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Paper No. 28

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today
(1) was not written for publication in a law journal and
(2) is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte REGINA E. BRIGELIUS-FLOHE, LEOPOLD FLOHE,
WOLFGANG HILLEN, GERD J. STEFFENS,
WOLFGANG STRASSBURGER and MARTIN R.F. WILHELM

MAILED

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Appeal No. 95-0475
Application 07/551,907¹

PAT & TM OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

HEARD May 7, 1996

Before SCHAFER, Vice Chief Administrative Patent Judge, WINTERS
and GRON, Administrative Patent Judges.

GRON, Administrative Patent Judge.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 of an examiner's
final rejections of Claims 1 to 3, 6, 8 to 10, 14 to 19, and 21
to 27, all claims pending in the application.

¹ Application for patent filed July 12, 1990

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1. Introduction

Claims 1, 3, 9, 15, 21, 22, 26 and 27 stand rejected under 35 U.S.C. § 103 as unpatentable over the combined teachings of Hibino² and Ernst³. All pending claims stand variously rejected under 35 U.S.C. § 103 over the combined teachings of Hibino, Ernst, and one or more of Stormo⁴, Belagaje⁵, Schollmeier⁶, Sato⁷, Holmes⁸, Brosius⁹, Brosius II¹⁰, Finnegan¹¹,

² Hibino, Y., et al., "Enhanced Expression of Human Pro-urokinase cDNA in Escherichia coli," Agric. Biol. Chem., Vol. 52, No. 2, pages 329 to 336 (May 3, 1988)

³ Ernst, J. F., "Codon Usage and Gene Expression," Trends in Biotechnology (TIBTECH), Vol. 6, pages 196 to 199 (1988)

⁴ Stormo, et al., Maximizing Gene Expression, Reznikoff, W., et al., eds., Butterworth, Stoneham, Mass., pages 203, 204 and 211 (1988)

⁵ Belagaje, R., et al., U.S. Patent 4,710,464, patented December 1, 1987

⁶ Schollmeier, K., et al., "A Bidirectionally Active Signal for Transcription Is Located Between tetA and orfL on Transposon Tn10," Nucleic Acids Res., Vol. 13, No. 12, pages 4227-4237 (1985)

⁷ Sato, T., et al., "New Approaches for the High-Level Expression of Human Interleukin-2 cDNA in Escherichia coli," J. Biochem., Vol. 101, pages 525-534 (1987)

⁸ Holmes, W. E., et al., "Cloning and Expression of the Gene for Pro-urokinase in Escherichia coli," Bio/Technology, Vol. 3, pages 923-929 (October 1985). This citation differs from the citation in the Examiner's Answer. Nevertheless, we believe this is the reference upon which the examiner relies. It is the only Holmes article in the application. The confusion appears to have originated when appellants entered the identical citation "Vol. 138, No. 3, pages 705-714 (June 1979)" improperly for the Holmes et al. article of the above title and then again properly for Jorgensen et al., "Organization of Structural and Regulatory (continued...)

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Winnacker¹², Vieira¹³ and Shepard¹⁴. Claims 1 to 3, 6, 9, 10, 15, 21, 22, 26, and 27 also stand rejected under 35 U.S.C. § 112, first paragraph, as based on a nonenabling disclosure.

All the examiner's rejections under 35 U.S.C. § 103 fall because the examiner has not established that the subject matter of Claims 1 and 3 would have been prima facie obvious to a person having ordinary skill in the art at the time the invention was made in view of the combined teachings of Hibino and Ernst. All the examiner's rejections under 35 U.S.C. § 112, first paragraph, fall because the examiner has not established that persons

^{*}(...continued)
Genes," Journal of Bacteriology, in Form PTO 1449 filed September 5, 1990 (Paper No. 3).

