In re Patrick H. O'FARRELL, Barry A. Polisky and David H. Gelfand.

United States Court of Appeals for the Federal Circuit
August 10, 1988
853 F.2d 894

J. Bruce McCubbrey, Fitch, Even, Tabin & Flannery, of San Francisco, Cal., argued for appellant. Virginia H. Meyer, Fitch, Even, Tabin & Flannery, of San Francisco, Cal., was on the brief for appellant.

Harris A. Pitlick, Associate Sol., of Arlington, Va., argued for appellee. With him on the brief were Joseph F. Nakamura, Sol. and Fred E. McKelvey, Deputy Sol.

Before MARKEY, Chief Judge, and RICH and NIES, Circuit Judges.

RICH, Circuit Judge.

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This appeal is from the decision of the United States Patent and Trademark Office Board of Patent Appeals and Interferences (board) affirming the patent examiner's final rejection of patent application Serial No. 180,424, entitled "Method and Hybrid Vector for Regulating Translation of Heterologous DNA in Bacteria." The application was rejected under 35 U.S.C. Sec. 103 on the ground that the claimed invention would have been obvious at the time the invention was made in view of a published paper by two of the three coinventors, and a publication by Bahl, Marians & Wu, 1 Gene 81 (1976) (Bahl). We affirm.

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The claimed invention is from the developing new field of genetic engineering. A broad claim on appeal reads:

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Claim 1. A method for producing a predetermined protein in a stable form in a transformed host species of bacteria comprising, providing a cloning vector which includes at least a substantial portion of a gene which is indigenous to the host species of bacteria and is functionally transcribed and translated in that species, said substantial portion of said indigenous gene further including the regulatory DNA sequences for RNA synthesis and protein synthesis but

lacking the normal gene termination signal, and linking a natural or synthetic heterologous gene encoding said predetermined protein to said indigenous gene portion at its distal end, said heterologous gene being in proper orientation and having codons arranged in the same reading frame as the codons of said indigenous gene portion so that readthrough can occur from said indigenous gene portion into said heterologous gene in the same reading frame, said heterologous gene portion further containing sufficient DNA sequences to result in expression of a fused protein having sufficient size so as to confer stability on said predetermined protein when said vector is used to transform said host species of bacteria.

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Illustrative embodiments are defined in more specific claims. For example:

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Claim 2. A method for producing a predetermined protein in a stable form in a transformed host species of bacteria, comprising, providing an E. coli plasmid having an operator, a promoter, a site for the initiation of translation, and at least a substantial portion of the beta-galactosidase gene of the E. coli lactose operon, said substantial portion of said beta-galactosidase gene being under the control of said operator, promoter and site for initiation of translation, said substantial portion of said beta-galactosidase gene lacking the normal gene termination signal, and linking a heterologous gene encoding said predetermined protein to said beta-galactosidase gene portion at its distal end, said heterologous gene being in proper orientation and having codons arranged in the same reading frame as the codons of the said beta-galactosidase gene portion so that readthrough can occur from said beta-galactosidase gene portion into said heterologous gene in the same reading frame, said heterologous gene portion further containing sufficient DNA sequences to result in expression of a fused protein having sufficient size so as to confer stability on said predetermined protein when said vector is used to transform said host species of bacteria.

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Claim 3. The method of Claim 2 wherein said E. coli plasmid comprises the plasmid designated pBGP120.

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Although the terms in these claims would be familiar to those of ordinary

skill in genetic engineering, they employ a bewildering vocabulary new to those who are not versed in molecular biology. An understanding of the science and technology on which these claims are based is essential before one can analyze and explain whether the claimed invention would have been obvious in light of the prior art.

I. Background[1]

[8]

Proteins are biological molecules of enormous importance. Proteins include enzymes that catalyze biochemical reactions, major structural materials of the animal body, and many hormones. Numerous patents and applications for patents in the field of biotechnology involve specific proteins or methods for making and using proteins. Many valuable proteins occur in nature only in minute quantities, or are difficult to purify from natural sources. Therefore, a goal of many biotechnology projects, including appellants' claimed invention, is to devise methods to synthesize useful quantities of specific proteins by controlling the mechanism by which living cells make proteins.

