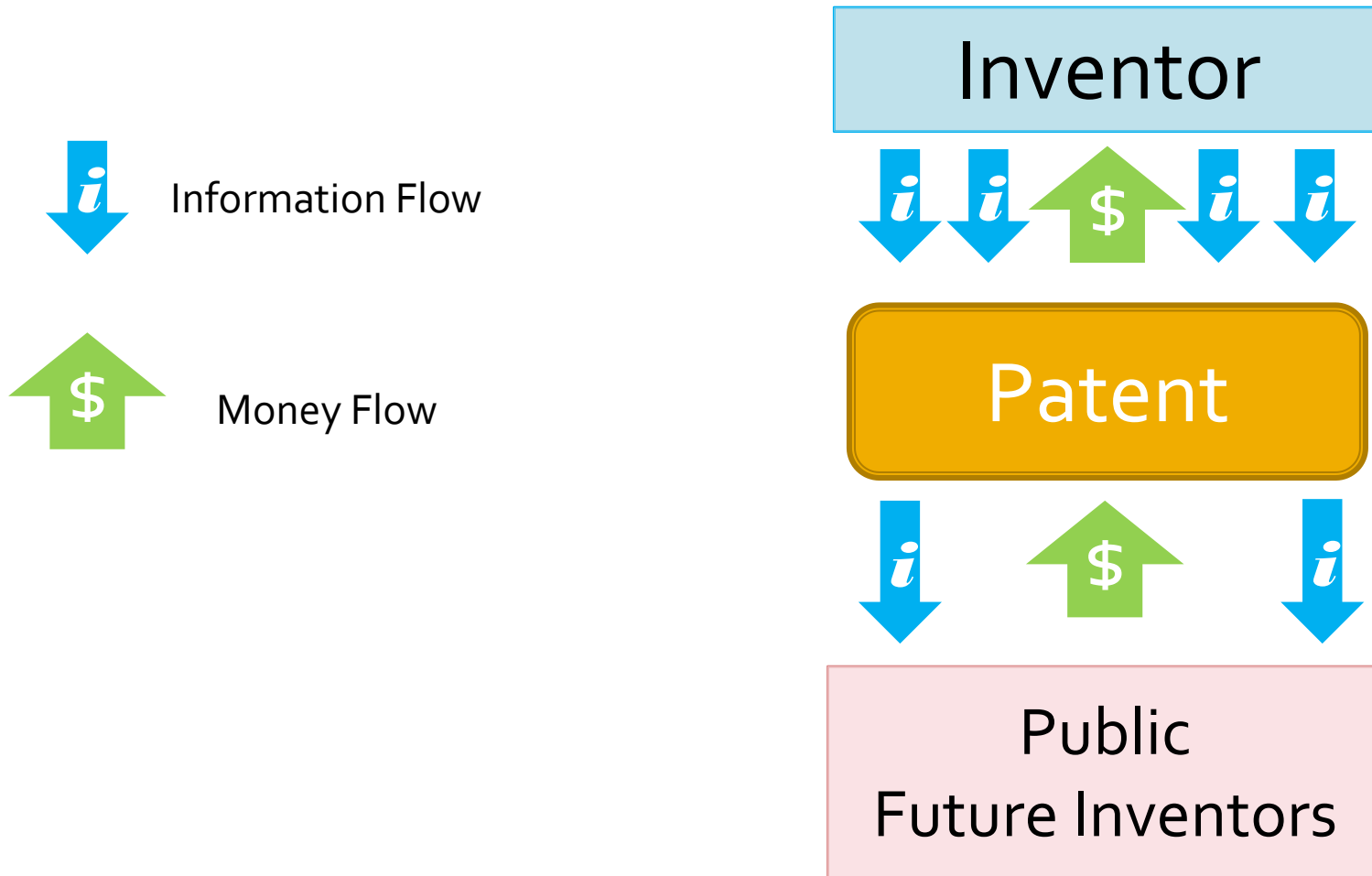


Peripheral Disclosure

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Conventional Disclosure Theory



