The opinion in support of the decision being entered today is *not* binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte MANSOOR MOHAMMED

Appeal 2007-3395
Application 10/260,733[1]
Technology Center 1600

Decided: 28 September 2007

Before RICHARD E. SCHAFER, TEDDY S. GRON and CAROL A. SPIEGEL, *Administrative Patent Judges.*

SPIEGEL, *Administrative Patent Judge.*

DECISION ON APPEAL

I. Introduction

Mansoor Mohammed (hereinafter "Appellant") seeks our review under 35 U.S.C. § 134 (2002) of the Examiner's final rejection of all pending claims in this Application, claims 1-3, 5-7, 13-14, 18-25, 28-31, 34-41, 46-

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[1] Application filed 27 September 2002. Applicant claims benefit under 35 U.S.C. § 119 of application 60/325,853, filed 27 September 2001. The real party-in-interest is said to be PerkinElmer LAS, Inc.

49, 54-58, 61-62 and 86. We have jurisdiction under 35 U.S.C. § 6(b) (2002). We REVERSE.

The subject matter on appeal is directed to a method of detecting the presence of two or more chromosomally distinct cell lines within a population of cells using array-based comparative genomic hybridization ("CGH"). Claim 1[2] is illustrative and reads as follows (emphasis added):

> 1. A method of detecting a degree of genetic mosaicism in a cell population by performing an array-based comparative genomic hybridization (CGH), wherein *an array* comprising a plurality of cloned genomic nucleic acid segments is provided in a plurality of identical replicas, each cloned segment immobilized to a discrete and known spot on a substrate surface to form the array, the cloned genomic nucleic acid segments *comprising a substantially complete first genome of a known first karyotype*, the method comprising:
>
> (a) contacting replicas of the array with mixtures of a first nucleic acid sample and a second nucleic acid sample and fractional dilutions of the second sample, wherein the *first sam*ple comprises a plurality of genomic nucleic acid segments *comprising a substantially complete complement of the first genome* labeled with a first detectable label, the *second sample* comprises a plurality of genomic nucleic acid segments *comprising a substantially complete complement of the second genome* labeled with a second detectable label, and the karyotype of the second sample is known and is different from that of the first sample;

[2] Amended Appeal Brief under 37 C.F.R. § 41.37 filed 27 November 2006 ("Br."), at 10.

> (b) contacting further replicas of the array with mixtures of the first nucleic acid sample and a third nucleic acid sample and fractional dilutions of the third sample, wherein the *third sample* comprises a genomic nucleic acid sample with an unknown karyotype and is labeled with the second detectable label, and the genomic nucleic acid of the third sample *comprises a substantially complete complement of genomic nucleic acid of a third genome* from a test cell or a tissue sample,
>
> wherein the contacting is under conditions wherein the nucleic acid in the mixtures of each of the first and second samples and the first and third samples can specifically hybridize to the genomic nucleic acid segments immobilized on the array;
>
> (c) measuring the amount of first label and second label on each spot for each respective contacted array and determining the karyotype of each dilution fraction by comparative genomic hybridization; and,
>
> (d) selecting which fractional dilution karyotype determination of the second sample most closely determines the known karyotype, and selecting data for the same fractional dilution of the third sample to determine the karyotype of the third sample, thereby determining the degree of genetic mosaicism in the cell population.

Claim 34 limits the array-immobilized genome of the method of claim 1 to a wild-type (normal) genome, while claim 35 limits the first sample of the method of claim 34 to a wild-type (normal) genome. Claim 37 requires the second sample of the method of claim 1 to have a mosaic karyotype. [Br. at 13.]

The Examiner has rejected claims 1-3, 5-7, 13-14, 18-25, 28-31, 34-41, 46-49, 54-58, 61-62 and 86 under 35 U.S.C. § 103(a) (Answer[3] at 3 and 7). The Examiner relies upon the following prior art[4] of record:

Bradley	US 6,048,695	Apr. 11, 2000
Boa	US 6,251,601 B1	Jun. 26, 2001

Kuukasjärvi, "Optimizing DOP-PCR for Universal Amplification of Small DNA Samples in Comparative Genomic Hybridization," *Genes, Chromosomes & Cancer*, Vol. 18, pp. 94-101 (1997).