[9]

The basic organization of all proteins is the same. Proteins are large polymeric molecules consisting of chains of smaller building blocks, called amino acids, that are linked together covalently.[2] The chemical bonds linking amino acids together are called peptide bonds, so proteins are also called polypeptides.[3] It is the exact sequence in which the amino acids are strung together in a polypeptide chain that determines the identity of a protein and its chemical characteristics.[4] Although there are only 20 amino acids, they are strung together in different orders to produce the hundreds of thousands of proteins found in nature.

[10]

To make a protein molecule, a cell needs information about the sequence in which the amino acids must be assembled. The cell uses a long polymeric molecule, DNA (deoxyriboneucleic acid), to store this information. The subunits of the DNA chain are called nucleotides. A nucleotide consists of a nitrogen-containing ring compound (called a base) linked to a 5-carbon sugar that has a phosphate group attached.[5] DNA is composed of only four nucleotides. They differ from each other in the base region of the molecule. The four bases of these subunits are adenine, guanine, cytosine, and thymine (abbreviated respectively as A, G, C and T). The sequence of these bases along

the DNA molecule specifies which amino acids will be inserted in sequence into the polypeptide chain of a protein.

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DNA molecules do not participate directly in the synthesis of proteins. DNA acts as a permanent "blueprint" of all of the genetic information in the cell, and exists mainly in extremely long strands (called chromosomes) containing information coding for the sequences of many proteins, most of which are not being synthesized at any particular moment. The region of DNA on the chromosome that codes for the sequence of a single polypeptide is called a gene.6 In order to express a gene (the process whereby the information in a gene is used to synthesize new protein), a copy of the gene is first made as a molecule of RNA (ribonucleic acid).

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RNA is a molecule that closely resembles DNA. It differs, however, in that it contains a different sugar (ribose instead of deoxyribose) and the base thymine (T) of DNA is replaced in RNA by the structurally similar base, uracil (U). Making an RNA copy of DNA is called transcription. The transcribed RNA copy contains sequences of A, U, C, and G that carry the same information as the sequence of A, T, C, and G in the DNA. That RNA molecule, called messenger RNA, then moves to a location in the cell where proteins are synthesized.

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The code whereby a sequence of nucleotides along an RNA molecule is translated into a sequence of amino acids in a protein (i.e., the "genetic code") is based on serially reading groups of three adjacent nucleotides. Each combination of three adjacent nucleotides, called a codon, specifies a particular amino acid. For example, the codon U-G-G in a messenger RNA molecule specifies that there will be a tryptophan molecule in the corresponding location in the corresponding polypeptide. The four bases A, G, C and U can be combined as triplets in 64 different ways, but there are only 20 amino acids to be coded. Thus, most amino acids are coded for by more than one codon. For example, both U-A-U and U-A-C code for tyrosine, and there are six different codons that code for leucine. There are also three codons that do not code for any amino acid (namely, U-A-A, U-G-A, and U-A-G). Like periods at the end of a sentence, these sequences signal the end of the polypeptide chain, and they are therefore called stop codons.

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The cellular machinery involved in synthesizing proteins is quite complicated, and centers around large structures called ribosomes that bind to the messenger RNA. The ribosomes and associated molecules "read" the information in the messenger RNA molecule, literally shifting along the strand of RNA three nucleotides at a time, adding the amino acid specified by that codon to a growing polypeptide chain that is also attached to the ribosome. When a stop codon is reached, the polypeptide chain is complete and detaches from the ribosome.

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The conversion of the information from a sequence of codons in an RNA molecule into the sequence of amino acids in a newly synthesized polypeptide is called translation. A messenger RNA molecule is typically reused to make many copies of the same protein. Synthesis of a protein is usually terminated by destroying the messenger RNA. (The information for making more of that protein remains stored in DNA in the chromosomes.)