⁹ Brosius, J., "Toxicity of an Overproduced Foreign Gene Product in Escherichia coli and Its Use in Plasmid Vectors for the Selection of Transcription Terminators," Gene, Vol. 27, pages 161-172 (1984)

¹⁰ Brosius, J., et al., "Regulation of Ribosomal RNA Promoters with a Synthetic lac Operator," Proc. Natl. Acad. Sci. USA, Vol. 81, pages 6929-6933 (November 1984)

¹¹ Finnegan, J., et al., "Plasmid ColE1 Conjugal Mobility: The Nature of hom, a Region Required in cis for Transfer," Mol. Gen. Genet., Vol. 185, pages 344-351 (1982)

¹² Winnacker, E.-L., Gene und Klone, VCH Verlagsgesellschaft, Weinheim, page 298 (1985)

¹³ Vieira, J., et al., "The pUC Plasmids, an M13mp7-derived System for Insertion Mutagenesis and Sequencing with Synthetic Universal Primers," Gene, Vol. 19, pages 259-268 (1982)

¹⁴ Shepard, H. M., et al., "Increased Synthesis in E. coli of Fibroblast and Leukocyte Interferons Through Alterations in Ribosome Binding Sites," DNA, Vol. 1, No. 2, pages 125-131 (1982)

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skilled in the art would not have been enabled by appellants' specification to make and use the operons of Claim 1 and the plasmids of Claim 3 without undue experimentation. Claims 1, 3 and 23 read (emphasis added):

1. An operon for use in the manufacture of the human single chain urinary plasminogen activator (recombinant scu-PA) in a strain of Escherichia coli, comprising in 5' to 3' order the following operatively linked elements:

a regulatable promotor selected from the group consisting of a Trp promotor and a Tac promotor;

a Shine-Dalgarno sequence effective as a ribosomal binding site,

a translational start codon,

a structural gene for single chain urinary plasminogen activator having the nucleotide sequence of Figure 15 and downstream of said structural gene

at least one transcription terminator,

wherein said Shine-Dalgarno sequence is separated from said start codon by from 6 to 12 nucleotides, and wherein further, said operon effects the synthesis in inclusion bodies of an inactive form of said single chain urinary plasminogen activator in a strain of Escherichia coli with an expression rate of from about 10 to about 25 percent by weight of the total protein produced.

3. A plasmid comprising the operon of claim 1, wherein said plasmid is suitable for expression of said operon in a strain of Escherichia coli.

23. A process for producing a plasmid according to claim 3 comprising the steps of:

(i) removing the nic/bom-region and mutationally inactivating the tetracycline resistance gene of pBr 322;

(ii) inserting a multi-cloning site having the nucleotide sequence of Figure 2 between the pBR 322 restriction sites Eco RI and Hind III; and

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(iii) inserting within said multi-cloning site a transcription terminator, a gene encoding scu-PA, and a synthetic Trp-promotor such that said gene is operatively linked to said promotor and to said terminator.

2. The invention

The underlined portions of Claims 1, 3, and 23 reproduced in the Introduction form the basis for our holding that all claims on appeal are directed exclusively to those operons (Claim 1 and dependent Claims 2, 6, 8 to 10, 21 and 22), plasmids (Claim 3 and dependent Claims 14 to 19), transformed hosts (Claim 26), and methods of making and using plasmids (Claims 23 and 27 and dependent Claims 24 and 25) which effect the synthesis of an inactive form of scu-PA in E. coli "with an expression rate of from about 10 to about 25 percent by weight of the total protein produced" (Claim 1).

3. The issues

Appellants do not acknowledge the examiner's argument (Examiner's Answer (Ans), pages 5 to 6, bridging ¶) that transformation of E. coli by operons comprising (1) a regulatable promotor selected from the group consisting of a Trp promotor and a Tac promotor, (2) a Shine-Dalgarno sequence effective as a ribosomal binding site, (3) a translational start codon, (4) a structural gene for single stranded inactive protein, and (5) at least one transcription terminator downstream of said structural gene, operatively linked in 5' to 3' order, to optimize or maximize production of any of a wide variety of

