comparison, repeating the experiment described above using non-amplified genomic DNA revealed that the amplification increased the sensitivity of detection by at least 1000 fold.

EXAMPLE 6

This example illustrates direct detection of a totally unpurified single copy gene in whole human DNA on gels without the need for a labeled probe.

Using the technique described in Example 3, a 110-bp fragment from a sequence in the first exon of the beta-globin gene was amplified from 10 micrograms of whole human DNA after 20 cycles. This 110-bp fragment produced after 20 cylces was easily visualized on gels stained with ethidium bromide.

The sequence was not amplified when it was first cut with the restriction enzyme DdeI unless, as in the beta-globin S allele, the sequence does not contain the restriction site recognized by the enzyme.

EXAMPLE 7

A. A total of 100 fmoles pBR328 containing a 1.9 kb insert from the human beta-globin A allele, 50 nmoles each alpha-32P-dNTP at 500 Ci/mole, and 1 nmole of

each of the primers used in Example 3 were dissolved in a solution containing 100 μl 30 mM Tris-acetate at pH 7.9, 60 mM sodium acetate, 100 mM dithiothreitol, and 10 mM magnesium acetate. This solution was brought to 100° C. for two minutes and cooled to 25° C. for one minute. A total of 1 μl containing 4.5 units Klenow fregment of E. coli DNA polymerase I and 0.09 units inorganic pyrophosphatase was added to prevent the possible build-up of pyrophosphate in the reaction mixture, and the reaction was allowed to proceed for two minutes at 25° C., after which the cycle of heating, cooling, adding enzyme, and reacting was repeated nine times. Ten-μl aliquots were removed and added to 1 μl 600 mM EDTA after each synthesis cycle. Each was analyzed on a 14% polyacrylamide gel in 90 mM Tris-borate and 2.5 mM EDTA at pH 8.3 and 24 volts/cm for 2.5 hours. The completed gel was soaked for 20 minutes in the same buffer with the addition of 0.5 μg/ml ethidium bromide, washed with the original buffer, and photographed in UV light using a red filter.

The 110-bp fragment produced was excised from the gel under ultraviolet light and the incorporated ^{32}p counted by Cerenkov radiation. An attempt to fit the data to an equation of the form: pmoles/10 μl=0.01 $[(1+y)^N-yN-1]$, where N represents the number of cycles and y the fractional yield per cycle, was optimal with y=0.619. This indicates that a significant amplification is occurring.

B. The above experiment was repeated except that 100 nmoles of each dNTP was added to a 100 μl reaction, no radiolabel was employed, and aliquots were not removed at each cycle. After 10 cycles the reaction was terminated by boiling for two minutes and rehybridization was performed at 57° C. for one hour. The sequence of the 110-bp product was confirmed by subjecting 8 μl aliquots to restriction analysis by addition of 1 μl bovine serum albumin (25 mg/ml) and 1 μl of the appropriate restriction enzyme (HinfI, MnlI, MstII, NcoI) and by reaction at 37° C. for 15 hours. PAGE was performed as described above.

EXAMPLE 8

This example illustrates the use of different primers to amplify various fragments of pBR328 and 322.

A. The experiment described in Example 7A was repeated except using the following primers: d(TTTGCTTCTGACACAACTGTGTTCACTAGC) and d(GCCTCACCACCAACTTCATCCACGTTCACC) to produce a 130-bp fragment of pBR328.

B. The experiment described in Example 7A was repeated except using the following primers: d(GGTTGGCCAATCTACTCCCAGG) and d(TGGTCTCCTTAAACCTGTCTTG) to produce a 262-bp fragment of pBR328. The reaction time was 20 minutes per cycle.

C. The experiment described in Example 8B was repeated except that 100 fmoles of an MstII digest of pBR328 containing a 1.9 kb insert from the human beta-globin S allele was used as initial template. This plasmid was cleaved several times by MstII but not inside the sequence to be amplified. In addition, the primers employed were as follows:

d(GGTTGGCCAATCTACTCCCAGG) and
d(TAACCTTGATACCAACCTGCCC)

to produce a 240-bp fragment.

D. The experiment described in Example 7B was repeated except that 100 fmoles of an NruI digest of pBR322 was used as template, 200 nmoles of each dNTP were used in the 100 μl reaction, and the primers were:

d(TAGGCGTATCACGAGGCCCT) and
d(CTTCCCCATCGGTGATGTCG) to produce a 500-bp fragment from pBR322. Reaction times were 20 minutes per cycle at 37° C. Final rehybridization was 15 hours at 57° C. Electrophoresis was on a 4% agarose gel.

EXAMPLE 9

This example illustrates the invention process wherein an in vitro mutation is introduced into the amplified segment.

A. A total of 100 fmoles of pBR322 linearized with NruI, 1 nmole each of the primers:

d(CGCATTAAAGCTTATCGATG) and
d(TAGGCGTATCACGAGGCCCT)

designed to produce a 75-bp fragment, 100 nmole each dNTP, in 100 μl 40 mM Tris at pH 8, 20 mM in $MgCl_2$, 5 mM in dithiothreitol, and 5 mg/ml bovine serum albumin were combined. The mixture was brought to 100° C. for one minute, cooled for 0.5 minutes in a water bath at 23° C., whereupon 4.5 units Klenow fragment and 0.09 units inorganic pyrophosphatase were added, and a reaction was allowed to proceed for three minutes. The cycle of heating, cooling, adding enzymes, and reacting was repeated nine times. The tenth reaction cycle was terminated by freezing and an 8-μl aliquot of the reaction mixture was applied to a 4% agarose gel visualized with ethidium bromide.