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The translation of messenger RNA begins at a specific sequence of nucleotides that bind the RNA to the ribosome and specify which is the first codon that is to be translated. Translation then proceeds by reading nucleotides, three at a time, until a stop codon is reached. If some error were to occur that shifts the frame in which the nucleotides are read by one or two nucleotides, all of the codons after this shift would be misread. For example, the sequence of codons [...C-U-C-A-G-C-G-U-U-A-C-C -A...] codes for the chain of amino acids [... leucine-serine-valine-threonine-...]. If the reading of these groups of three nucleotides is displaced by one nucleotide, such as [...C-U-C-A-G-C-G-U-U-A-C-C-A...], the resulting peptide chain would consist of [...serine-alanine-leucine-proline...]. This would be an entirely different peptide, and most probably an undesirable and useless one. Synthesis of a particular protein requires that the correct register or reading frame be maintained as the codons in the RNA are translated.

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The function of messenger RNA is to carry genetic information (transcribed from DNA) to the protein synthetic machinery of a cell where its information is translated into the amino acid sequence of a protein. However, some kinds of RNA have other roles. For example, ribosomes contain several large strands of RNA that serve a structural function (ribosomal RNA). Chromosomes contain

regions of DNA that code for the nucleotide sequences of structural RNAs and these sequences are transcribed to manufacture those RNAs. The DNA sequences coding for structural RNAs are still called genes even though the nucleotide sequence of the structural RNA is never translated into protein.

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Man, other animals, plants, protozoa, and yeast are eucaryotic (or eukaryotic) organisms: their DNA is packaged in chromosomes in a special compartment of the cell, the nucleus. Bacteria (procaryotic or prokaryotic organisms) have a different organization. Their DNA, usually a circular loop, is not contained in any specialized compartment. Despite the incredible differences between them, all organisms, whether eucaryote or procaryote, whether man or mouse or lowly bacterium, use the same molecular rules to make proteins under the control of genes. In all organisms, codons in DNA are transcribed into codons in RNA which is translated on ribosomes into polypeptides according to the same genetic code. Thus, if a gene from a man is transferred into a bacterium, the bacterium can manufacture the human protein. Since most commercially valuable proteins come from man or other eucaryotes while bacteria are essentially little biochemical factories that can be grown in huge quantities, one strategy for manufacturing a desired protein (for example, insulin) is to transfer the gene coding for the protein from the eucaryotic cell where the gene normally occurs into a bacterium.

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Bacteria containing genes from a foreign source (heterologous genes) integrated into their own genetic makeup are said to be transformed. When transformed bacteria grow and divide, the inserted heterologous genes, like all the other genes that are normally present in the bacterium (indigenous genes), are replicated and passed on to succeeding generations. One can produce large quantities of transformed bacteria that contain transplanted heterologous genes. The process of making large quantities of identical copies of a gene (or other fragment of DNA) by introducing it into procaryotic cells and then growing those cells is called cloning the gene. After growing sufficient quantities of the transformed bacteria, the biotechnologist must induce the transformed bacteria to express the cloned gene and make useful quantities of the protein. This is the purpose of the claimed invention.

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In order to make a selected protein by expressing its cloned gene in bacteria,

several technical hurdles must be overcome. First the gene coding for the specific protein must be isolated for cloning. This is a formidable task, but recombinant DNA technology has armed the genetic engineer with a variety of techniques to accomplish it.7 Next the isolated gene must be introduced into the host bacterium. This can be done by incorporating the gene into a cloning vector. A cloning vector is a piece of DNA that can be introduced into bacteria and will then replicate itself as the bacterial cells grow and divide. Bacteriophage (viruses that infect bacteria) can be used as cloning vectors, but plasmids were the type used by appellants. A plasmid is a small circular loop of DNA found in bacteria, separate from the chromosome, that replicates like a chromosome. It is like a tiny auxilliary chromosome containing only a few genes. Because of their small size, plasmids are convenient for the molecular biologist to isolate and work with. Recombinant DNA technology can be used to modify plasmids by splicing in cloned eucaryotic genes and other useful segments of DNA containing control sequences. Short pieces of DNA can even be designed to have desired nucleotide sequences, synthesized chemically, and spliced into the plasmid. One use of such chemically synthesized linkers is to insure that the inserted gene has the same reading frame as the rest of the plasmid; this is a teaching of the Bahl reference cited against appellants. A plasmid constructed by the molecular geneticist can be inserted into bacteria, where it replicates as the bacteria grow.