Bradley, Bao and Kuukasjärvi qualify as prior art under 35 U.S.C. § 102(b). According to the Examiner, claims 1-3, 5-7, 13-14, 18-25, 28-31, 34-41, 46, 54-58, 61-62 and 86 would have been obvious over the combined teachings of Bao and Kuukasjärvi; and, claims 47-49 would have been obvious over the combined teaches of Bao, Kuukasjärvi, and Bradley.

According to Appellant, the patentability of all the claims on appeal stand or fall with claim 1 (Br. at 5 and 8). Therefore, we decide this appeal on the basis of claim 1. 37 C.F.R. § 41.37(c)(1)(v).

II. Findings of Fact ("FF")

The following findings of fact are supported by a preponderance of the evidence of record.

A. Appellant's specification

[1] CGH is said to be a molecular cytogenetics approach that can be used to detect regions in a genome undergoing quantitative changes, e.g., gains or losses of sequence or copy numbers (Specification at 28, ¶ 85).

[2] According to the specification, the

[3] Examiner's Answer mailed 12 March 2007 ("Answer").
[4] No references to *et al*. are made in this opinion.

> principal of the array CGH approach is simple. Equitable amounts of total genomic DNA from cells of a test sample and a reference sample (e.g., a sample from cells known to be free of chromosomal aberrations) are differentially labeled with fluorescent dyes and co-hybridized to the array of BACs [bacterial artificial chromosomes], which contain the cloned genomic DNA fragments that collectively cover the cell's genome. The resulting co-hybridization produces a fluorescently labeled array, the coloration of which reflects the competitive hybridization of sequences in the test and reference genomic DNAs to the homologous sequences within the arrayed BACs. Theoretically, the copy number ratio of homologous sequences in the test and reference genomic DNA samples should be directly proportional to the ratio of their respective fluorescent signal intensities at discrete BACs within the array. [Specification at 2, ¶ 4, bracketed text added.]

[3] Further according to the specification, "[t]he methods of the invention are used to determine the karyotype of a cell population, which includes an [sic] determination of the genetic mosaicism of a cell population, including the number of karyotype subpopulations in a sample and the percent of the cell population having a particular karyotype" (Specification at 18, ¶ 55).

[4] Genetic mosaicism is said to be defined as "the presence of two or more chromosomally distinct cell lines or cell lineages within a sample or a reference population of cells. For example, a solid

tumor's ('a sample') genetic make-up can be 50% 47,XXX and 50% 45 X,-X cells." (Specification at 2, ¶ 5).[5]

[5] Determining the presence or degree of genetic mosaicism in a cell population can be helpful in determining the cause of a disease (e.g., cancer or an inherited disease) or for diagnosing or prognosing its cause (Specification at 2, ¶ 5).

[6] Instead of analyzing the chromosomes from individual cells, karyotyping using array-based CGH analyzes the DNA sequence copy number of the total genomic DNA extracted from the cells (Specification at 2, ¶ 6).

[7] From a DNA copy number perspective, the genome profile of a tumor made up of 50% 47,XXX cells and 50% 45 X,-X cells is said to be no different from a reference population of 100% 46,XX cells (Specification at 2-3, ¶ 6).

[8] However, "the genetic mosaicisms observed in clinical samples will likely only rarely involve cell populations whose combined genetic profiles completely mask the presence of a mosaic population" (Specification at 3, ¶ 7).

[9] The methods described in Appellant's specification are said to be "sufficiently sensitive to detect clonally distinct (by karyotypic criteria) cell populations within a more dominant background cell population" (Specification at 14, ¶ 42).