B. The experiment described in Example 9A was repeated except that the oligonucleotide primers employed were:

d(CGCATTAAAGCTTATCGATG) and
d(AATTAATACGACTCACTATAAGG-GAGATAGGCGTATCACGAGGCCCT).

These primers are designed to produce a 101-bp fragment, 26 nucleotides of which (in the second listed primer) are not present in pBR322. These nucleotides represent the sequence of the T7 promoter, which was appended to the 75-bp sequence from pBR322 by using the primer with 20 complementary bases and a 26-base 5′ extension. The procedure required less than two hours and produced two picomoles of the relatively pure 101-bp fragment from 100 fmoles of pBR322.

The T7 promoter can be used to initiate RNA transcription. T7 polymerase may be added to the 101-bp fragment to produce singlestranded RNA.

C. The experiment described in Example 8D was repeated except that the oligonucleotide primers employed were as follows:

d(TAGGCGTATCACGAGGCCCT) and
d(CCAGCAAGACGTAGCCCAGC)

to produce a 1000-bp fragment from pBR322.

D. The experiment described in Example 9C was repeated except that the oligonucleotide primers employed were as follows:

d(TAGGCGTATCACGAGGCCCT) and
d(AATTAATACGACTCACTATAGG-GAGATAGGCGTATCACGAGGCCCT)

so as to produce a 1026-bp fragment, 26 nucleotides of which (in the second listed primer) are not present in pBR322 and represent the T7 promoter described above. The promoter has been inserted adjacent to a 1000-bp fragment from pBR322.

The results indicate that a primer which is not a perfect match to the template sequence but which is none-

theless able to hybridize sufficiently to be enzymatically extended produces a long product which contains the sequence of the primer rather than the corresponding sequence of the original template. The long product serves as a template for the second primer to introduce an in vitro mutation. In further cycles this mutation is amplified with an undiminished efficiency, because no further mispaired primings are required. In this case, a primer which carries a non-complementary extension on its 5′ end was used to insert a new sequence in the product adjacent to the template sequence being copied.

E. Because the reaction with polymerase generates pyrophosphate and is theoretically reversible (Kornberg, A., DNA Replication, W. H. Freeman, San Francisco, 1980), the effect of including an inorganic pyrophosphatase to avoid potential pyrophosphorolysis of the product was examined. Qualitative polyacrylamide gel electrophoresis examination of reactions plus and minus pyrophosphatase demonstrated a minor but significant increase in homogeneity of product as a result of the inclusion of this enzyme.

EXAMPLE 10

This example illustrates employing nested sets of primers to decrease the background in the amplification of single copy genes.

Whole human DNA homozygous for the wild-type betaglobin allele was subjected to twenty cycles of amplification as follows: A total of 10 μg DNA, 200 picomoles each of the primers:

d(ACACAACTGTGTTCACTAGC) and
d(CAACTTCATCCACGTTCACC)

and 100 nanomoles each dNTP in 100 μl of 30 mM Tris-acetate pH 7.9, 60 mM sodium acetate, 10 mM dithiothreitol, and 10 mM magnesium acetate were heated to 100° C. for one minute, cooled to 25° C. for one minute, and treated with 2 units Klenow fragment for two minutes. The cycle of heating, cooling and adding Klenow was repeated 19 times. A ten-μl aliquot was removed from the reaction mixture and subjected to a further ten cycles of amplification using each of the primers:

d(CAGACACCATGGTGCACCTGACTCCTG)
and
d(CCCCACAGGGCAGTAACG-GCAGACTTCTCC),

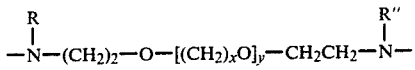
which amplify a 58-bp fragment contained within the 110-bp fragment produced above. This final ten cycles of amplification was accomplished by diluting the 10-μl aliquot into 90 μl of the fresh Tris-acetate buffer described above containing 100 nanomoles each dNTP and 200 pmoles of each primer. Reaction conditions were as above. After ten cycles a 10-μl aliquot (corresponding to 100 nanograms of the original DNA) was applied to a 6% NuSieve (FMC Corp.) agarose gel and visuallized using ethidium bromide.

FIG. **10** illustrates this gel illuminated with UV light and photographed through a red filter as is known in the art. Lane 1 is molecular weight markers. Lane 2 is an aliquot of the reaction described above. Lane 3 is an aliquot of a reaction identical to that described above, except that the original wild-type DNA was cleaved with DdeI prior to amplification. Lane 4 is an aliquot of a reaction identical to that described above, except that human DNA homozygous for the sickle betaglobin allele was treated with DdeI prior to amplification (the sickle allele does not contain a DdeI site in the fragment being amplified here). Lane 5 is an aliquot of a reaction identical to that described above, except that salmon sperm DNA was substituted for human DNA. Lane 6 is an aliquot of a reaction identical to that described above, except that the aliquot was treated with DdeI after amplification (DdeI should convert the 58-bp wild-type product into 27-and 31-bp fragments). Lane 7 is an aliquot of the Lane 4 material treated with DdeI after amplification (the 58-bp sickle product contains no DdeI site).

Detection of a 58-bp fragment representative of a singlecopy gene from one microgram of human DNA using only ethidium bromide staining of an agarose gel requires an amplification of about 500,000-fold. This was accomplished by using the two nested sets of oligonucleotide primers herein. The first set amplifies the 110-bp fragment and the inner nested set amplifies a sub-fragment of this product up to the level of convenient detection shown in FIG. **10**. This procedure of using primers amplifying a smaller sequence contained within the sequence being amplified in the previous amplification process and contained in the extension products of the other primers allows one to distinguish the wild-type from the sickle allele at the betaglobin locus without resorting to either radiosotopic or otherwise cumbersome methodology such as that of Conner et al., Proc. Natl. Acad. Sci. U.S.A., 80:278 (1983) and Leary et al., Proc. Natl. Acad. Sci. U.S.A., 80:4045 (1983).