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single stranded inactive proteins, would have been obvious to persons having ordinary skill in the art in view of the prior art of record. However, appellants also do not deny that the cited prior art teachings, e.g., Hibino, Holmes, and Ernst, reasonably would have taught persons having ordinary skill in the art how and why to transform E. coli to produce proteins such as scu-PA at least at marginally acceptable expression rates with operons comprising (1) a known regulatable promotor of which the Trp and Tac promoters are two, (2) an art-recognized Shine-Dalgarno sequence, (3) an ATG start codon, (4) a native structural gene encoding production of a single stranded inactive protein such as scu-PA or one modified for E. coli codon expression bias, and (5) at least one known transcription terminator downstream of said structural gene, operatively linked in 5' to 3' order. Rather, appellants emphasize (Brief, pages 2 and 10; Reply Brief, page 6) that the inventions they claim are patentably distinct from those operons, plasmids, transformed hosts, and transformation processes in the public domain because the particular combination of elements which form the operons they claim unexpectedly effect synthesis of an inactive form of scu-PA in E. coli "with an expression rate of from about 10 to about 25 percent by weight of the total protein produced." At the same time, appellants urge that persons skilled in the art would have been enabled by their specification to transform E. coli to produce scu-PA at the expression rate

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they claim without undue experimentation. See appellants' specification at page 17, lines 20 to 24, page 21, lines 1 to 10, and pages 29 to 30, the bridging paragraph; Brief at pages 2, 4 to 6, and 13 to 14; and Reply Brief at pages 8 to 9. In this case, we need not determine whether transformation of E. coli by operons to optimize production of scu-PA at maximum expression rates would have been obvious to persons having ordinary skill in the art. Appellants claim operons, plasmids, transformed hosts, and methods of making and using plasmids which effect the synthesis of an inactive form of scu-PA in E. coli "with an expression rate of from about 10 to about 25 percent by weight of the total protein produced" (Claim 1). Therefore, the issues to be resolved in this case are (1) whether the combined prior art teachings would have led persons having ordinary skill in the art to reasonably expect to transform E. coli to produce scu-PA at an expression rate of from about 10 to about 25 percent by weight of the total protein produced using "well-defined" operons modified for E. coli expression bias, and (2) whether appellants' specification satisfies the enablement requirement of 35 U.S.C. § 112, first paragraph, for the full scope of the claims.

4. Discussion

A. Rejections under 35 U.S.C. § 103

The examiner does not argue that expression of scu-PA in E. coli at the rates from about 10 to about 25 percent by weight of the total protein produced which appellants achieved

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reasonably would have been expected using transformation plasmids comprising the operons claimed. Based on the collective prior art teachings, we find that persons skilled in the art reasonably would not have predicted that E. coli could be transformed to synthesize human scu-PA at expression rates as high as 10 percent by weight of the total protein produced simply by replacing the codons in known expression elements of the operons of expression plasmids in conformance with recognized E. coli codon expression bias.

Hibino "tried to produce a large quantity of pro-UK⁽¹⁵⁾ in Escherichia coli" (Hibino, page 329, column 1). Hibino formed E. coli expression plasmids pMUT4L (modified N-terminal sequence) and pMUT7L (native N-terminal sequence) which carry structural cDNA sequences which encode human scu-PA by operatively linking each structural sequence to a lac promoter and translational signal including three "well-defined" features, i.e., "(i) ATG, (ii) the distance between Shine-Dalgarno (SD) sequence and ATG, and (iii) the complementarity to the 3' end of 16S rRNA" (Hibino, page 329, columns 1 and 2; page 330, column 2; page 331, columns 1 and 2, bridging ¶; page 332, column 2). Hibino's cDNA sequences differed from those previously reported by use of the CTC codon for CTA encoding Leu and the AAG codon for AAA encoding Lys (Hibino, page 331, column 1). The native N-terminal sequence

¹⁵ Pro-UK is scu-PA (Specification, page 2, lines 6 to 8).

