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BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte RICHARD CONRAD and EMILY ZERINGER

Appeal 2019-003093
Application 15/162,373
Technology Center 1600

Before DONALD E. ADAMS, TIMOTHY G. MAJORS, and MICHAEL A. VALEK, *Administrative Patent Judges*.

VALEK, *Administrative Patent Judge.*

DECISION ON APPEAL

Appellant[1] submits this appeal under 35 U.S.C. § 134(a) involving claims to methods of isolating RNA and determining an amount of full-length RNA from a fixed tissue sample. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

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[1] We use the word "Appellant" to refer to "applicant" as defined in 37 C.F.R. § 1.42(a). Appellant identifies Applied Biosystems, LLC as the real party in interest. Appeal Br. 1. Herein, we refer to the Final Action mailed August 3, 2018 ("Final Act."); Appellant's Appeal Brief filed October 29, 2018 ("Appeal Br."); Examiner's Answer mailed January 31, 2019 ("Ans."); and Appellant's Reply Brief filed March 7, 2019 ("Reply Br.").

STATEMENT OF THE CASE

The Specification explains that "RNA is often isolated from fixed tissue however, due to the processes involved in fixing tissue, such as the use of formaldehyde, the RNA obtained is fragmented." Spec. 1. According to the Specification, the present "invention is effective for obtaining RNA by isolating, extracting, and enriching for RNA, including full-length and substantially full-length RNA, from a sample using a digestion buffer or solution that includes a polyanion." *Id.* at 3. "It is thus contemplated that methods . . . of the invention can be employed to obtain a better yield of RNA from a sample." *Id.*

Claims 1, 4–6, 8, 10, 13, 16, 20, 33, 36–39, 42, and 43 are on appeal and can be found in the Claims Appendix of the Appeal Brief. Claims 1 and 39 are independent and representative of the claims on appeal. They read as follows:

> 1. A method for isolating RNA from a fixed tissue sample comprising:
>
> (a) contacting the fixed tissue sample with a digestion buffer comprising a polycarboxylate selected from sodium citrate, 1,4-cyclohexanedicarboxylic acid, 1,3,5-cyclohexanetriccarboxylic acid, isocitric acid, succinic acid or a combination thereof, and a protease to produce a lysate wherein the digestion buffer lacks guanidinium and lacks Mg2+;
>
> (b) adding guanidinium and a sodium salt to the lysate from (a); and
>
> (c) extracting RNA from the lysate from (b) using solid-phase extraction.
>
> 39. A method for determining an amount of full-length RNA from a fixed tissues sample comprising:
>
> (a) contacting the fixed tissue sample with a digestion buffer comprising a polycarboxylate and a

> protease to produce a lysate wherein the digestion buffer lacks guanidinium and lacks Mg2+;
>
> (b) adding an alcohol solution to the lysate;
>
> (c) applying the lysate to a mineral support;
>
> (d) eluting the full-length RNA from the mineral support with an elution solution; and
>
> (e) amplifying the eluted RNA.

Appeal Br. 11, 13.

Appellant seeks review of the following rejections:

I. Claims 1, 4, 6, 8, 10, 16, 20, 33, 36–39, 42, and 43 under 35 U.S.C. § 103 as unpatentable over Roche[2] in view of Light[3] and Colpan[4]; and

II. Claims 5 and 13 under 35 U.S.C. § 103 as unpatentable over Roche in view of Light, Colpan, Goelz,[5] Masuda,[6] and Ebeling.[7]

Appeal Br. 3–9. Appellant does not argue claims 4, 6, 8, 10, 16, 20, 33, 36–38, 42, and 43 separately from the independent claims so those claims stand or fall with claims 1 and 39. 37 C.F.R. § 41.37 (c)(1)(iv).

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[2] Roche Molecular Biochemicals, *High Pure RNA Paraffin Kit* Instruction Manual, Cat. No. 3 270 289 (2001) ("Roche").

[3] WO 01/29265 A1; published April 26, 2001 ("Light").

[4] US 6,383,393 B1; issued May 7, 2002 ("Colpan").

[5] Goelz et al., *Purification of DNA from Formaldehyde Fixed and Paraffin Embedded Human Tissue*, Biochemical and Biophysical Research Comm'n, Vol. 130(1), 118–126 (1985) ("Goelz").

[6] Norikazu Masuda et al., *Analysis of Chemical Modification of RNA from Formalin-Fixed Samples and Optimization of Molecular Biology Applications for Such Samples*, Nucleic Acids Research, Vol. 27(22), 4436–4443 (1999) ("Masuda").

[7] Wolfgang Ebeling et al., *Proteinase K from Tritirachium Album Limber*, Eur. J. Biochem. Vol. 47, 91–97 (1974) ("Ebeling").

The issue is: Does the preponderance of evidence of record support Examiner's conclusion that the cited prior art renders the claimed methods obvious?