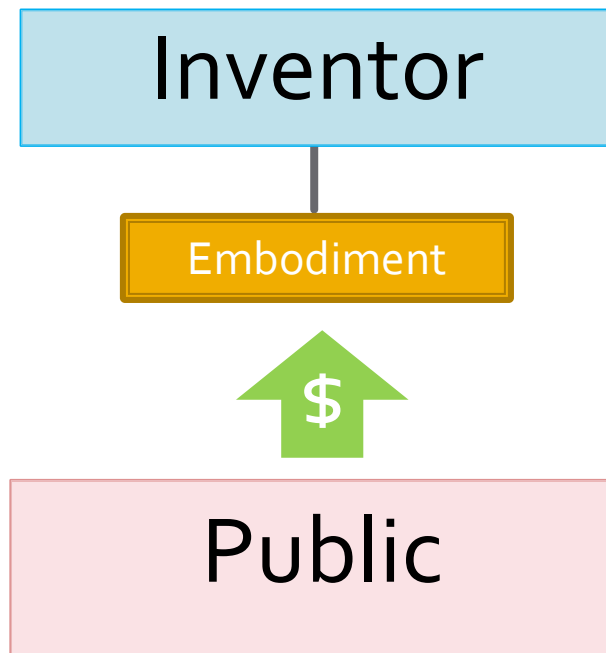
A World Without Patents



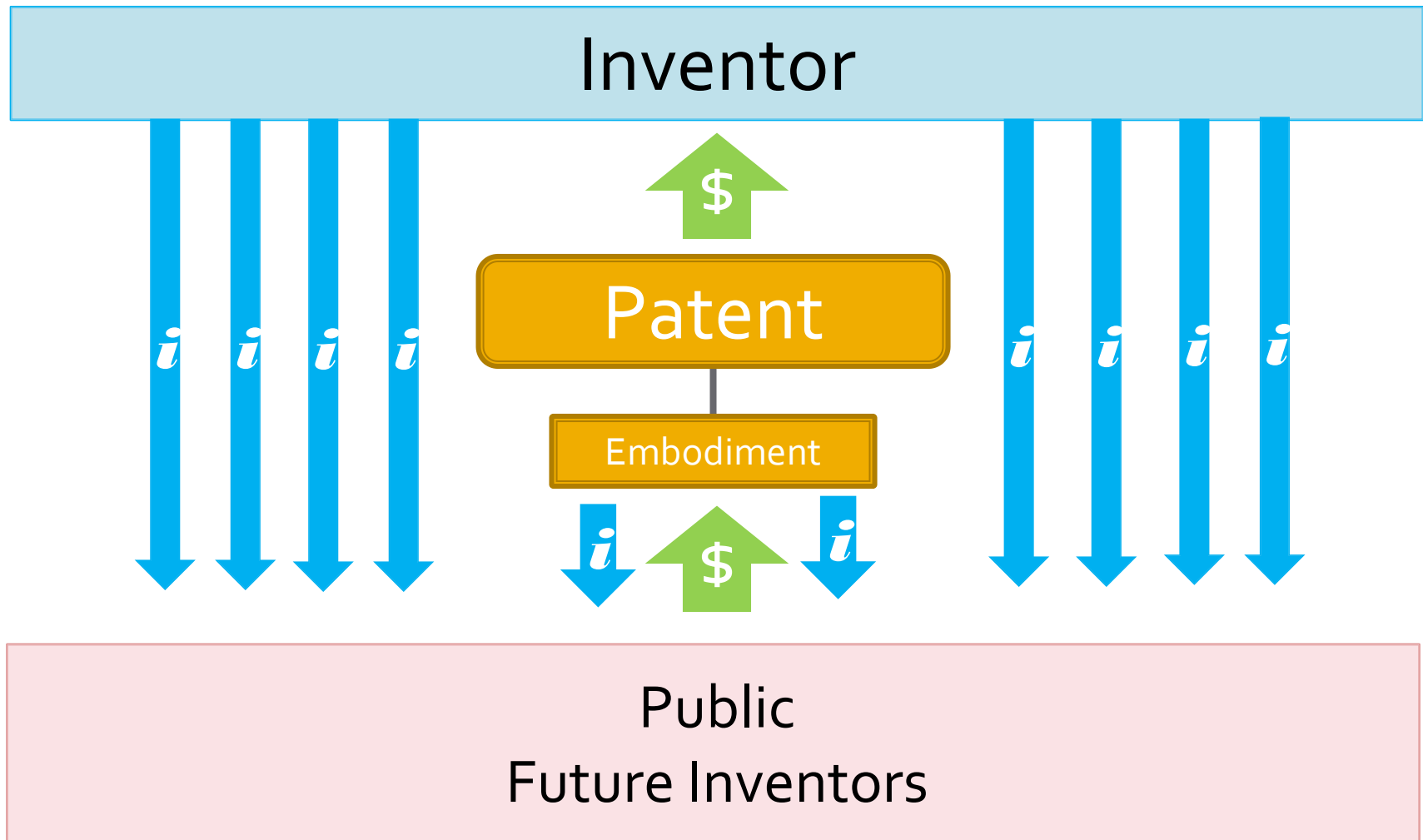
Information Flow



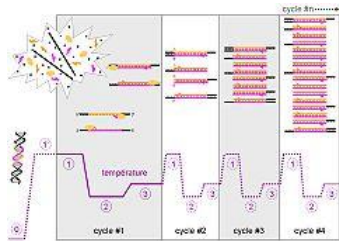
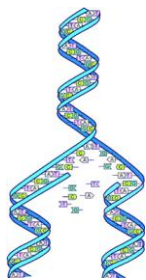
Money Flow



Peripheral Disclosure



Patents Allow Inventors to Publish About their Inventions



RESEARCH ARTICLE

Enzymatic Amplification of β -Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia

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Glenn T. Horn, Henry A. Erlich, Norman Arheim

Recent advances in recombinant DNA technology have made possible the molecular analysis and prenatal diagnosis of several human genetic diseases. Fetal DNA obtained by amniocentesis or chorionic villus sampling can be analyzed by restriction enzyme digestion, with subsequent electrophoresis, Southern transfer, and specific hybridization to cloned gene or oligonucleotide probes. With

This disease results from homozygosity of the sickle-cell allele (β^S) at the β -globin gene locus. The S allele differs from the wild-type allele (β^A) by substitution of an A in the wild-type to a T at the second position of the sixth codon of the β chain gene, resulting in the replacement of a glutamic acid by a valine in the expressed protein. For the prenatal diagnosis of sickle cell anemia, DNA ob-

lessen the complexity of prenatal diagnosis for sickle cell anemia, they may also be generally applicable to the diagnosis of other genetic diseases and in the use of DNA probes for infectious disease diagnosis.

Sequence amplification by polymerase chain reaction. We use a two-step procedure for determining the β -globin genotype of human genomic DNA samples. First, a small portion of the β -globin gene sequence spanning the polymorphic Dde I restriction site diagnostic of the β^S allele is amplified. Next, the presence or absence of the Dde I restriction site in the amplified DNA sample is determined by solution hybridization with an end-labeled complementary oligomer followed by restriction endonuclease digestion, electrophoresis, and autoradiography.

The β -globin gene segment was amplified by the polymerase chain reaction (PCR) procedure of Mullis and Falcoona (2) in which we used two 20-base oligonucleotide primers that flank the region to be amplified. One primer, PC04, is complementary to the (+) strand and the other, PC03, is complementary to the (-) strand (Fig. 1). The annealing of PC04 to the (+) strand of denatured genomic DNA followed by extension with the Klenow fragment of *Escherichia coli* DNA polymerase I and deoxynucleotide triphosphates results in the synthesis of a (+) strand fragment containing the target sequence. At the same time, a similar reaction occurs with PC03, creating a new (+) strand. Since these newly synthesized DNA strands are themselves template for the PCR primers, repeated cycles of denaturation, primer annealing, and extension result in the exponential accumulation of the 110-base pair region defined by the primers.