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had the codons AAT for Asn, GAA for Glu, CAT for His, CAA for Gln, and CCA for Pro. The modified N-terminal sequence had the codons AAC for Asn, GAG for Glu, CTC for His, CAC for Gln, and CCG for Pro.¹⁶ Hibino suspected that the N-terminal mutation "possibly diminishes the hairpin structure, which might render the SD domain accessible to ribosomes" (Hibino, page 334, column 1). Hibino measured the percentage of insoluble E. coli protein produced by the recombinant pro-UK gene "by separating the proteins by SDS-Page, staining them with Coomassie Brilliant Blue and scanning the stained gel with a Helena Lab. Quick Scan II" (Hibino, pages 331 to 332, bridging sentence). Hibino found (Hibino, page 332):

This method showed that the pro-UK protein accounts for 15% of insoluble E. coli protein (2% of the total bacterial protein) in the case of [E. coli strain] JM103 containing pMUT4L [(modified)], but to a lesser extent in the case of JM103 containing pMUT7L [(native)]. These results agree with the previous expression data.

Hibino analyzed the results and suggested possibilities for further study (Hibino, page 334, column 1, to page 335, column 2 (including Table I); citations omitted):

The mutation increased the amount of bacterial pro-UK expressed in E. coli by 3,4-fold compared with that of the native type. . . . The expression level, however, was not

¹⁶ We note that certain codons in the N-terminal sequences selected for use in Hibino's modified plasmid correspond to codons for which Ernst suggests E. coli shows an expression bias while others do not. Similarly, certain codons in the N-terminal sequences of Hibino's native plasmid correspond to codons for which Ernst suggests E. coli shows an expression bias while others do not. See Ernst, page 197, Table 1.

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increased dramatically by introducing the mutation described by Hall et al. This might indicate that other factors still influence the expression level, such as the sequence upstream from the SD-spacing-ATG signal [(sic, signal)] or the nucleotide composition of the sequence between SD and the initial codon.

We find that while Hibino suggests various experimental paths that persons skilled in the art might take to try to improve the expression level of DNA encoding human scu-PA in E. coli, the guidance he provides would have been inadequate to bridge the gap between "obvious-to-try" and obviousness within the meaning of 35 U.S.C. § 103. See In re Eli Lilly & Co., 902 F.2d 943, 945, 14 USPQ2d 1741, 1743 (Fed. Cir. 1990):

An "obvious-to-try" situation exists when a general disclosure may pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claimed result would be obtained if certain directions were pursued.

We agree with the examiner that Ernst suggests many possibilities for improving the expression levels of Hibino's plasmids in E. coli. Ernst tabulates the relative bias E. coli shows for various codons in gene expression (Ernst, page 197, Table 1). Ernst reports that some prior art attempts to predict the level of expression of foreign genes in E. coli on the basis of this codon bias have succeeded while others have failed, both to varying degrees. For example, Ernst indicates that the effect of codon bias is controversial. It varies with the protein the gene being expressed encodes, and the expression rate is limited

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by the stage of gene expression least subject to the codon bias. In short, the effect of codon bias is not understood and left unexplained. For example, Ernst states:

Experiments testing the effects of codon usage on translation elongation have led to controversial results. Data are often difficult to interpret since, along with codon usage, other parameters (including the structure and rates of synthesis and degradation of mRNA) are changed simultaneously. . . . [M]any translational pauses were found to be unrelated to codon usage. . . . Petersen . . . found a less than two fold difference of translation rates between a chromosomal gene with high codon usage and a plasmid-encoded gene with low bias. Hoekema . . . substituted rare codons for up to 39% of the optimal codons in the highly expressed yeast PYK1 gene and observed that steady-state protein levels were reduced tenfold, but steady-state mRNA levels were reduced threefold. Thus, there was only a threefold decrease of translation, and even this may be an overestimation . . . [(Ernst, page 197, columns 2 and 3, bridging ¶; citations omitted);]

No clear causal relationship between codon usage and gene expression was found for expression of heterologous genes: several heterologous genes with low codon bias have been expressed to very high levels in E. coli and yeast. . . . Similarly, a comparison of differently biased versions of another heterologous gene (somatomedin C) revealed that, although alterations in codon usage can affect gene expression, there is no causal relationship between 'optimal' codon usage and gene expression. Conversely, optimization of codon usage improves expression of interferon- γ in yeast [(Ernst, page 197, column 3, first full ¶; citations omitted);]

. . . .