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Even after a cloned heterologous gene has been successfully inserted into bacteria using a plasmid as a cloning vector, and replicates as the bacteria grow, there is no guarantee that the gene will be expressed, i.e., transcribed and translated into protein. A bacterium such as E. coli (the species of bacterium used by appellants) has genes for several thousand proteins. At any given moment many of those genes are not expressed at all. The genetic engineer needs a method to "turn on" the cloned gene and force it to be expressed. This is the problem appellants worked to solve.

II. Prior art

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Appellants sought to control the expression of cloned heterologous genes inserted into bacteria. They reported the results of their early efforts in a publication, the three authors of which included two of the three coinventor-appellants (the Polisky reference8), that is undisputed prior art against them.

Their strategy was to link the foreign gene to a highly regulated indigenous gene. Turning on expression of the indigenous gene by normal control mechanisms of the host would cause expression of the linked heterologous gene.

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As a controllable indigenous gene, the researchers chose a gene in the bacterium
E. coli that makes beta-galactosidase. Beta-galactosidase is an enzyme needed to digest the sugar, lactose (milk sugar). When E. coli grows in a medium that contains no lactose, it does not make beta-galactosidase. If lactose is added to the medium, the gene coding for beta-galactosidase is expressed. The bacterial cell makes beta-galactosidase and is then able to use lactose as a food source. When lactose is no longer available, the cell again stops expressing the gene for beta galactosidase.

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The molecular mechanisms through which the presence of lactose turns on expression of the beta-galactosidase gene has been studied in detail, and is one of the best understood examples of how gene expression is regulated on the molecular level. The beta-galactosidase gene is controlled by segments of DNA adjacent to the gene. These regulatory DNA sequences (the general term used in Claim 1) include the operator and promoter sequences (specified in Claim 2).9 The researchers constructed a plasmid containing the beta-galactosidase gene with its operator and promoter. This gene (with its regulatory sequences) was removed from the chromosome of E. coli where it is normally found and was transplanted to a plasmid that could be conveniently manipulated.

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Restriction endonucleases are useful tools in genetic engineering. These enzymes cut strands of DNA, but only at places where a specific sequence of nucleotides is present. For example, one restriction endonuclease, called EcoRI, cuts DNA only at sites where the nucleotide sequence is [...-G-A-A-T-T-C-...]. With restriction enzymes the genetic engineer can cut a strand of DNA at very specific sites into just a few pieces. With the help of "repair" enzymes, other pieces of DNA can be spliced onto the cut ends. The investigators found that the plasmid which they had constructed contained only two sequences that were cut by EcoRI. They were able to eliminate one of these sites that was unwanted. They were then left with a plasmid containing the beta-

galactosidase gene with its regulatory sequences, and a single EcoRI site that was within the beta-galactosidase gene and close to its stop codon. They named this plasmid that they had constructed pBGP120.

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The next step was to cut the plasmid open at its EcoRI site and insert a heterologous gene from another organism. The particular heterologous gene they chose to splice in was a segment of DNA from a frog that coded for ribosomal RNA. The frog gene was chosen as a test gene for reasons of convenience and availability. The new plasmid created by inserting the frog gene was similar to pBGP120, but its beta-galactosidase gene was incomplete. Some codons including the stop codon were missing from its end, which instead continued on with the sequence of the frog ribosomal RNA gene. The investigators named this new plasmid pBGP123. They inserted this plasmid back into E. coli and grew sufficient quantities for study. They then fed the E. coli with lactose. As they had intended, the lactose turned on transcription of the beta-galactosidase gene in the plasmid. RNA polymerase moved along the plasmid producing a strange new kind of RNA: Each long strand of RNA first contained codons for the messenger RNA for beta-galactosidase and then continued without interruption with the codons for the frog ribosomal RNA. Thus, there was readthrough transcription in which the RNA polymerase first transcribed the indigenous (beta-galactosidase) gene and then "read through," i.e., continued into and through the adjacent heterologous (frog ribosomal RNA) gene. Although the RNA produced was a hybrid, it nevertheless contained a nucleotide sequence dictated by DNA from a frog. The researchers had achieved the first controlled transcription of an animal gene inside a bacterium.