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[5] The standard way of describing karyotypic information is to (a) give the total number of chromosomes, (b) identify the sex chromosomes and (c) identify any other abnormalities that may be present. A normal male karyotype, 46,XY, means the cell contains 46 chromosomes, including one X and one Y.

[10] The methods described in Appellant's specification are said to determine "the number of karyotype subpopulations in a sample and the percent of the cell population having a particular karyotype" (Specification at 18, ¶ 55).

[11] In one embodiment, the specification describes a method comprising

> (a) providing an array comprising a plurality of cloned genomic nucleic acid segments, wherein each genomic nucleic acid segment is immobilized to a discrete and known spot on a substrate surface to form an array and the cloned genomic nucleic acid segments comprise a substantially complete first genome of a known karyotype; (b) providing a first sample, wherein the sample comprises a plurality of genomic nucleic acid segments comprising a substantially complete complement of the first genome labeled with a first detectable label; (c) providing a second sample, wherein the sample comprises a plurality of genomic nucleic acid labeled with a second detectable label, and the genomic nucleic acid sample comprises a substantially complete complement of genomic nucleic acid of a cell or a tissue sample, and the karyotype of the second sample is known and is different from that of the first sample of step (b); (d) providing a third sample, wherein the sample comprises a genomic nucleic acid sample with an unknown karyotype labeled with the second detectable label, and the genomic nucleic acid comprises a substantially complete complement of genomic nucleic acid of a cell or a tissue sample; (e) preparing serial dilution fractions of the samples of steps (c) and (d); (f) contacting the sample of step (b) separately with each serial dilution fraction of the sample of step (c) with the array of step (a) under conditions wherein the nucleic acid in the samples can specifically hybridize to the genomic nucleic acid segments

> immobilized on the array; (g) measuring the amount of first and second fluorescent label on each spot after the contacting of step (f) for each serial dilution fraction and determining the karyotype of each serial dilution fraction by comparative genomic hybridization; (h) contacting the sample of step (b) and serial dilution fractions of the sample of step (d) with the array of step (a) under conditions wherein the nucleic acid in the samples can specifically hybridize to the genomic nucleic acid segments on the array; (i) measuring the amount of first and second fluorescent label on each spot after the contacting of step (h) for each serial dilution fraction and determining the karyotype of each serial dilution fraction by comparative genomic hybridization; and, (j) selecting which dilution fraction karyotype determination of step (g) most closely determined the known karyotype, and selecting the same serial dilution measurement in step (i) to determine the karyotype of the sample of step (d), thereby determining the degree of genetic mosaicism in a cell population. [Specification at 4-5, ¶ 12.]

[12] The array-immobilized genome may comprise a normal genome or karyotype, and the first sample may have a normal genome or karyotype (Specification at 6-7, ¶ 21).

[13] The second sample may have a mosaic karyotype comprising two or more cell subpopulations (Specification at 7, ¶ 22).

B. Bao

[14] Chromosomal abnormalities, which may or may not involve a change in DNA sequence copy number from the normal one copy per chromosome, are said to be involved in various human pathologies (Bao at col. 1, ll. 24-32).

[15] One form of aggressive breast cancer is said to result from gene amplification and overexpression of the Her-2/neu gene located on chromosome 17 at band q12 (Bao at col. 1, ll. 35-38).

[16] Overexpression of the Her-2 gene, prior to gene amplification, is said to occur at an earlier, less aggressive stage of the disease (Bao at col. 1, ll. 42-46).

[17] Thus, proper management of breast cancer is said to require both measurement of Her-2 gene expression and Her-2 gene copy number, i.e., two separate tests on a tissue sample (Bao at col. 1, ll. 46-49).

[18] Bao describes methods for simultaneously measuring gene expression and chromosome abnormalities in the same tissue sample using array-based CGH (Bao at col. 2, l. 57 to col. 3, l. 3; col. 6, ll. 23-28).

[19] The Bao method comprises cohybridizing first, second and at least one reference nucleic acid populations, each labeled with a different fluorescent marker, to an array of nucleic acid target elements immobilized on a solid substrate and measuring the presence and intensity of each marker at each target element of the array (Bao at col. 2, l. 66 through col. 3, l. 12; col. 3, ll. 31-55).

[20] The nucleic acid target elements may comprise total genomic DNA[6] (Bao at col. 7, l. 33 through col. 8, l. 26, esp. col. 7, ll. 63-66; col. 34, ll. 1-31).

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[6] Genomic DNA is all the DNA sequences comprising the total genetic information (genome) of a cell or organism. Since cDNA is DNA copied from an mRNA by reverse transcription, cDNA lacks the introns present in genomic DNA. *See e.g.*, MOLECULAR CELL BIOLOGY, 5th ed., W.H. Freeman and Company, New York, NY (2004), at G-3 and G-9.

[21] Various control nucleic acid target elements may also be included on the array, e.g. total genomic DNA or total genomic or cDNA from a tissue with known abnormalities (Bao at col. 11, ll. 13-18).

[22] The first nucleic acid population comprises a mixture of mRNA or its complementary cDNA, which is representative of gene expression in the tissue sample and is labeled with a first marker, e.g., a red fluorescent dye (Bao at col. 6, ll. 34-38; col. 17, ll. 5-9).

[23] The second nucleic acid population comprises a mixture of genomic DNA which is representative of the tissue sample's total genomic, i.e., chromosomal, DNA and is labeled with a second marker, e.g., a green fluorescent dye (Bao at col. 6, ll. 34-40; col. 17, ll. 5-9).

[24] The third nucleic acid population comprises a reference population labeled with a third marker, e.g., an orange fluorescent dye (Bao at col. 6, ll. 40-42; col. 17, ll. 5-9).

[25] The reference population may be total human genomic DNA from normal tissue (Bao at col. 3, ll. 60-62; col. 6, ll. 46-49; col. 12, ll. 33-36).

[26] The array of target elements is contacted, under hybridization conditions, with the three labeled nucleic acid populations; the presence and intensity of each marker is measured at each target element; and, the ratios of the markers (e.g., first and third markers and second and third markers) are determined for each target element (Bao at col. 3, ll. 3-8 and 31-59; col. 6, ll. 22-55).

[27] Comparison of the ratios at a particular target element is said to provide the copy number for the genomic DNA sequence and for

cDNA sequences which are complementary to that target element (Bao at col. 6, ll. 51-55).

C. Kuukasjärvi

[28] According to Kuukasjärvi, a major limitation in analyzing genetic changes in the early stages of tumorigenesis is that the higher number of normal cells in a tissue sample often mask genetic alterations in premalignant and small malignant tumors (Kuukasjärvi at 94).

[29] While standard CGH[7] allows genome-wide screening for DNA sequence copy number abnormalities, it is said to require 0.5 to 1 µg of genomic DNA, corresponding to roughly 50,000 to 100,000 diploid cells from the tumor sample (Kuukasjärvi, ¶ bridging 94-95).

[30] Microdissected tumor samples are said to be too small to provide enough DNA for standard CGH and, therefore, the DNA must be amplified using PCR with degenerate probes (DOP-PCR) prior to CGH analysis (Kuukasjärvi at 95).

[31] Kuukasjärvi describes an improved DOP-PCR method which includes incorporating fluorescent labeled nucleotides directly into the PCR reaction (Kuukasjärvi at 95).

[32] To test the sensitivity of DOP-PCR CGH, Kuukasjärvi made serial dilutions of a known concentration of DNA extracted from the MCF-7 breast cancer cell line and used the dilutions as starting material in DOP-PCR (Kuukasjärvi at 97).

[33] According to Kuukasjärvi, DOP-PCR could amplify template DNA from concentrations as low as 25 pg DNA (Kuukasjärvi at 97).

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[7] Standard CGH uses chromosomes as the target elements for hybridization (see e.g., Kuukasjärvi at 96 and Figure 3).

[34] Further according to Kuukasjärvi, "CGH analysis showed that 50 pg of DNA (corresponding roughly to two hypertetrapoloid MCF-7 cells) was sufficient to produce copy number profiles, which were identical to those obtained from a standard CGH protocol" (Kuukasjärvi at 97).

[35] PCR-based incorporation of fluorescent markers (labeled nucleotides) was said "to be very effective and resulted in a high signal intensity in CGH" (Kuukasjärvi at 100).

[36] Kuukasjärvi "concludes that CGH can now be efficiently used to analyze DNA sequence gains and losses in small subpopulations of cells from, e.g., premalignant and early lesions" (Kuukasjärvi at 100).

D. Rejections over the Prior Art and Rebuttal

[37] The Examiner finds that Bao teaches the method of claim 1 but for use of dilution fractions of the second and third samples (Answer at 3-4 and 6).

[38] Specifically, the Examiner finds that Bao teaches claim 1,

> (a) contacting replicas of the array with a first nucleic acid and a second nucleic acid sample . . . , wherein the first nucleic acid is labeled with a first detectable label and the second sample with a second detectable label and each comprise substantially complete complements of the first genome (reference nucleic acids) and second genome (*cDNA sequences complementary to expressed gene sequences*) and karyotype of first and second genome is known . . . ,
>
> (b) contacting further replicas of the arrays with a third sample (test or genomic nucleic acids or tumor comprising substantially complete complement of genomic nucleic acid of a third genome . . . [Answer at 3-4, citations to Bao omitted, emphasis added.]

[39] The Examiner finds that Kuukasjärvi teaches "the use of serial dilutions of DNA in CGH analysis to increase the sensitivity of the assay to detect the genetic variation in a dilution factor comprising a single cell that aid in efficient detection of genetic variation in small sub populations of cells . . ." (Answer at 6, citations to Kuukasjärvi omitted).

[40] The Examiner concludes that it would have been obvious to modify the method of Bao with a step of including dilution fractions of the DNA sample as taught by Kuukasjärvi for the purpose of increasing the sensitivity of the method down to a single cell fraction as taught by Kuukasjärvi (Answer at 6).

[41] Appellant contends that the cDNA population disclosed by Bao does not constitute a substantially complete genome because cDNAs do not include introns and, therefore, Bao does not teach or suggest a method using three populations of labeled probes comprising substantially complete complements of genomes as defined by claim 1 (Br. at 6-7; Reply Br.[8] at 8-10).

[42] Appellant further contends that Bao only discloses (1) a mixture of mRNA or its complementary cDNA and (2) a mixture of genomic DNA, both of which are merely representative of (1) gene expression in the tissue sample and (2) genomic status of the tissue sample, respectively; and, neither is a substantially complete complement of genomic nucleic acid (Reply Br. at 9).

[43] The Examiner responds that the scope of a “substantially” complete genome or its complement was not defined in Appellant’s

[8] Reply Brief filed 11 May 2007 (“Reply Br.”).

specification and "could permit some variation in a genome resulting in a broad range of percentage complementarity representing a genome" (Answer at 9).

[44] According to the Examiner, "claim 1 is broad and can represent a bacterial genome which do not contain intronic sequences, thus the cDNA population [of Bao] does read on bacterial genome and the assertions drawn to labeled cDNA genome" (Answer at 9).

[45] Appellant also argues that Kuukasjärvi teaches using dilutions to obtain a suitable concentration of DNA to be used as a starting material for DOP-PCR and, therefore, does not cure the deficiencies of Bao (Br. at 7).

[46] The Examiner responds that the motivation in obviousness rejections need not be the same as Appellant's (Answer at 10).

[47] According to the Examiner, Kuukasjärvi teaches that serial dilutions of the starting DNA template for PCR amplification shows that small amounts of DNA could be used to accurately detect genetic mosaicism in CGH assays and provides a reasonable expectation of success of detecting genetic variation in each cell type of a cell population (Answer at 10-11).

Other findings of fact are cited as necessary below.