EXAMPLE 11

The present process is expected to be useful in detecting, in a patient DNA sample, a specific sequence associated with an infectious disease such as, e.g., Chlamydia using a biotinylated hybridization probe spanning the desired amplified sequence and using the process described in U.S. Pat. No. 4,358,535, supra. The biotinylated hybridization probe may be prepared by intercalation and irradiation of a partially double-stranded DNA with a 4′-methylene substituted 4,5′-8-trimethylpsoralen attached to biotin via a spacer arm of the formula:



where R is —H or a —CHO group, R″ is —H, x is a number from 1 to 4, and y is a number from 2 to 4, as described in U.S. Pat. No. 4,582,789 issued Apr. 15, 1986 to K. Mullis et al., the disclosure of which is incorporated herein by reference. Detection of the biotinyl groups on the probe may be accomplished using a streptavidin-acid phosphatase complex commercially obtainable from Enzo Biochemical using the detection procedures suggested by the manufacturer in its brochure. The hybridized probe is seen as a spot of precipitated stain due to the binding of the detection complex, and the subsequent reaction catalyzed by acid phosphatase, which produces a precipitable dye.

Deposit of Materials

The cell line SC-1 (CTCC #0082) was deposited on Mar. 19, 1985 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852 USA, with ATCC Accession No. CRL#8756, The deposit of SC-1 was made pursuant to a contract between the ATCC and the assignee of this patent application, Cetus Corporation. The contract with ATCC

provides for permanent availability of the progeny of this cell line to the public on the issuance of the U.S. patent describing and identifying the deposit or the publications or upon the laying open to the public of any U.S. or foreign patent application, whichever comes first, and for availability of the progeny of this cell line to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 CFR §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638). The assignee of the present application has agreed that if the cell line on deposit should die or be lost or destroyed when cultivated under suitable conditions, it will be promptly replaced on notification with a viable culture of the same cell line.

In summary, the present invention is seen to provide a process for amplifying one or more specific nucleic acid sequences using a chain reaction in which primer extension products are produced which can subsequently act as templates for further primer extension reactions. The process is especially useful in detecting nucleic acid sequences which are initially present in only very small amounts.

Other modifications of the above described embodiments of the invention which are obvious to those of skill in the area of molecular biology and related disciplines are intended to be within the scope of the following claims.

What is claimed is:

1. A process for amplifying at least one specific nucleic acid sequence contained in a nucleic acid or a mixture of nucleic acids wherein each nucleic acid consists of two separate complementary strands, of equal or unequal length, which process comprises:

(a) treating the strands with two oligonucleotide primers, for each different specific sequence being amplified, under conditions such that for each different sequence being amplified an extension product of each primer is synthesized which is complementary to each nucleic acid strand, wherein said primers are selected so as to be sufficiently complementary to different strands of each specific sequence to hybridize therewith such that the extension product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of the extension product of the other primer;

(b) separating the primer extension products from the templates on which they were synthesized to produce single-stranded molecules; and

(c) treating the single-stranded molecules generated from step (b) with the primers of step (a) under conditions that a primer extension product is synthesized using each of the single strands produced in step (b) as a template.

2. The process of claim **1**, wherein steps (b) and (c) are repeated at least once.

3. The process of claim **1**, wherein said step (b) is accomplished by denaturing.

4. The process of claim **3**, wherein said denaturing is caused by heating.

5. The process of claim **1**, wherein said step (b) is accomplished using the enzyme helicase.

6. The process of claim **1**, wherein steps (a) and (c) are accomplished using an enzyme.

7. The process of claim **6**, wherein said enzyme is selected from the group consisting of *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, reverse transcriptase wherein the template is RNA on DNA and the extension product is DNA, and an enzyme that after being exposed to a temperature of about 65°–90° C. forms said extension products at the temperature of reaction during steps (a) and (c).

8. The process of claim **7**, wherein said nucleic acid is double stranded and its strands are separated by denaturing before or during step (a).

9. The process of claim **1**, wherein said nucleic acid is DNA and said primers are oligodeoxyribonucleotides.

10. The process of claim **1**, wherein said nucleic acid is messenger RNA.

11. The process of claim **1** wherein said mixture of nucleic acids used in step (a) is the product of step (c).

12. The process of claim **11**, wherein the primers employed are different from the primers employed in the process for producing the product of step (c) used in step (a).

13. The process of claim **12**, wherein the primers employed result in the amplification of a smaller sequence contained within the sequence being amplified in the process for producing the product of step (c) used in step (a).

14. The process of claim **1**, wherein said steps are carried out simultaneously above room temperature using an enzyme that after exposed to a temperature of about 65°–90° C. forms said extension products at the temperature of reaction during steps (a) and (c).

15. The process of claim **1**, wherein the two primers in steps (a) and (c) are each present in a molar ratio of at least 1000:1 primer:complementary strand.

16. The process of claim **1**, wherein the nucleic acid sequence(s) to be modified is contained in a mixture of nucleic acids resulting from a chemical synthesis.

17. The process of claim **1**, wherein at least one primer contains at least one nucleotide which is not complementary to the specific sequence to be amplified.

18. The process of claim **17** wherein one primer comprises an oligonucleotide with 20 complementary nucleotides and, at its 5′ end, a T7 promoter containing 26 noncomplementary nucleotides.