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The researchers had used a gene coding for a ribosomal RNA as their heterologous test gene. Ribosomal RNA is not normally translated into protein. Nevertheless, they were obviously interested in using their approach to make heterologous proteins in bacteria. They therefore examined the beta-galactosidase made by their transformed bacteria. Patrick O'Farrell, who was not a coauthor of the Polisky paper but was to become a coinventor in the patent application, joined as a collaborator. They found that beta-galactosidase from the transformed bacteria had a higher molecular weight than was normal. They concluded that the bacteria must have used their strange new hybrid RNA like any other messenger RNA and translated it into protein. When the

machinery of protein synthesis reached the premature end of the sequence coding for beta-galactosidase it continued right on, three nucleotides at a time, adding whatever amino acid was coded for by those nucleotides, until a triplet was reached with the sequence of a stop codon. The resulting polypeptide chains had more amino acids than normal beta-galactosidase, and thus a higher molecular weight. The researchers published their preliminary results in the Polisky article. They wrote:

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[I]f the normal translational stop signals for [beta]-galactosidase are missing in pBGP120, in-phase translational readthrough into adjacent inserted sequences might occur, resulting in a significant increase in the size of the [beta]-galactosidase polypeptide subunit. In fact, we have recently observed that induced cultures of pBGP123 contain elevated levels of [beta]-galactosidase of higher subunit molecular weight than wild-type enzyme (P. O'Farrell, unpublished experiments). We believe this increase results from translation of Xenopus [frog] RNA sequences covalently linked to [messenger] RNA for [beta]-galactosidase, resulting in a fused polypeptide.

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Polisky at 3904.

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Since ribosomal RNA is never translated in normal cells, the polypeptide chain produced by translating that chain was not a naturally occuring, identified protein. The authors of the Polisky paper explicitly pointed out that if one were to insert a heterologous gene coding for a protein into their plasmid, it should produce a "fused protein" consisting of a polypeptide made of beta-galactosidase plus the protein coded for by the inserted gene, joined by a peptide bond into a single continuous polypeptide chain:

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It would be interesting to examine the expression of a normally translated eukaryotic sequence in pBGP120. If an inserted sequence contains a ribosome binding site that can be utilized in bacteria, production of high levels of a readthrough transcript might allow for extensive translation of a functional eukaryotic polypeptide. In the absence of an independent ribosome binding site, the eukaryotic sequence would be translated to yield a peptide covalently linked to [beta]-galactosidase. The extent of readthrough translation under lac control will depend on the number of translatable codons between the EcoRI

site and the first in-phase nonsense [i.e., stop] codon in the inserted sequence.

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Id.

III. The Claimed Invention

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Referring back to Claims 1 through 3, it can be seen that virtually everything in the claims was present in the prior art Polisky article. The main difference is that in Polisky the heterologous gene was a gene for ribosomal RNA while the claimed invention substitutes a gene coding for a predetermined protein. Ribosomal RNA gene is not normally translated into protein, so expression of the heterolgous gene was studied mainly in terms of transcription into RNA. Nevertheless, Polisky mentioned preliminary evidence that the transcript of the ribosomal RNA gene was translated into protein. Polisky further predicted that if a gene that codes for a protein were to be substituted for the ribosomal RNA gene, "a readthrough transcript might allow for extensive translation of a functional eukaryotic polypeptide." Thus, the prior art explicitly suggested the substitution that is the difference between the claimed invention and the prior art, and presented preliminary evidence suggesting that the method could be used to make proteins.

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Appellants reduced their invention to practice some time in 1976 and reported their results in a paper that was published in 1978.10 During 1977 they communicated their results to another group of researchers who used the readthrough translation approach to achieve the first synthesis of a human protein in bacteria.11 Appellants filed an application to patent their invention on August 9, 1978, of which the application on appeal is a division.