An example of the degree of specific gene amplification achieved by the PCR method is shown in Fig. 2A. Samples of DNA (1 μ g) were amplified for 20 cycles and a fraction of each sample, equivalent to 36 ng of the original DNA, was subjected to alkaline gel electrophoresis and transferred to a nylon filter. The filter was then hybridized with a 32 P-labeled 40-base oligonucleotide probe, R306, which is complementary to the target sequence (Fig. 1A) but not to the PCR primers. The results, after a 2-hour autoradiographic exposure, show that a fragment hybridizing with the R306 probe

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SCIENCE, VOL. 230

United States Patent [19]
Mullis et al.

Patent Number: 4,683,195
Date of Patent: Jul. 28, 1987

[54] PROCESS FOR AMPLIFYING, DETECTING, AND/OR CLONING NUCLEIC ACID SEQUENCES

[75] Inventors: Kary B. Mullis, Kensington; Henry A. Erlich, Oakland; Norman Arheim, Woodland Hills; Glenn T. Horn, Emeryville; Randall K. Saki, Richmond; Stephen J. Scharf, Berkeley, all of Calif.

[73] Assignee: Cetus Corporation, Emeryville, Calif.
[1*] Notice: The portion of the term of this patent subsequent to Jul. 28, 2004 has been disclaimed.

[21] Appl. No. 828,144
[22] Filed: Feb. 7, 1986

Related U.S. Application Data
[60] Continuation-in-part of Ser. No. 824,044, Jan. 30, 1986, abandoned, which is a division of Ser. No. 791,308, Oct. 23, 1985, which is a continuation-in-part of Ser. No. 716,075, Mar. 28, 1985, abandoned.

[51] Int. Cl.⁴ C12Q 1/68; C12P 19/34; C12N 1/00; G01N 15/00; G01N 33/68; G01N 33/00; G01N 33/566; G01N 33/564; C07H 21/02; C07H 21/04

[52] U.S. Cl. 435/6; 435/91; 435/172.3; 435/317; 436/63; 436/96; 436/201; 436/208; 536/27; 536/28; 536/29; 935/17; 935/18; 935/23; 935/77; 935/78

[58] Field of Search 435/91; 172.3; 317; 435/6; 536/27; 28; 29; 935/17; 18; 78; 77; 76; 436/93; 94; 501; 508

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Primary Examiner—James Harnwell
Attorney, Agent, or Firm—Janet E. Haak; Albert P. Hallinan

[57] ABSTRACT
The present invention is directed to a process for amplifying and detecting any target 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, extending the primers to form complementary primer extension products which act as templates for synthesizing the desired nucleic acid sequence, and detecting the sequence so amplified. The steps of the reaction may be carried out stepwise or simultaneously and can be repeated as often as desired.

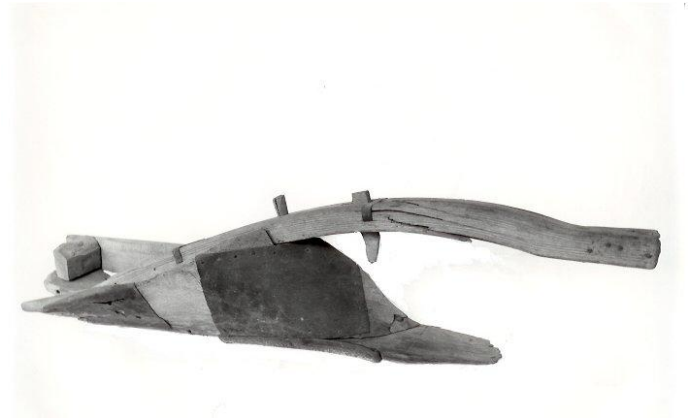
In addition, a specific nucleic acid sequence may be cloned into a vector by using primers to amplify the sequence, which contain restriction sites on their non-complementary ends, and a nucleic acid fragment may be prepared from an existing shorter fragment using the amplification process.

26 Claims, 12 Drawing Figures

Patents Allow Inventors to Invest in the Creation of Self-Disclosing Inventions



VS.



Peripheral Disclosure

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