19. A process for amplifying a specific nucleic acid sequence contained in double-stranded DNA which process comprises:

(a) separating the strands of the DNA by physical, chemical or enzymatic means;

(b) treating the single strands with two oligodeoxyribonucleotide primers, in a molar excess of primer: its complementary strand, under conditions such that an extension product of each primer is synthesized, using *E. coli* DNA polymerase I or Klenow fragment thereof, which extension product is complementary to each DNA strand, wherein said primers are selected so as to be sufficiently complementary to different strands of each specific sequence to hybridize therewith such that the extension product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of the extension product of the other primer;

(c) separating the primer extension products from the templates on which they are synthesized to produce single-stranded molecules by physical, chemical or enzymatic means; and

(d) treating the single-stranded molecules generated from step (c) with the two primers of step (b), in a

molar excess of primer: its complementary molecule, under conditions such that a primer extension product is synthesized, using *E. coli* DNA polymerase I or Klenow fragment thereof, and using each of the single strands produced in step (c) as a template.

20. The process of claim **19**, wherein steps (c) and (d) are repeated at least once.

21. The process of claim **1**, wherein, due to the degeneracy of the genetic code, a collection of primers is employed for each complementary strand, the sequence of one of which primers is exactly complementary to said complementary strand over the length of the primer.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,683,202
DATED : July 28, 1987
INVENTOR(S) : Kary B. Mullis

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,

After [*] Notice, please replace "The portion of the term of this patent subsequent to Jul. 28, 2004 has been disclaimed." with -- This patent is subject to a terminal disclaimer. --

Signed and Sealed this

First Day of July, 2003

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,683,202
APPLICATION NO. : 06/791308
DATED : July 28, 1987
INVENTOR(S) : Kary B. Mullis

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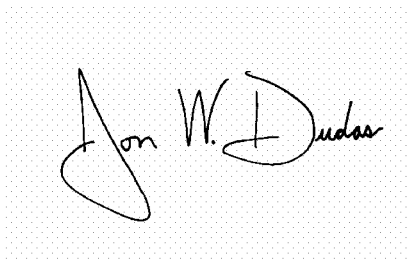
Fifth Day of June, 2007



JON W. DUDAS
Director of the United States Patent and Trademark Office

REEXAMINATION CERTIFICATE (1388th)

United States Patent [19]

Mullis

[11] **B1 4,683,202**

[45] Certificate Issued * **Nov. 27, 1990**

[54] **PROCESS FOR AMPLIFYING NUCLEIC ACID SEQUENCES**

[75] Inventor: **Kary B. Mullis**, Kensington, Calif.

[73] Assignee: **Cetus Corporation**

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[51] **Int. Cl.**[5] **C12P 19/34**; C12N 15/00; C12N 1/00; C07H 21/04

[52] **U.S. Cl.** **435/91**; 435/91; 435/172.3; 435/317.1; 536/27; 536/28; 536/29; 935/17; 935/18; 935/16

[58] **Field of Search** 435/91, 172.3, 317.1; 536/27, 28, 29; 935/17, 18

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,555,486	11/1985	Bahl et al.	435/91
4,582,788	4/1986	Erlich	435/6
4,737,462	4/1988	Mark et al.	435/252.33
4,853,332	8/1989	Mark et al.	435/252.33

OTHER PUBLICATIONS

Khorana, Progress Report, Grant No. CA11981-02, 09-01-1971 through 08-31-1972.
Zoller et al; DNA 3: 479 (1984).
BioLogicals Advertisement; Science, Dec. 11, 1981.
New England Biolabs Catalog, 1983/84, p. 29.
Molecular Cloning A Laboratory Manual, 1982, Maniatis et al, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 113-132 and 230-238.
Kaledin et al; Biokhimiya 45(4), 644 (1980), published by Plenum Publishing Corporation.
Chien et al; J. Bacteriol. 127: 1550 (1976).
Anonymous; Nature 341: 570 (1989).
Kleppe et al; J. Mol. Biol. 56: 341 (1976).
Khorana et al; J. Mol. Biol. 72: 209 (1972).
Panet et al; J. Biol. Chem. 249: 5213 (1974).
Besmer et al., 1972, *J. Mol. Biol.* 72:503-522.

Primary Examiner—James Martinell

[57] **ABSTRACT**

The present invention is directed to a process for amplifying any desired specific nucleic acid sequence contained in a nucleic acid or mixture thereof. The process comprises treating separate complementary strands of the nucleic acid with a molar excess of two oligonucleotide primers, and extending the primers to form complementary primer extension products which acts as templates for synthesizing the desired nucleic acid sequence. The steps of the reaction may be carried out stepwise or simultaneously and can be repeated as often as desired.

REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

NO AMENDMENTS HAVE BEEN MADE TO THE PATENT

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

The patentability of claims 1-21 is confirmed.

* * * * *

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United States Patent [19]

Salman

[11] Patent Number: 5,213,428

[45] Date of Patent: May 25, 1993

[54] **BIODEGRADABLE TOOTHBRUSH**

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[73] Assignee: **Elisabetta Molari**, Cesena, Italy

[21] Appl. No.: **878,550**

[22] Filed: **May 5, 1992**

[51] **Int. Cl.**[5] **A46B 5/04**

[52] **U.S. Cl.** **401/7**; 15/227; 15/167.1

[58] **Field of Search** 401/6-8; 132/308, 309; 15/227, 167.1

[56] **References Cited**

U.S. PATENT DOCUMENTS

D. 283,761	5/1986	Chen .	
463,309	11/1891	Schulze .	
1,811,833	6/1931	Simon	15/167.1 X
1,894,413	1/1933	Nenning et al.	401/7 X
1,947,720	2/1934	Laub	401/7
2,075,681	3/1937	Welker	401/7
2,077,540	4/1937	Welker	15/227 X
2,966,691	11/1958	Cameron .	
3,902,509	9/1975	Tundermann et al. .	
3,934,299	1/1976	Regester .	
4,251,897	2/1981	Alam	15/227 X
4,292,705	10/1981	Stouffer	132/308 X
4,335,731	6/1982	Bora, Jr. .	
4,432,114	2/1984	Goudsmit	15/167.1 X
4,617,694	10/1986	Bori .	
4,875,247	10/1989	Berg .	