III. Obviousness

A claimed invention is not patentable if the subject matter of the claimed invention would have been obvious to a person having ordinary skill in the art. 35 U.S.C. § 103(a); *KSR Int'l Co. v. Teleflex, Inc.*, 127 S.Ct. 1727, 82 USPQ2d 1385 (2007); *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966). Facts relevant to a determination of obviousness include (1)

scope and content of the prior art, (2) any differences between the claimed invention and the prior art, (3) the level of ordinary skill in the art, and (4) relevant objective evidence of obviousness or non-obviousness. *KSR*, 127 S.Ct. at 1734, 82 USPQ2d at 1388; *Graham*, 383 U.S. at 17-18. All limitations of claimed invention must be taught or suggested by the prior art to establish *prima facie* obviousness. *In re Royka*, 490 F.2d 981, 985, 180 USPQ 580, 583 (CCPA 1974).

The dispositive issue here is whether Bao teaches or suggests a method comprising contacting an array of nucleic acid target elements with three populations of differentially labeled nucleic acid populations or segments, each of which comprises a substantially complete complement of its respective genome. Bao describes cohybridizing first, second and at least one reference nucleic acid populations, each labeled with a different fluorescent marker, to an array of nucleic acid target elements immobilized on a solid substrate (FF 19). Bao teaches that the nucleic acid target elements comprise total genomic DNA (FF 20). The first nucleic acid population comprises a mixture of cDNA complementary to the mRNA representative of gene expression in the tissue sample and is labeled with a first marker, e.g., a red fluorescent dye (FF 22). The Examiner found that this population of cDNA sequences reads on the second sample of step (a) of claim 1 (FF 38). However, the method of claim 1, step (a) requires a second sample comprising a plurality of *genomic* nucleic acid segments comprising a substantially complete complement of the second genome. Since cDNA is DNA copied from an mRNA by reverse transcription, cDNA lacks the introns. Therefore, as argued by Appellant (FF 41), the cDNA population used in Bao's method cannot be genomic DNA because it lacks introns.

Realizing this, the Examiner, for the first time,[9] construes claim 1 as broad enough to encompass a bacterial genome, which does not contain intronic sequences (FF 44). However, Bao is directed to measuring gene expression and chromosome abnormalities in the same *tissue sample* using array-based CGH (FF 18). A unicellular bacterium cannot have multicellular tissues. Hence, the Examiner's response is contradictory to the teachings of Bao. Bao teaches a method using only *two* of the *three* genomic nucleic acid samples required by the method of claim 1 (FF 22-24). Kuukasjärvi does not cure the deficiencies of Bao. Since the Examiner has not established that the prior art (Bao and Kuukasjärvi) teach or suggest all of the limitations of claim 1, we will reverse the rejections of (i) claims 1-3, 5-7, 13-14, 18-25, 28-31, 34-41, 46, 54-58, 61-62 and 86 under § 103(a) as obvious over Bao and Kuukasjärvi, and (ii) claims 47-49 under § 103(a) as obvious over Bao, Kuukasjärvi and Bradley.

IV. Summary

In view of the record and for the reasons given, it is

ORDERED that the rejection of claims 1-3, 5-7, 13-14, 18-25, 28-31, 34-41, 46, 54-58, 61-62 and 86 under 35 U.S.C. § 103(a) as obvious over the combined teachings of Bao and Kuukasjärvi is REVERSED;

FURTHER ORDERED that the rejection of claims 47-49 under 35 U.S.C. § 103 (a) as obvious over the combined teaches of Bao, Kuukasjärvi, and Bradley is REVERSED; and

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[9] The Board's review of the claims on appeal is not an independent analysis in the first instance. The Examiner should set forth sufficient factual findings and reasoning supporting claim construction in the first instance, to permit a meaningful evaluation of the claimed invention vis-à-vis the applied prior art.

FURTHER ORDERED that this application is returned to the Examiner for action consistent with the views espoused herein.

REVERSED

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