Nevertheless, the lack of a correlation between codon usage and gene expression in some experiments suggests that, in addition to tRNA selection, the structure of the mRNA influences translation. . . . Bains observed a correlation between gene length and conservation of specific codons in gene families, a phenomenon that cannot be explained by tRNA selection. . . . Although Robinson . . . showed that

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replacing rare codons with more common ones could improve expression at high expression levels, this could not be verified in other studies. More complex explanations must be sought [(Ernst, page 198, column 1, second ¶; citations omitted; emphasis added)].

Ernst ultimately concludes that (Ernst, page 199, column 1, first full ¶; emphasis added):

(1) codon usage and codon context can significantly influence gene expression . . . ;

(2) the effects of changes in the average codon usage of a coding region on gene expression can be small, or even absent; and

(3) the magnitude of the codon-usage effect is not yet predictable.

The issue before us is not merely whether the combined teachings of Hibino and Ernst would have led persons having ordinary skill in the art to make and use appellants' plasmids to produce or even to optimize production of scu-PA in E. coli with reasonable expectation of success. The issue is whether the combined teachings of Hibino and Ernst would have led persons having ordinary skill in the art to make and use appellants' plasmids with reasonable expectation of effecting the synthesis of an inactive form of scu-PA in E. coli "with an expression rate of from about 10 to about 25 percent by weight of the total protein produced" (Claim 3). We must consider whether the evidence as a whole supports the examiner's conclusion that "the claimed invention" would have been obvious. In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1532 (Fed. Cir. 1988).

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The prior art the examiner cited in this case does not support the examiner's conclusion that "the claimed invention" would have been obvious under 35 U.S.C. § 103. Rather, the combined references merely suggest that persons skilled in the art follow a general experimental approach in their attempts to improve heterologous gene expression in E. coli. The strategy may work. It may not. Ernst does not teach or even suggest that high expression levels will be achieved if his conceptual steps are followed (Ernst, page 198, columns 1 to 2). Certainly, the combined prior art teachings reasonably would have suggested that to improve the expression rate of any foreign structural gene in E. coli, Ernst's suggestions with regard to codon bias would be "obvious-to-try." In re O'Farrell, 853 F.2d 894, 904, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988).

We are well aware that the codon preferences which appellants list on page 11 of the specification and apparently followed in constructing their claimed operons are identical to those codon preferences indicated in Ernst's Table 1 (Ernst, page 197). However, even if we were to presume that the combined teachings of Hibino and Ernst would have led persons having ordinary skill in the art reasonably to expect to improve the expression levels for scu-PA in E. coli, the same artisan reasonably would not have predicted expression rates as high as 10 percent by weight of the total protein produced by E. coli using operons encompassed by appellants' Claim 1. Certainly, it

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would have been unreasonable for the artisan to expect or predict that an improved expression rate of about 10 to 25 percent could be realized using what otherwise would have appeared to be a "well-defined" technique. See appellants' Figures 10 and 12.

The examiner acknowledges that appellants' invention would have been obvious "barring unexpected results" (Ans12, lines 10 to 11). We hold that appellants' claims, because they are directed to operons, plasmids, and hosts transformed therewith and methods of making and using operons and plasmids which effect expression levels far in excess of those which reasonably would have been expected by persons having ordinary skill in the art, would not have been obvious over the combined prior art teachings the examiner cites. Therefore, all the examiner's rejections of Claims 1 to 3, 6, 8 to 10, 14 to 19, and 21 to 27 under 35 U.S.C. § 103 are reversed.