Primary Examiner—Danton D. DeMille

Attorney, Agent, or Firm—Jacobson, Price, Holman & Stern

[57] **ABSTRACT**

A disposable toothbrush formed of a biodegradable material such as biodegradable plastic. The toothbrush would fit, preferably, over the index finger of a user. By using the index finger, it is easy to reach any part of the teeth by locating the toothbrush so as to apply a brushing action to the upper and lower teeth, with the toothbrush located on the outside or inside of the index finger. A toothbrush cap provides an opening for receipt of an index finger of a user. On a flattened portion of the toothbrush cap, are defined two to four rows of short bristles, preferably 3/16 inches in height. These bristles may be formed integrally with the toothbrush cap or include bristles imbedded in the cap. These bristles are impregnated with dehydrated toothpaste which bonds with the bristles to form a defined layer on top of the bristles. Upon contact with water, the impregnated toothpaste would hydrolyze to aid in brushing of the teeth for a single use. Upon completion of brushing, the toothbrush would be disposed of in its entirety.

11 Claims, 1 Drawing Sheet

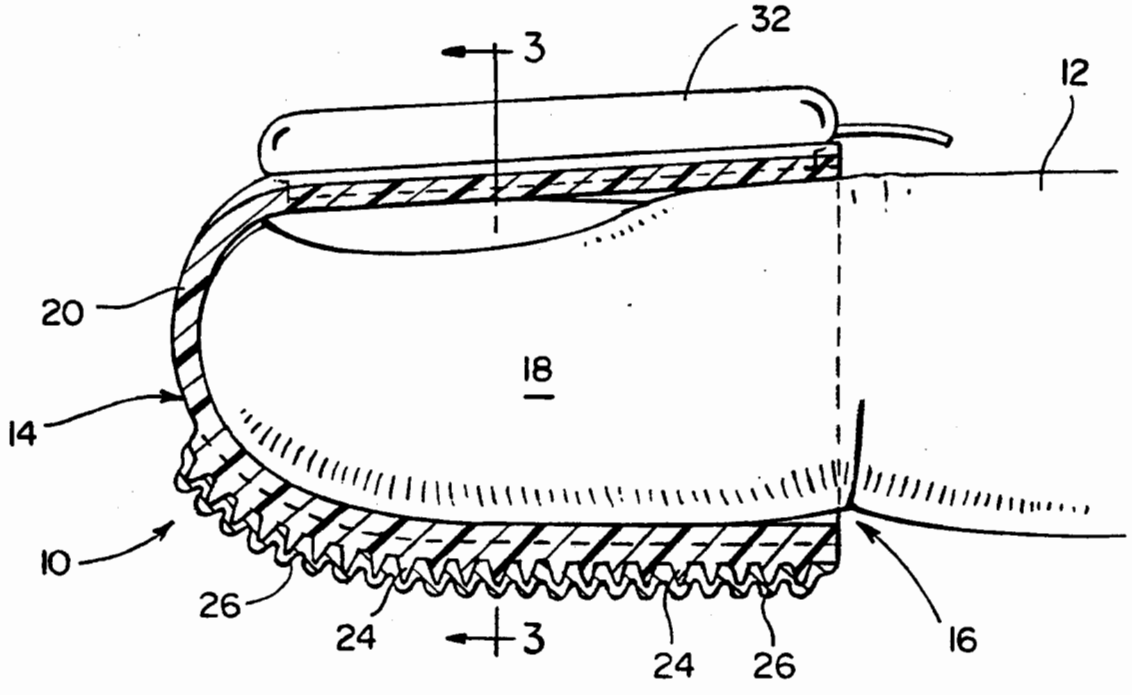


FIG. 1

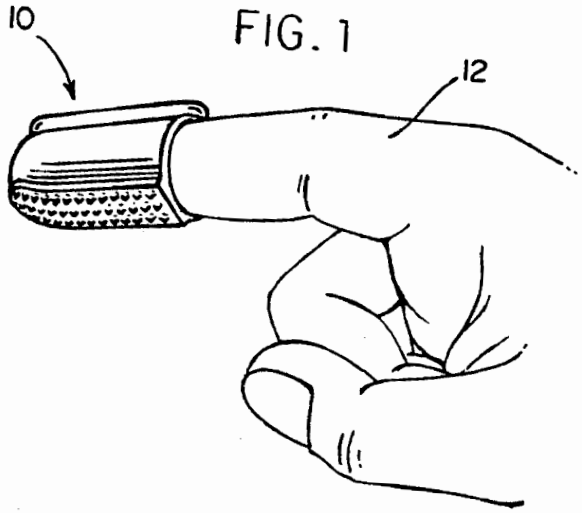


FIG. 5

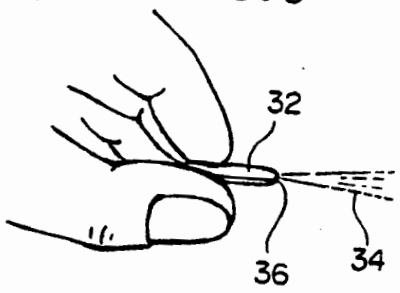
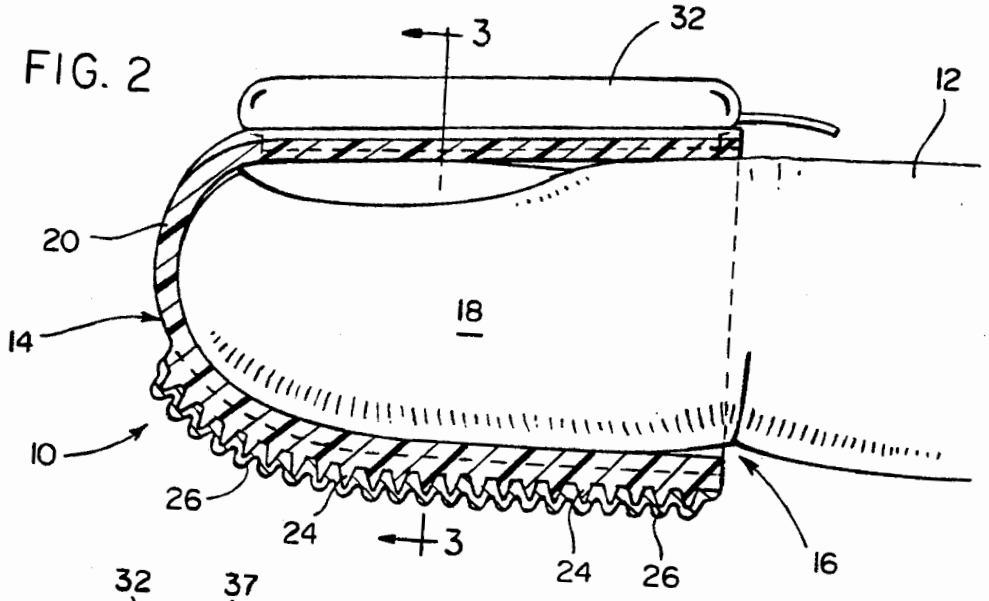


FIG. 2



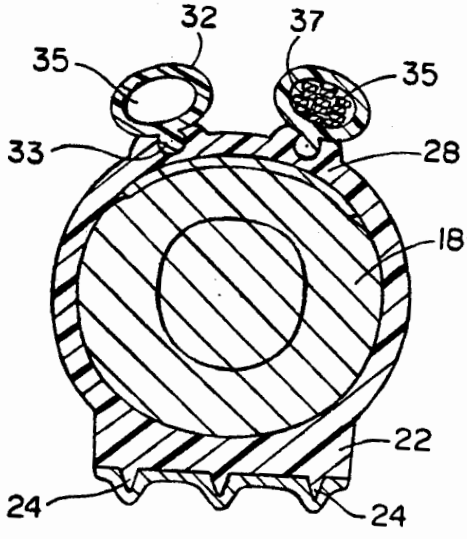
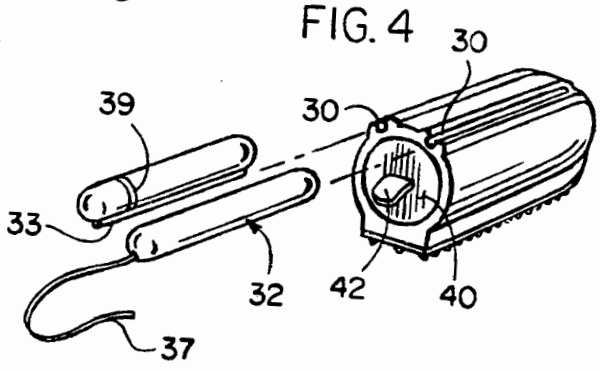


FIG. 3

FIG. 4



BIODEGRADABLE TOOTHBRUSH

FIELD OF THE INVENTION

This invention relates to a disposable, biodegradable toothbrush which is placed over the index finger of a user and includes a series of short bristles impregnated with dehydrated toothpaste and which includes accessory items stored on a periphery of the toothbrush, on a side of the toothbrush opposite to that of the bristles.