Findings of Fact

FF1. Roche teaches a procedure for isolating RNA from fixed tissue sections. Roche 7–9. According to this procedure, the tissue is contacted with a digestion buffer containing Proteinase K, i.e., a protease, and incubated overnight. *Id.* at 8. This digestion buffer does not contain guanidinium or magnesium. *See id.* at 3, 6 (describing components in "Tissue Lysis Buffer" and "Proteinase K working solution"). After incubation, a binding buffer comprising guanidinium and ethanol is added to the lysate and the solution is pipetted into a filter tube where it selectively binds to a solid glass fiber surface therein. *Id.* After washing, the RNA is eluted using an elution buffer and further purified. *See id.* at 8–9. Roche teaches that the RNA isolated using this procedure is suitable for amplification by RT-PCR. *Id.* at 4.

FF2. Light teaches in situ hybridization techniques that include pretreatment with a digestion buffer comprising proteinase K and 2X SSC at 7.0 pH. Light 15. Light teaches that digestion with this buffer "permit[s] penetration of the labeled nucleic acid probes" and "reduce[s] non-specific backgrounds which may be necessary for a given specimen." *Id.* at 9.

FF3. Colpan teaches a method for the "purification and separation of nucleic acid mixtures by chromatography" that involves "absorbing the nucleic acids to be separated and purified from a solution with a high concentration of salts (ionic strength) and/or a high concentration of alcohol

on a substrate." Colpan, Abstr. Colpan teaches that suitable salts for this method include both sodium and guanidine salts. *Id.* at 5:20–32.

FF4. Goelz teaches a method for extracting DNA from fixed tissue involving contacting the tissue with a digestion buffer, i.e., "TE9 (500 mM Tris, 20 mM EDTA, 10 mM NaCl, pH 9.0) containing 1% SDS and 500 μg/ml proteinase K" to digest the tissue before isolating the DNA. Goelz 119–20.

FF5. Masuda teaches the extraction of RNA from fixed tissue using a digestion buffer comprising "200 mM Tris-HCl, 200 mM NaCL, 1.5 mM $MgCl_2$, 2% SDS, pH 7.5) with 500 μg proteinase K" with a 1 hour incubation period. Masuda 4437.

Analysis

I. Rejection of Claims 1, 4, 6, 8, 10, 16, 20, 33, 36–39, 42, and 43

Examiner finds that Roche teaches the elements of claims 1 and 39 other than the presence of a polycarboxylate in the digestion buffer. Final Act. 3–8. Examiner further finds that Light teaches a digestion buffer comprising a polycarboxylate because "SSC is NaCl and sodium citrate."[8] *Id.* at 4. Examiner determines it would have been obvious "to substitute the lysis buffer of Roche with the lysis buffer of Light since a person of ordinary skill in the art would recognize the lysis buffer of [L]ight is [an] art-accepted suitable alternative for proteinase K digestion." *Id.* Examiner further determines it would be obvious to add a sodium salt, along with the guanidinium and alcohol already taught in Roche's protocol, after digestion

[8] Appellant does not dispute Examiner's finding that "SSC," as taught in Light, includes sodium citrate, i.e., one of the polycarboxylate's recited in the Markush group of claim 1. *See* FF2.

because Colpan teaches that adding those components "increases the specificity of the absorption after digestion" by "altering specificity of binding to the support." *Id.* at 6.

Appellant argues that Examiner's rejection should be reversed for two reasons. First, Appellant argues that Light teaches in situ hybridization and therefore is "specifically directed to retaining the nucleic acid in its location in the specimen but allowing the hybridization probe to enter so it can bind with the target nucleic acid." Appeal Br. 4. For this reason, Appellant contends "Light is directed to an entirely different method than Roche, and therefore, one skilled in the art would not be motivated to" use Light's digestion buffer in Roche's method "as there is no reasonable expectation that the modification would be successful." *Id.* at 3; *see also* Reply Br. 2–4. Second, Appellant argues that Colpan teaches "combining the reagents necessary for digestion (where a lystate is produced) and the chaotropic agents, such as guanidinium, high salt concentration, etc., which allow for absorption of the nucleic acids on the solid support" at the same time. *Id.* at 6. Thus, urges Appellant, Colpan teaches away from "adding guanidinium and a sodium salt (or an alcohol solution) to the lysate <u>after digestion</u>, as required in claims 1 and 39." *Id.*

We are not persuaded by Appellant's arguments and agree with Examiner's statement of the rejection and responses to Appellant's arguments in the Answer and Final Action, which we adopt and incorporate by reference. We further address Appellant's arguments below.

We are not persuaded by Appellant's argument that it would not be obvious to use Light's digestion buffer in Roche's method. Both Light and Roche teach the use of a digestion buffer containing the same protease, i.e.,

Proteinase K, for the same purpose, i.e., digesting tissue. FF1; FF2. "When a patent simply arranges old elements with each performing the same function it had been known to perform and yields no more than one would expect from such an arrangement, the combination is obvious." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 417 (2007) (internal quotations and citation omitted). The fact that Light's method is directed to a different end (in situ hybridization as opposed to RNA isolation/quantification) does not suggest that Light's buffer would be unsuitable for Roche's method. To the contrary, Light teaches that its Proteinase K digestion buffer is sufficient to digest tissue in a deparaffinized tissue specimen similar to that in Roche's method. *See* Light 15 (Ex. 1). Accordingly, we agree with Examiner that a skilled artisan would reasonably expect that Light's digestion buffer would be successfully used to digest tissue in Roche's method. *See, e.g.*, *In re Mayne*, 104 F.3d 1339, 1340 (Fed. Cir. 1997) ("Because the applicants merely substituted one element known in the art for a known equivalent, this court affirms [the rejection for obviousness].").