B. Rejections under 35 U.S.C. § 112, first paragraph

The examiner suggests that if persons having ordinary skill in the art would not have expected the results appellants exemplify, then the same examples would not have enabled persons skilled in the art to make and use the full scope of the subject matter claimed without undue experimentation as 35 U.S.C. § 112, first paragraph, requires. In re Vaeck, 947 F.2d 488, 496, 20 USPQ2d 1438, 1445 (Fed. Cir. 1991). Therefore, the examiner held that appellants have not satisfied the enablement requirements of 35 U.S.C. § 112, first paragraph.

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Even though appellants appear to be first to show that high expression levels are a real possibility and first to explain how to attain high expression levels, the examiner denies patentability because appellants (1) have not explained why they achieved their unexpected results, and (2) have not shown that their examples would have led persons skilled in the art reasonably to expect the same high expression levels for the full scope of operons, plasmids, and transformed hosts claimed. We reverse.

First, the examiner concedes that "[t]he reference teachings supply all the art needed to make any possible modifications needed concerning expression of the structural gene in the instant invention" (Ans12). Second, "[t]hat some experimentation may be required is not fatal; the issue is whether the amount of experimentation required is undue." In re Vaeck, 947 F.2d at 495, 20 USPQ2d at 1444. Third, appellants should not be required to test every combination encompassed by the claims. This would tend to discourage inventors from filing patent applications in an unpredictable art. In re Angstadt, 537 F.2d 498, 504, 190 USPQ 214, 218 (CCPA 1976). Fourth, the examiner does not deny that the specification would have been sufficient to enable persons having ordinary skill in the art to determine without undue experimentation whether or not an unexemplified plasmid falls within the scope of appellants' claims. Methods for determining whether E. coli has been

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transformed to effect the synthesis in inclusion bodies of an inactive form of scu-PA "with an expression rate of from about 10 to about 25 percent by weight of the total protein produced" (Claim 1) are well-known in the art (Specification, pages 3 to 4). See Hibino, pages 331 to 332, bridging ¶. Specifications need not and preferably do not disclose what is well-known in the art. In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir.), cert. denied, 495 U.S. 932 (1991). Fifth, to satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph, it is not necessary for an inventor to explain, or even know, why the invention works. Newman v. Quigg, 877 F.2d 1575, 1582, 11 USPQ2d 1340, 1345 (Fed. Cir. 1989). Sixth, where the specification contains teaching of the manner of making and using the invention in terms corresponding in scope with those of the claims, compliance with the enablement requirement of the first paragraph of 35 U.S.C. § 112 is presumed. The examiner has the burden to provide reasons why he doubts the objective truth of statements in the specification supporting enablement. In re Vaeck, 947 F.2d at 496 n.23, 20 USPQ2d at 1444-45 n.23; In re Marzochi, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971). On this record, we conclude that the examiner has not met his burden.

Appellants have provided persons skilled in the art with the knowledge that DNA encoding human scu-PA can be expressed at "an expression rate of from about 10 to about 25 percent by weight of

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the total protein produced." Further, appellants have placed within the public's grasp means to effect the synthesis in inclusion bodies of an inactive form of scu-PA in E. coli at that expression rate. Here, as in In re Angstadt, 537 F.2d at 504, 190 USPQ at 219 (emphasis added):

[a]ppellants have enabled those in the art to see . . . a real possibility, which is commendable frankness in a disclosure. Without undue experimentation or effort or expense the combinations which do not work will readily be discovered and, of course, nobody will use them and the claims do not cover them. . . . [T]o make everything predictable in advance . . . is impracticable and unreasonable.

The examiner's rejections of Claims 1 to 3, 6, 9, 10, 15, 21, 22, 26, and 27 under 35 U.S.C. § 112, first paragraph, are reversed.

5. Conclusion

A. The examiner's rejections of Claims 1 to 3, 6, 8 to 10, 14 to 19, and 21 to 27 under 35 U.S.C. § 103 are reversed.

B. The examiner's rejections of Claims 1 to 3, 6, 9, 10, 15, 21, 22, 26, and 27 under 35 U.S.C. § 112, first paragraph, are reversed.

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REVERSED

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

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Sherman D. Winters
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BOARD OF PATENT
APPEALS AND
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