BACKGROUND OF THE INVENTION

There have been various attempts to provide an inexpensive, finger applied toothbrush device. Some of these prior attempts are as follows:

U.S. Pat. No. 463,309 to Schulze discloses a tooth cleaner composed of a piece of textile fabric impregnated with a tooth powder.

U.S. Pat. No. 2,966,691 to Cameron discloses a tooth cleaner formed of paper and elastic threads to cause the paper to contract and snugly fit around a finger of a user. Upon wetting the paper, the paper is permitted to shrink and firmly grip the fingertip of a user, having a puckered or pebbled configuration. A dentifrice is incorporated in the paper itself to produce an additional cleansing effect when the paper is wet and the dentifrice becomes active.

U.S. Pat. No. 3,902,509 to Tundermann et al. discloses a disposable device for cleaning teeth formed of a high wet strength material which is shaped and sealed in the form of a pocket or flat thimble. Adhered to the outer surface of the device is encapsulated polishing agent.

U.S. Pat. No. 3,934,299 to Regester discloses a tooth cleaning device adapted to be worn on the finger for cleaning the teeth, having an outer surface provided with a fabric texture and the fabric impregnated with a dentifrice material.

U.S. Pat. No. 4,335,731 to Bora, Jr., discloses a device for oral hygiene care composed of a flexible, soft honeycomb sheet. Bristles of the sheet extend outward from the exposed surface of the sheet. The sheet is secured over the finger to clean the user's gums and teeth. A dentifrice is impregnated in the sheet.

U.S. Pat. No. 4,617,694 to Bori discloses a finger-mounted device for cleaning teeth having pick means for cleaning between the teeth, a non-slip finger engaging means, and means for providing a length of dental floss.

U.S. Pat. No. 4,875,247 to Berg discloses a disposable tooth cleaning and polishing apparatus including a sheet of thin, flexible material such as paper cloth or synthetic foam material which also may be formed and contoured.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a toothbrush improved over the noted prior toothbrushes.

By the present invention, a versatile toothbrush enables people to have the availability of a toothbrush at any time and at any place without the need to carry any of the standard, more common toothbrushes. It would not be necessary to separately carry a tube of toothpaste or other dental accessories such as toothpick, dental floss, mouth medication and/or mouth freshener.

The toothbrush of the present invention is disposable and entirely formed of a biodegradable material such as biodegradable plastic. The toothbrush would fit, preferably, over the index finger of a user. By using the index finger, it is easy to reach any part of the teeth by locating the brush so as to apply a brushing action to the upper and lower teeth, with the toothbrush positioned to be located on the outside or inside of the index finger.

Moreover, by using the index finger, one can easily feel and verify whether or not the furthest portion of the teeth located at the back of the mouth have been brushed. The index finger provides a direct and instantaneous communication between finger nerve tissues of the sensory function which communicate with the brain to sense and ascertain complete and thorough brushing. Further, the index finger can be curved to all appropriate angles required for brushing of the teeth. It therefore, would no longer be necessary to carry a conventional toothbrush when travelling away from home. The present invention is ideally suited for hospitals, hotels, camping, air travel, etc., where a disposable toothbrush is necessary and not presently available.

It is also important that the present invention be made available to the elderly and sick, especially those with arthritis, Parkinson's disease and/or other forms of disease, where the age or sickness of the individuals makes it difficult for them to brush their teeth, due to a lack of ability to grab a utensil. Presently, this segment of society depends on others to brush their teeth and maintain appropriate dental hygiene. However, by the present invention, locating of the inventive toothbrush on the index finger of an individual enables that individual to maintain proper dental hygiene, thereby increasing their self-esteem and decreasing their susceptibility to disease and infections. Such disease or infections usually attribute directly to the deterioration of their already weakened state and thereby would improve the general state of their health.

Economically, the present invention allows a large portion of the population improved dental hygiene maintenance. For old or sick individuals, the savings produced by a reduction of disease and infection caused by an availability of a usable toothbrush, would produce large savings in their annual budgets attributed to health needs. Therefore, this invention would enable society and individual countries to provide a better quality of life for their inhabitants. Further, all age groups would benefit from the use of the present invention by contributing to the ease within which teeth could be brushed and thereby improve the dental hygiene of an increased cross-section of society.

By the toothbrush of the invention a cap provides an opening for receipt of an index finger of a user. On a flattened portion of the toothbrush cap, are defined two to four rows of short bristles, preferably 3/16 inches in height. These bristles may be formed integrally with the toothbrush cap o include bristles imbedded in the cap. These bristles are impregnated with dehydrated toothpaste which bonds with the bristles to form a defined layer on top of the bristles. Upon contact with water, the impregnated toothpaste would hydrolyze to aid in brushing of the teeth for a single use. Upon completion of brushing, the toothbrush would be disposed of in its entirety.

Located on an opposite side of the toothbrush cap from the bristles is a groove or a hole for mating with a capsule, including any one of different oral hygiene products. For example, a capsule could include 1 to 2 feet of dental floss, breath spray, a sterilized toothpick

or oral medication. It is also considered part of the present invention that these elements sealed in capsules may also be sealed in voids formed in the toothbrush cap so that upon gaining entry into the sealed voids of the toothbrush cap, access would then be obtained to dental floss, toothpick, breath spray, etc.

All of the materials used for the invention would be disposable, biodegradable material. Optionally, the tip of the toothbrush cap could be open to allow the tip of the finger to protrude through the toothbrush cap. All edges are rounded to prevent the possibility of any cuts being caused in the mouth during brushing. The toothbrush would be sterile and may be sealed within a biodegradable plastic enclosure for distribution purposes.

Typical measurements of the overall toothbrush would be approximately ¾ inch by 1½ inches. The bristle area may be as small as ¼ inch wide by 7/8 inch long.

It is therefore an object of the present invention to provide a disposable, biodegradable toothbrush cap which fits over a finger of the user for brushing of the teeth with impregnated toothpaste which hydrolyzes upon contact with water.

It is another object of the present invention to provide a disposable, biodegradable toothbrush cap which fits over a finger of the user for brushing of the teeth with impregnated toothpaste which hydrolyzes upon contact with water with the bristles being very short and projecting from one side of the toothbrush cap.

It is yet another object of the present invention to provide a disposable, biodegradable toothbrush cap which fits over a finger of the user for brushing of the teeth with impregnated toothpaste which hydrolyzes upon contact with water with the bristles being very short and projecting from one side of the toothbrush cap, with dental hygiene accessories stored inside the cap, and in or on the side wall of the cap on an opposite side of the cap from the bristles.

These and other objects of the invention, as well as many of the intended advantages thereof, will become more readily apparent when reference is made to the following description taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the disposable biodegradable toothbrush of the present invention located on the index finger of a user.

FIG. 2 is a longitudinal sectional view of the toothbrush cap as applied to the finger of a user.

FIG. 3 is a sectional view taken along line 3—3 of FIG. 2.

FIG. 4 is an exploded view of two capsules includes dental accessories which are separated from the toothbrush cap.

FIG. 5 illustrates application of a mouthwash spray from a capsule for a dental hygiene product.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In describing a preferred embodiment of the invention illustrated in the drawings, specific terminology will be resorted to for the sake in clarity. However, the invention is not intended to be limited to the specific terms so selected, and it is to be understood that each specific term includes all technical equivalents which operate in a similar manner to accomplish a similar purpose.

With reference to the drawings, in general, and to FIGS. 1 and 2, in particular, a disposable biodegradable toothbrush embodying the teachings of the subject invention is generally designated as **10**. With reference to its orientation in FIG. **1**, the toothbrush **10** is shown located on an index finger **12** of a user at the tip **18** of the index finger.

In FIG. **2**, the toothbrush is shown as including a cap **14** on a finger **12**, having an opening **16** into which the tip **18** of the index finger **12** is inserted. End **20** of the cap **14** may optionally be open so that the tip **18** of the index finger passes through the cap.

On one side **22** of the cap, an shown in FIG. **3**, is located a relatively flat portion from which projects bristles **24**, which in the embodiment shown includes three rows of bristles. It is also possible that two or four rows of bristles be included on the side **22** of the cap.

The bristles **24** are shown as being formed integrally with the cap. Optionally, the bristles may be formed from rigid plastic embedded in the cap **14**.

The bristles project from the outermost surface of side **22** for a distance of approximately 3/16 of an inch. Located on top of the bristles and the flat side **22** of the cap **14** is a dehydrated toothpaste layer **26**. This layer is hydrolyzed upon contact with water to provide a toothpaste aid during brushing.

Located on an opposite side **28** of the cap **14**, from the side **22**, are at least one groove **30**, as shown in FIG. **4**, for receipt of a dental hygiene accessory capsule **32**. The capsule is made of a disposable, biodegradable material such as biodegradable plastic. In FIG. **4**, two grooves **30** are shown for receipt of two capsules **32** which include complementary shaped projections **33** for slidingly mounting the capsules **32** on the cap **14**.

Capsule **32** may include a length of dental floss, a toothpick, mouthwash spray, oral medicine, or other dental hygiene products. The capsules **32** include a void **35** so that upon gaining access of the void by removing one end of the capsule as shown in FIG. **5**, upon application of pressure to the capsule, a liquid, such as a breath spray **34**, or oral medication, may be released under pressure from the open end **36** of the capsule **32**. Similarly, access to the interior of the capsule could be obtained, possibly by biting the end of the capsule to obtain access to dental floss **37** or other products sealed within the void **35** of the capsule. Alternately, a groove **39** is formed in side walls of the capsule so that the capsule is broken easily.

In addition a capsule **40** for holding mouthwash may be located in the opening formed by the cap for the receipt of a finger tip **18**. Handle projection **42** facilitates removal of capsule **40** for access of finger tip **18** into the cap **14**.

Optionally, the dental hygiene accessory products may be located in voids formed directly in side **28** of the cap **14** for access to the accessories either before or after brushing of the teeth with the bristles **24**. For brushing, the fingertip **18** is inserted in opening **16**, the layer **26** wetted, and the finger **12** moved along the teeth with application of slight pressure.

Improved dental hygiene would be more readily obtainable by a wider segment of the population through distribution of disposable, biodegradable toothbrushes as embodied by the invention. Further, those presently unable to brush their own teeth would have a better chance of brushing their teeth by locating the subject invention upon a finger of the hand and using their finger as the motive force as well as the applicator

for ensuring complete and improved brushing of their teeth.

The advantages obtained by the present invention are numerous. For example, one size toothbrush can fit men, women and children. A savings on water consumption is obtained by the invention by reduced rinsing over that required for a permanent-type toothbrush since this toothbrush is merely disposed of. Further, chemicals used for keeping the toothpaste moist for use on permanent-type toothbrushes are no longer needed. Bathroom sink mess from excess toothpaste is avoided by premeasured toothpaste impregnated on the disposable toothbrush. In addition, toothbrush holders are no longer necessary.

Having described the invention, many modifications thereto will become apparent to those skilled in the art to which it pertains without deviation from the spirit of the invention as defined by the scope of the appended claims.

I claim:

1. A disposable toothbrush comprising:

a cap including an opening for receipt of a fingertip,

a flat surface located on one side of said cap having bristles projecting therefrom for brushing of teeth,

a layer of dehydrated toothpaste being located on said bristles, and

at least one dental hygiene accessory located on a side of said cap opposite to said one side,

said at least one dental hygiene accessory being located within a capsule slidably mounted on said cap.

2. A disposable toothbrush as claimed in claim 1, wherein said capsule surrounds said at least one dental hygiene accessory.

3. A disposable toothbrush as claimed in claim 1, wherein said cap is biodegradable.

4. A disposable toothbrush as claimed in claim 1, wherein said bristles are integral with said cap.

5. A disposable toothbrush as claimed in claim 1, wherein said cap includes a groove for receipt of a projection extending from said capsule.

6. A disposable toothbrush as claimed in claim 1, wherein said capsule includes a void for storing of said dental hygiene accessory.

7. A disposable toothbrush comprising:

a cap including an opening for receipt of a fingertip,

a flat surface defined by one side of said cap and having bristles projecting therefrom for brushing of teeth,

a layer of dehydrated toothpaste embedded on said bristles, and

a dental hygiene accessory located on said cap,

said dental hygiene accessory being located on a side of said cap opposite to said one side and within a capsule slidably mounted on said cap.

8. A disposable toothbrush as claimed in claim 7, wherein said dental hygiene accessory is a liquid located in said capsule housed within said cap.

9. A disposable toothbrush as claimed in claim 8, wherein said liquid is released upon opening of a capsule having stored said liquid.

10. A disposable toothbrush as claimed in claim 9, wherein said cap includes a groove for receipt of a projection extending from said capsule.

11. A disposable toothbrush as claimed in claim 7, wherein said cap is biodegradable.

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