IV. The Obviousness Rejection

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The application was rejected under 35 U.S.C. Sec. 103. The position of the examiner and the Board is, simply, that so much of the appellant's method was revealed in the Polisky reference that making a protein by substituting its gene for the ribosomal RNA gene in Polisky (as suggested by Polisky) would have been obvious to one of ordinary skill in the art at the time that the invention was made.

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The claims specify that the heterologous gene should be inserted into the plasmid in the same orientation and with the same reading frame as the preceding portion of the indigenous gene. In view of this limitation, the Sec. 103 rejection was based either on Polisky alone (supplemented by the fact that the importance of orientation and reading frame was well known in the prior art) or in combination with the Bahl reference which describes a general method for inserting a piece of chemically synthesized DNA into a plasmid. Bahl teaches that this technique could be used to shift the sequence of DNA inserted into a plasmid into the proper reading frame.

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Appellants argue that at the time the Polisky article was published, there was significant unpredictability in the field of molecular biology so that the Polisky article would not have rendered the claimed method obvious to one of ordinary skill in the art. Even though there was speculation in the article that genes coding for proteins could be substituted for the ribosomal RNA gene and would be expressed as readthrough translation into the protein, this had never been done. Appellants say that it was not yet certain whether a heterologous protein could actually be produced in bacteria, and if it could, whether additional mechanisms or methods would be required. They contend that without such certainty the predictions in the Polisky paper, which hindsight now shows to have been correct, were merely invitations to those skilled in the art to try to make the claimed invention. They argue that the rejection amounts to the application of a standard of "obvious to try" to the field of molecular biology, a standard which this court and its predecessors have repeatedly rejected as improper grounds for a Sec. 103 rejection. E.g., In re Fine, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1599 (Fed.Cir.1988); In re Geiger, 815 F.2d 686, 688, 2 USPQ2d 1276, 1278 (Fed.Cir.1987); In re Merck & Co., Inc., 800 F.2d 1091, 1097, 231 USPQ 375, 379 (Fed.Cir.1986); In re Antonie, 559 F.2d 618, 620, 195 USPQ 6, 8 (CCPA 1977).

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Obviousness under Sec. 103 is a question of law. Panduit Corp. v. Dennison Mfg. Co., 810 F.2d 1561, 1568, 1 USPQ2d 1593, 1597 (Fed.Cir.), cert. denied, --- U.S. ----, 107 S.Ct. 2187, 95 L.Ed.2d 843 (1987). An analysis of obviousness must be based on several factual inquiries: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3)

the level of ordinary skill in the art at the time the invention was made; and (4) objective evidence of nonobviousness, if any. Graham v. John Deere Co., 383 U.S. 1, 17-18, 86 S.Ct. 684, 693-94, 15 L.Ed.2d 545, 556-57, 148 USPQ 459, 467 (1966). See, e.g., Custom Accessories, Inc. v. Jeffrey-Allan Indus., 807 F.2d 955, 958, 1 USPQ2d 1196, 1197 (Fed.Cir.1986). The scope and content of the prior art and the differences between the prior art and the claimed invention have been examined in sections II and III, supra. Appellants say that in 1976 those of ordinary skill in the arts of molecular biology and recombinant DNA technology were research scientists who had "extraordinary skill in relevant arts" and "were among the brightest biologists in the world." Objective evidence of nonobviousness was not argued.

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With the statutory factors as expounded by Graham in mind and considering all of the evidence, this court must determine the correctness of the board's legal determination that the claimed invention as a whole would have been obvious to a person having ordinary skill in the art at the time the invention was made. We agree with the board that appellants' claimed invention would have been obvious in light of the Polisky reference alone or in combination with Bahl within the meaning of Sec. 103. Polisky contained detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting that it would be successful.

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Appellants argue that after the publication of Polisky, successful synthesis of protein was still uncertain. They belittle the predictive value of the observation that expression of the transcribed RNA in Polisky produced beta-galactosidase with a greater than normal molecular weight, arguing that since ribosomal RNA is not normally translated, the polypeptide chains that were added to the end of the beta-galactosidase were "junk" or "nonsense" proteins. This characterization ignores the clear implications of the reported observations. The Polisky study directly proved that a readthrough transcript messenger RNA had been produced. The preliminary observation showed that this messenger RNA was read and used for successful translation. It was well known in the art that ribosomal RNA was made of the same nucleotides as messenger RNA, that any sequence of nucleotides could be read in groups of three as codons, and that reading these codons should specify a polypeptide chain that would elongate until a stop codon was encountered. The preliminary

observations thus showed that codons beyond the end of the beta-galactosidase gene were being translated into peptide chains. This would reasonably suggest to one skilled in the art that if the codons inserted beyond the end of the beta-galactosidase gene coded for a "predetermined protein," that protein would be produced. In other words, it would have been obvious and reasonable to conclude from the observation reported in Polisky that since nonsense RNA produced nonsense polypeptides, if meaningful RNA was inserted instead of ribosomal RNA, useful protein would be the result. The relative shortness of the added chains is also not a source of uncertainty, since one skilled in the art would have known that a random sequence of nucleotides would produce a stop codon before the chain got too long.12

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Appellants complain that since predetermined proteins had not yet been produced in transformed bacteria, there was uncertainty as to whether this could be done, and that the rejection is thus founded on an impermissible "obvious to try" standard. It is true that this court and its predecessors have repeatedly emphasized that "obvious to try" is not the standard under Sec. 103. However, the meaning of this maxim is sometimes lost. Any invention that would in fact have been obvious under Sec. 103 would also have been, in a sense, obvious to try. The question is: when is an invention that was obvious to try nevertheless nonobvious?

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The admonition that "obvious to try" is not the standard under Sec. 103 has been directed mainly at two kinds of error. In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. E.g., In re Geiger, 815 F.2d at 688, 2 USPQ2d at 1278; Novo Industri A/S v. Travenol Laboratories, Inc., 677 F.2d 1202, 1208, 215 USPQ 412, 417 (7th Cir.1982); In re Yates, 663 F.2d 1054, 1057, 211 USPQ 1149, 1151 (CCPA 1981); In re Antonie, 559 F.2d at 621, 195 USPQ at 8-9. In others, what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1532 (Fed.Cir.1988); Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367,

1380, 231 USPQ 81, 90-91 (Fed.Cir.1986), cert. denied, --- U.S. ----, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987); In re Tomlinson, 363 F.2d 928, 931, 150 USPQ 623, 626 (CCPA 1966). Neither of these situations applies here.

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Obviousness does not require absolute predictability of success. Indeed, for many inventions that seem quite obvious, there is no absolute predictability of success until the invention is reduced to practice. There is always at least a possibility of unexpected results, that would then provide an objective basis for showing that the invention, although apparently obvious, was in law nonobvious. In re Merck & Co., 800 F.2d at 1098, 231 USPQ at 380; Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co., 730 F.2d 1452, 1461, 221 USPQ 481, 488 (Fed.Cir.1984); In re Papesch, 315 F.2d 381, 386-87, 137 USPQ 43, 47-48 (CCPA 1963). For obviousness under Sec. 103, all that is required is a reasonable expectation of success. In re Longi, 759 F.2d 887, 897, 225 USPQ 645, 651-52 (Fed.Cir.1985); In re Clinton, 527 F.2d 1226, 1228, 188 USPQ 365, 367 (CCPA 1976). The information in the Polisky reference, when combined with the Bahl reference provided such a reasonable expectation of success.

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Appellants published their pioneering studies of the expression of frog ribosomal RNA genes in bacteria more than a year before they applied for a patent. After providing virtually all of their method to the public without applying for a patent within a year, they foreclosed themselves from obtaining a patent on a method that would have been obvious from their publication to those of ordinary skill in the art, with or without the disclosures of other prior art. The decision of the board is

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AFFIRMED.

1 Basic background information about molecular biology and genetic engineering, can be found in Alberts, Bray, Lewis, Raff, Roberts & Watson, The Molecular Biology of the Cell, 1-253, 385-481 (1983) [hereinafter The Cell]; Watson, Hopkins, Roberts, Steitz & Weiner, The Molecular Biology of the Gene, Vol. 1 (4th ed., 1987) 3-502 [hereinafter The Gene]. These standard textbooks were used to supplement the information in the glossary supplied by appellants. The description here is necessarily simplified and omits important facts and concepts that are not necessary for the analysis of this case

2 There are twenty amino acids: alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, aspartic acid, glutamic acid, lysine, arginine, and histidine

3 Proteins are often loosely called peptides, but technically proteins are only the larger peptides with chains of at least 50 amino acids, and more typically hundreds of amino acids. Some proteins consist of several polypeptide chains bound together covalently or noncovalently. The term "peptide" is broader than "protein" and also includes small chains of amino acids linked by peptide bonds, some as small as two amino acids. Certain small peptides have commercial or medical significance

4 Polypeptide chains fold up into complex 3-dimensional shapes. It is the shape that actually determines many chemical properties of the protein. However, the configuration of a protein molecule is determined by its amino acid sequence. The Cell at 111-12; The Gene at 50-54

5 The sugar in DNA is deoxyribose, while the sugar in RNA, infra, is ribose. The sugar and phosphate groups are linked covalently to those of adjacent nucleotides to form the backbone of the long unbranched DNA molecule. The bases project from the chain, and serve as the "alphabet" of the genetic code

DNA molecules actually consist of two chains tightly entwined as a double helix. The chains are not identical but instead are complementary: each A on one chain is paired with a T on the other chain, and each C has a corresponding G. The chains are held together by noncovalent bonds between these complementary bases. This double helical structure plays an essential role in the replication of DNA and the transmission of genetic information. See generally The Cell at 98-106; The Gene at 65-79. However, the information of only one strand is used for directing protein synthesis, and it is not necessary to discuss the implication of the double-stranded structure of DNA here. RNA molecules, infra, are single stranded.

6 Chromosomes also contain regions of DNA that are not part of genes, i.e., do not code for the sequence of amino acids in proteins. These include sections of DNA adjacent to genes that are involved in the control of transcription, infra, and regions of unknown function

7

See The Cell at 185-194; The Gene at 208-10

8 Polisky, Bishop & Gelfand, A plasmid cloning vehicle allowing regulated

expression of eukaryotic DNA in bacteria, 73 Proc.Nat'l Acad.Sci. USA 3900 (1976)

9 The promoter is a sequence of nucleotides where the enzyme that synthesizes RNA, RNA polymerase, attaches to the DNA to start the transcription of the beta-galactosidase gene. The operator is an overlapping DNA sequence that binds a small protein present in the cell, the lactose repressor protein. The lactose repressor protein binds to the operator and physically blocks the RNA polymerase from properly attaching to the promoter so that transcription cannot proceed. Lactose molecules interact with the lactose repressor protein and cause it to change its shape; after this change in shape it moves out of the way and no longer prevents the RNA polymerase from binding to the promoter. Messenger RNA coding for beta-galactosidase can then be transcribed. See generally The Cell at 438-39; The Gene at 474-80

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> O'Farrell, Polisky & Gelfand, Regulated expression by readthrough translation from a plasmid-encoded beta-galactosidase, 134 J. Bacteriol. 645 (1978). The heterologous genes expressed in these studies were not predetermined, but were instead unidentified genes of unknown origin. The authors speculated that they were probably genes from E. coli that were contaminants in the source of beta-galactosidase genes. Id. at 648

11 Itakura, Hirose, Crea, Riggs, Heynecker, Bolivar & Boyer, Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin, 198 Science 1056 (1977). A pioneering accomplishment of the Itakura group is that the gene was not from a human source, but instead was entirely synthesized in the laboratory using chemical methods. It is not clear whether the appellants communicated only the results reported in the Polisky publication or whether they communicated the complete claimed invention

12 The patent application indicates that chains as long as 60 amino acids were added, which is hardly a trivial length of polypeptide