We are also unpersuaded by Appellant's argument that Colpan teaches away from the claimed method. Colpan teaches that digestion may occur "in a per se known manner" and "[a]fter larger cell constituents . . . have been removed by centrifugation or filtration" the solution containing the nucleic acids from the digested sample is "contacted with a mineral substrate material in order to absorb the nucleic acids . . . on the mineral substrate." Colpan 2:39–50. Colpan further describes a "modification" of this method wherein "digestion of the nucleic acids [is performed] directly within the buffer system employed for the adsorption." *Id.* at 2:51–55. It is this "modification" that Appellant relies upon for its argument that Colpan

teaches away from adding guanidinium and sodium salt to the lysate after digestion. *See* Appeal Br. 6 (quoting Colpan 2:51–55). But Colpan teaches that the absorption step can occur either concurrently or "[a]fter" digestion. Thus, the premise of Appellant's argument is flawed, because Colpan, in fact, teaches the addition of guanidinium, sodium salt and alcohol to improve binding *after* digestion of the sample.[9] FF3. That Colpan additionally teaches an alternative embodiment where the digestion occurs concurrently and thus the digestion buffer includes these components does not "teach away" from the method in claims 1 and 39. *See Merck & Co. v. Biocraft Labs., Inc.*, 874 F.2d 804, 807 (Fed. Cir. 1989) ("[I]n a section 103 inquiry, 'the fact that a specific [embodiment] is taught to be preferred is not controlling, since all disclosures of the prior art, including unpreferred embodiments, must be considered.'") (quoting *In re Lamberti*, 545 F.2d 747, 750 (CCPA 1976)).

For these reasons, we determine that the preponderance of the evidence supports Examiner's rejection of claims 1, 4, 6, 8, 10, 16, 20, 33, 36–39, 42, and 43 as obvious over Roche in view of Light and Colpan.

II. Rejection of Claims 5 and 13

Examiner's rejection of claims 5 and 13 is premised on the same findings concerning Roche, Light, and Colpan discussed above. Ans. 9. In addition, Examiner points to components of digestion buffers taught in Goelz and Masuda and the one hour incubation time taught in Masuda as

[9] Appellant's argument also appears to assume that claims 1 and 39 exclude the addition of sodium salt and alcohol until after digestion is complete, but there is no such requirement in claim language. Indeed, claim 5 depends from claim 1 and recites the presence of "about 200 mM NaCl" in the digestion buffer itself.

evidence that the limitations recited in claims 5 and 13 would be obvious. *See id.*

Appellant advances that the same arguments against Examiner's rejection of claims 5 and 13 as for the other claims. Appeal Br. 9 (arguing that "none of Goelz, Masuda, or Ebeling, either alone or in combination, cures the above noted deficiencies of Roche, Light, and Colpan"). As explained above, we are not persuaded by those arguments. In addition, with respect to claim 5, Appellant argues that Colpan teaches a higher (1 M) salt concentration than the claimed concentration of "about 200 mM NaCl." *Id.*; *see also* Reply Br. 9–10.

We are unpersuaded by Appellant's additional argument concerning claim 5. The cited art teaches digestion buffers comprising the various components recited in claim 5. FF1, FF2, FF4, and FF5. Indeed, Masuda teaches a Proteinase K digestion buffer containing the same concentration of NaCl (200 mM) as that in claim 5. As Examiner explains, "where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Ans. 9–10 (quoting MPEP § 2144.05(II) (citing *In re Aller*, 220 F.2d 454, 456 (CCPA 1955)). Here, the record supports that the general conditions, i.e., digestion buffers comprising the various components recited in claim 5 and the incubation time recited in claim 13, were known in the art. Appellant has not presented any evidence to the contrary. *See, e.g.*, *Merck*, 874 F.2d at 806–09 (explaining "[p]atentability may be imparted . . . if the results achieved at the designated concentrations are 'unexpectedly good'") (quoting *In re Antonie*, 559 F.2d 618, 620 (CCPA 1977)). Accordingly, we

determine that Examiner's rejection of claims 5 and 13 is supported by the preponderance of the evidence.

DECISION SUMMARY

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
1, 4, 6, 8, 10, 16, 20, 33, 36–39, 42, 43	103	Roche, Light, Colpan	1, 4, 6, 8, 10, 16, 20, 33, 36–39, 42, 43	
5, 13	103	Roche, Light, Colpan, Goelz, Masuda, Ebeling	5, 13	
Overall Outcome			1, 4–6, 8, 10, 13, 16, 20, 33, 36–39, 42, 43	

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED