

Filed on behalf of:

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UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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INTELLIGENT BIO-SYSTEMS, INC.

Petitioner,

v.

ILLUMINA CAMBRIDGE LTD.

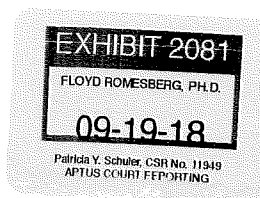
Patent Owner

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**Case IPR2013-00266 (LMG)**  
Patent 8,158,346

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**SECOND DECLARATION OF FLOYD ROMESBERG, PH.D.**



Columbia Exhibit 2081  
Illumina, Inc. v. The Trustees  
of Columbia University in the  
City of New York  
IPR2018-00291, 318, 322, 385 & 797

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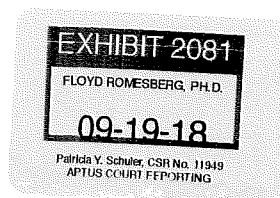
Patent Owner

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**Case IPR2013-00266 (LMG)**  
Patent 8,158,346

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**SECOND DECLARATION OF FLOYD ROMESBERG, PH.D.**



**Illumina Ex. 2037**  
IBS v. Illumina  
Case IPR2013-00266

1. I, Floyd Romesberg, Ph.D., have been retained by Knobbe, Martens, Olson & Bear, LLP, counsel for Illumina Cambridge Limited (“Illumina”). I understand that the U.S. Patent and Trademark Office Patent Trial and Appeal Board has instituted an inter partes review of the patentability of the claims of U.S. Patent No. 8,158,346 (“the ’346 patent,” Ex. 1001) based upon a petition filed by Intelligent Bio-Systems, Inc. (“IBS”).

2. I previously submitted a declaration (Ex. 2004) that provided my opinion as to why the proposed claims submitted in Illumina’s motion to amend are novel and would have been nonobvious.

3. I submit this declaration in support of Illumina’s Reply to IBS’ opposition to Illumina’s motion to amend.

#### **I. MATERIALS CONSIDERED**

4. I have considered and reviewed at least the following in connection with providing this declaration:

- a. Second declaration of Dr. Bruce Branchaud (Ex. 1021);
- b. Ruby *et al.*, Methods in Enzymology, 181:97-121, 1990 (“Ruby,” Ex. 2016);
- c. U.S. 4,772,691 (“Herman,” Ex. 2019);
- d. U.S. 6,664,079 (“Ju,” Ex. 1002);

- e. Letsinger et al., J. Org. Chem., 29:2615-2618 (1964) (“Letsinger,” Ex. 1036);
- f. Canard et al., Gene, 148:1-6 (1994) (“Canard,” Ex. 1028);
- g. Welch et al., Nucleosides & Nucleotides, 18:197-201 (1999) (Ex. 2012);
- h. Welch et al., Chem. Eur. J., 5:951-960 (1999) (Ex. 2045);
- i. Welch et al., Chem. Eur. J., 11:7145 (2005) (Ex. 2046);
- j. Metzker et al., Nucleic Acids Research, 22:4259-4267 (1994) (“Metzker,” Ex. 2011);
- k. Wu et al., Nucleic Acids Research 35:6339-6349, (2007) (“Wu,” Ex. 2047);
- l. Taylor et al., Virus Research, 2:175-182 (1985) (“Taylor,” Ex. 2048);
- m. Watson et al., Molecular Biology of the Gene, Fifth Edition, Chapter 6 (2004) (“Watson,” Ex. 2049);
- n. Shen et al., FASEB J., 9:1023-1033 (1995) (“Shen,” Ex. 2050);
- o. Holtzman et al., Proc. Natl. Acad. Sci. USA, 79:310-314 (1982) (“Holtzman,” Ex. 2051);
- p. Pugliese et al., Journal of Molecular Biology, 231:698-710 (1993) (“Pugliese,” Ex. 2052);

- q. Fersht, Proc. Natl. Acad. Sci. USA, 76:4946-4950 (1979) (“Fersht 1,” Ex. 2053);
- r. Fersht et al., Proc. Natl. Acad. Sci. USA, 78:4251-4255 (1981) (“Fersht 2,” Ex. 2054);
- s. Bebenek et al., Proc. Natl. Acad. Sci. USA, 87:4946-4950 (1990) (“Bebenek 1,” Ex. 2055);
- t. Bebenek et al., J. Biol. Chem., 267:3589-3596 (1992) (“Bebenek 2,” Ex. 2056); and
- u. Greene and Wuts, Protective Groups in Organic Synthesis, 3rd ed., Chapter 1 (1999) (Ex. 2057).

**II. Ruby et al., Methods In Enzymology, 181:97-121, 1990 (“Ruby,” Ex. 2016)**

5. Ruby teaches that a disulfide linker can be cleaved from biotinylated anchor RNA 3'-21 using dithiothreitol (DTT). Ruby at p. 117, FIG. 4 (Ex. 2016).

Figure 4 is reproduced below:

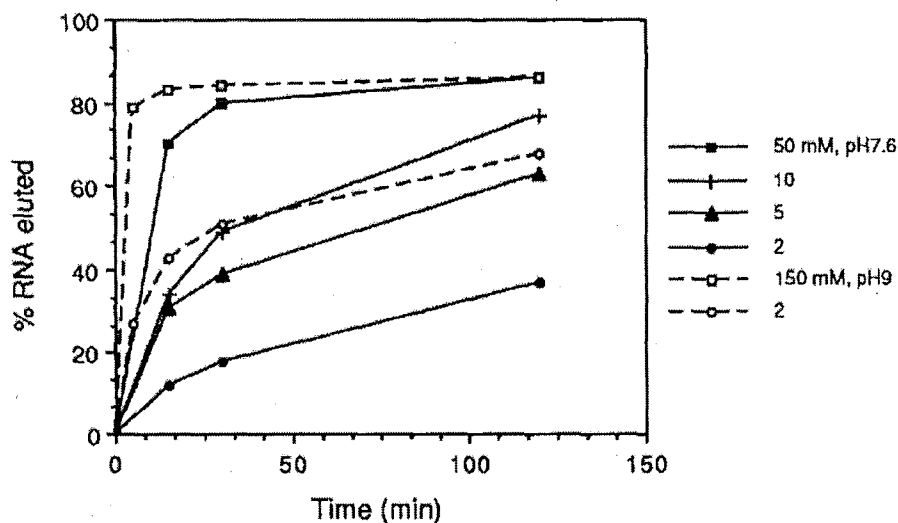
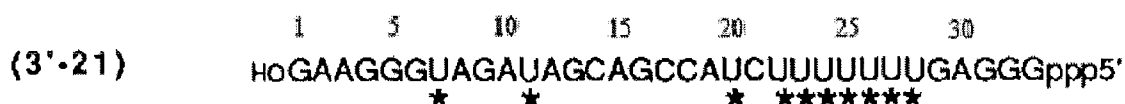


FIG. 4. Eluting biotinylated anchor RNA. Biotinylated anchor RNA 3'-21 was bound to biotin-acyl with succinylavidin as described in Methods. The amounts of RNA eluted with various concentrations of DTT at pH 7.6 (solid lines) or with 2 or 150 mM DTT at pH 9 (dashed lines) were measured as a function of time of incubation in the elution buffers.

The disulfide linker cleavage reported by Ruby provides a maximum recovery of Anchor RNA 3'-21 of approximately 86% after more than 100 minutes.

6. The sequence of Ruby's Anchor RNA 3'-21 that was used for the elution experiment in Fig. 4 is shown in Fig. 3.



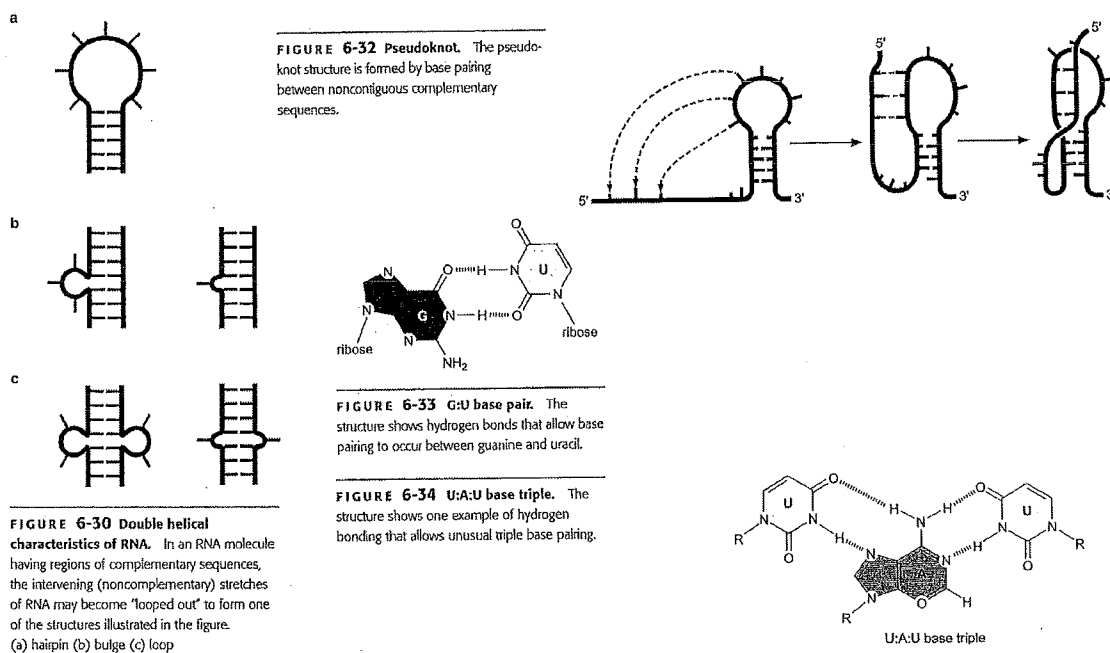
Ruby at 113, Fig. 3 (nucleotide numbers added for reference purposes). As shown in Fig. 3, Anchor RNA 3'-21 has 21 nucleotides that can pair with complementary nucleotides of another strand of RNA (in this case "a pre-mRNA strand"). Anchor RNA 3'-21 has 10 uridine residues that can be biotinylated. Dr. Branchaud calculated a 98.5% disulfide cleavage rate by assuming that all 10 uridine residues

were biotinylated, that all 10 disulfide linkers were bound to Ruby's solid support, and thus, that all 10 disulfide linkers must be cleaved to elute Anchor RNA 3'-21. *See* Second declaration of Dr. Bruce Branchaud ¶ 37 (Ex. 1021). Dr. Branchaud also calculated a 97% disulfide cleavage efficiency by assuming that 5 biotinylated uridine residues were bound and cleaved to elute the RNA. *Id.* As discussed below, it is highly unlikely that 5 or 10 biotinylated uridines were bound to Ruby's solid support. In fact, Ruby's data suggests that only one biotinylated uridine was bound to the solid support.

A. Dimensions Of Anchor RNA 3'-21

7. The Anchor RNA 3'-21 shown in Fig. 3 of Ruby is hybridized to exon2 and is represented as the expected hybrid duplex. Ruby at 113, Fig. 3. There are 22 nucleotides between the uridine residue closest to the 3'-end (identified as residue #7 in ¶ 6) and the uridine residue closest to the 5'-end (identified as residue #28 in ¶ 6). The greatest possible length of these 22 nucleotides would be approximately 6.2 nm (*i.e.*, the rise per base in helical RNA is 0.28 nm; and  $22 \text{ nucleotides} * 0.28 \text{ nm} = 6.2 \text{ nm}$ ; *see, e.g.*, Taylor et al., *Virus Research*, 2:175-182 at 175 (1985) (Ex. 2048)), and that would only be possible with helix formation through both double and single stranded regions (*i.e.*, if the 5' single stranded region also formed a helix). Otherwise the length will be less. But the Anchor RNA 3'-21 that was used for the elution experiment in Fig. 4 was not

hybridized to another strand of RNA; the Anchor RNA 3'-21 was single stranded. See Ruby at 117, caption to Fig. 4. A single stranded RNA chain is expected to form base-paired segments by folding back upon itself into various secondary structures, such as duplex, stem-loop and/or bulges. Further base-pairing can form between noncontiguous complementary sequences. Several exemplary RNA base-pairing structures are shown below:

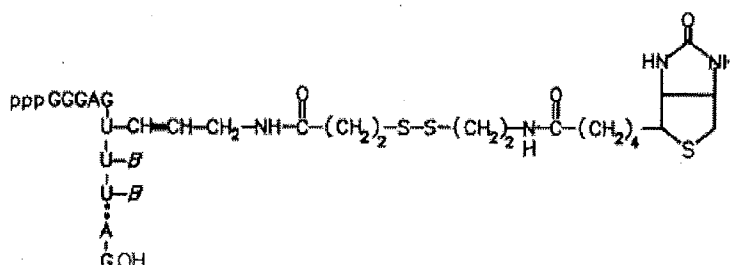


The above figures are reproduced from Watson et al., *Molecular Biology of the Gene*, Fifth Edition at 123-124 (Ex. 2049). These structures are also discussed in Shen et al., *FASEB J.*, 9:1023-1033 (1995) (Ex. 2050). The formation of such structures limits the potential length of the RNA and the 3-dimensional degrees of freedom of the constituent nucleotide residues. Thus, the Anchor RNA 3'-21 used



in the elution experiment shown in Fig. 4 would have adopted secondary stem-loop structures that would have made the overall length of the Anchor RNA 3'-21 significantly smaller than 6 nm. Based on standard principles of RNA folding and structure, which were well known prior to 2002, I estimate that the length of the Anchor RNA 3'-21 was about 4 nm.

8. Each biotinylated uridine in Anchor RNA 3'-21 has a 12 atom chain that links the biotin to the uracil base.



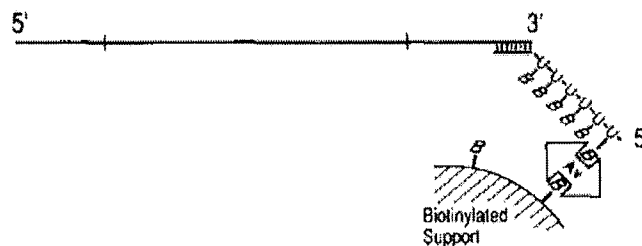
Ruby at 100, Fig. 2. The length of the 12 atom chain is approximately 2 nm. The linkers could extend each biotin up to about 2 nm away from the Anchor RNA 3'-21 structure. But the linker has lipophilic and hydrophilic parts that will fold back and interact with Anchor RNA 3'-21. As a result, the secondary structure adopted by the Anchor RNA 3'-21 and the length of the linker affect the possible geometric orientations of the biotins.

9. Once one biotin of Anchor RNA 3'-21 binds to an avidin on Ruby's solid support the remainder of the anchor RNA becomes significantly constrained in space. This significantly restricts the spatial freedom of any additional biotins

that may be attached to the anchor RNA. Therefore, the first biotin/avidin binding event for any given Anchor RNA 3'-21 greatly reduces the spatial freedom of other biotins to reach and potentially bind any other binding sites, which, as discussed in the next paragraph, are also spatially restricted.

### B. Dimensions of Avidin

10. Whether the spatial restrictions discussed in paragraphs 7-9 will preclude Anchor RNA 3'-21 from binding via more than a single biotin to the solid support depends upon the dimensions and orientations of the avidin. Avidin is a cylindrical protein that has a diameter of 5.5 nm and a height of 4.1 nm (i.e., avidin is larger in size than the estimated size of Ruby's Anchor RNA 3'-21). *See* Holtzman et al., Proc. Natl. Acad. Sci. USA, 79:310-314 at 313 (1982) (Ex. 2051). Avidin is a tetramer that has four biotin binding sites. *See, e.g.*, Ruby at 98. In Ruby's elution experiment, one of avidin's binding sites is occupied by a biotin that is bound to the solid support, as illustrated by Fig. 1A of Ruby:



Therefore, only three of avidin's biotin binding sites remain available to bind a biotinylated uridine residue on the Anchor RNA 3'-21. Each of the binding sites

are approximately 2-3 nm from each other and located deep within the core of the protein. Pugliese et al., *Journal of Molecular Biology*, 231:698-710 at 698 & 709 (1993) (Ex. 2052). Moreover, the binding sites are diametrically opposed on opposite faces of the tetramer. Thus, the relationship of the biotin binding sites on the avidin tetramer severely restricts simultaneous engagement by a single RNA molecule. A second binding event would cause the Anchor RNA 3'-21 to contort and curl around the tetramer, pass through an 0.8 nm wide channel that is partly occluded by a tryptophan residue, and reach the biotin binding site located deep within the protein core. *Id.* at 698, 707. This contortion would potentially require the RNA to unravel from its favored secondary structure.<sup>1</sup> Furthermore, not all binding sites within a given tetramer are expected to be competent to bind biotin, this is not only due to imperfect protein preparation (as is the case with any protein) but also because the avidin is succinylated (and thus, Ruby refers to it as succinyl avidin), which modifies lysine residues (some of which interfere with binding). Ruby at 115. These constraints greatly reduce the probability of any

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<sup>1</sup> Ruby shows that the biotinylated anchor RNA 3'-21 is able to bind to the solid support when it is hybridized to the pre-mRNA. See, e.g., Ruby at 114, Table 1. The hybrid and anchor RNAs are bound and eluted at similar percentages to each other. *Id.*; see also *infra* ¶ 14. The similarity between binding and elution data suggests that Anchor RNA 3'-21 does not contort around avidin because such contortion should disrupt the hybrid RNA structure and result in different binding and elution percentages for hybrid versus anchor RNA.

given avidin molecule binding a second biotin on any individual Anchor RNA 3'-21 molecule.

11. If the avidins on Ruby's solid support were packed close enough together that a single Anchor RNA 3'-21 molecule could span biotin binding sites on two different avidin tetramers, then there would be a possibility for a single Anchor RNA 3'-21 molecule to bind to two different avidins. Given that the size of an avidin tetramer is 5.5 nm by 4.1 nm (Holtzman at 313 (Ex. 2051)), the avidins would need to be packed very closely together for the Anchor RNA 3'-21 to be able to span the length between two avidins. Moreover, the more closely that avidins are packed on a surface, the more likely that two out of the four biotin binding sites will become sterically occluded from binding by neighboring avidins.

12. Considering all the various constraints described above in ¶¶ 7-11, the structure, rigidity, and conformational constraints imposed by a first biotin/avidin binding event greatly reduce the possibility that any individual Anchor RNA 3'-21 molecule can undergo a second biotin binding.

13. Furthermore, not all of the uridine residues in Anchor RNA 3'-21 are biotinylated because some uridine residues are labeled with [<sup>32</sup>P]. *See, e.g.*, Ruby at 105 (copolymerization with allylamine-UTP and [<sup>32</sup>P]UTP); *id.* at 108 ("The two eluents are combined and counted to determine the total number of eluted cpm"); *id.* at 114, note c ("Biotinylated anchor RNA 3'-21 was <sup>32</sup>P labeled to a specific

activity of  $1.9 \times 10^3$  Cerenkov cpm/pmol.”). Ruby’s radiolabeling reduces the biotinylation of Anchor RNA 3’-21. Therefore, even if a second uridine residue on a given Anchor RNA 3’-21 molecule were positioned such that an attached biotin might access a binding site, that uridine residue might not be biotinylated. As a result, Ruby’s use of [ $^{32}\text{P}$ ] labeled uridine residues further reduces the probability of a second biotin/avidin binding event for an Anchor RNA 3’-21 molecule.

C. Data from Table 1 suggests that only one biotin is bound to the support

14. Below is a comparison of Ruby’s binding and elution percentages using Hybrid and Anchor RNA with succinyl avidin and various solid supports.

Binding method	RNA	Avidin type	Solid support		RNA bound (%)	RNA eluted (%)
Bridge	Hybrid	Suc. avidin	+	EY biotin-acryl	70-80	>80
Bridge	Anchor	Suc. avidin	+	EY biotin-acryl	70-85	>75
Bridge	Hybrid	Suc. avidin	+	EY biotin-agarose	40-80	>75
Bridge	Anchor	Suc. avidin	+	EY biotin-agarose	40-60	>75
Bridge	Hybrid	Suc. avidin	+	Pierce biotin-agarose	>90	<5
Bridge	Anchor	Suc. avidin	+	Pierce biotin-agarose	85-95	<10
Bridge	Hybrid	Suc. avidin	+	Pierce biotin-cellulose	30-50	>85
Bridge	Anchor	Suc. avidin	+	Pierce biotin-cellulose	20-30	>85
Bridge	Hybrid	Suc. avidin	+	Sigma biotin-agarose	70-90	>70
Bridge	Anchor	Suc. avidin	+	Sigma biotin-agarose	80-95	>75

Data from p. 114, Table 1 of Ruby (Ex. 2016).

A comparison of the hybrid and anchor RNA data shows that very similar percentages of RNA are bound and eluted for the hybrid and anchor RNAs. The double stranded portion of the hybrid RNA will have an extended helical structure,

while the anchor RNA will adopt a different secondary base-pairing structure. *Supra* ¶ 7. The different structures of the hybrid and anchor RNAs will result in very different orientations of biotins for the hybrid RNA versus the anchor RNA alone. If the anchor RNA had multiple biotins bound to the solid support, then the different orientations of biotins for the hybrid RNA would be expected to add or eliminate one or more biotin interactions as compared to the anchor RNA. But the binding and elution percentages are essentially identical for the hybrid and anchor RNAs. Therefore, Ruby's results suggest that the hybrid RNA and anchor RNA have the same number of biotins bound to the solid support, and the most reasonable interpretation of Ruby's data is that only one biotin is bound per anchor RNA. *See supra* ¶¶ 9-11.

### **III. U.S. 4,772,691 ("Herman," Ex. 2019)**

15. U.S. 4,772,691 ("Herman," Ex. 2019) reports the binding of two different forms of the biotinylated DNA to an avidin-agarose support. Herman at col. 10, l. 47 to col. 12, l. 17 (Ex. 2019). The first was duplex DNA, which is rigid and straight. And the second was DNA of a nucleosome particle, which is a highly specialized structure with the DNA wrapped around histone proteins. Therefore, the biotinylated nucleosomes will have a dramatically different structure than the biotinylated duplex DNA. As a result, the possible orientations of the biotins in the nucleosomal DNA will be dramatically different than in the duplex DNA.

Thus, Herman's biotinylated DNA and biotinylated nucleosomes will present biotins in an even more dramatically different manner than Ruby's hybrid and anchor RNAs (discussed above).

16. Herman states that "nonbiotinylated and biotinylated nucleosomes behaved in the same way as their DNA counterparts." Herman at col. 11, ll. 65-67 (Ex. 2019). If the biotinylated DNA had multiple biotins bound to the solid support, then the different orientations of biotins for the biotinylated nucleosomes would be expected to add or eliminate one or more biotin interactions as compared to the biotinylated duplex DNA. But Herman reports that biotinylated nucleosomes displayed the same recovery behavior as the biotinylated duplex DNA. Therefore, Herman's results suggest that the biotinylated nucleosomes and biotinylated DNA had the same number of biotins bound to the avidin-agarose support. As discussed above for Ruby, the most reasonable interpretation of Herman's data is that only one biotin is bound per biotinylated DNA.

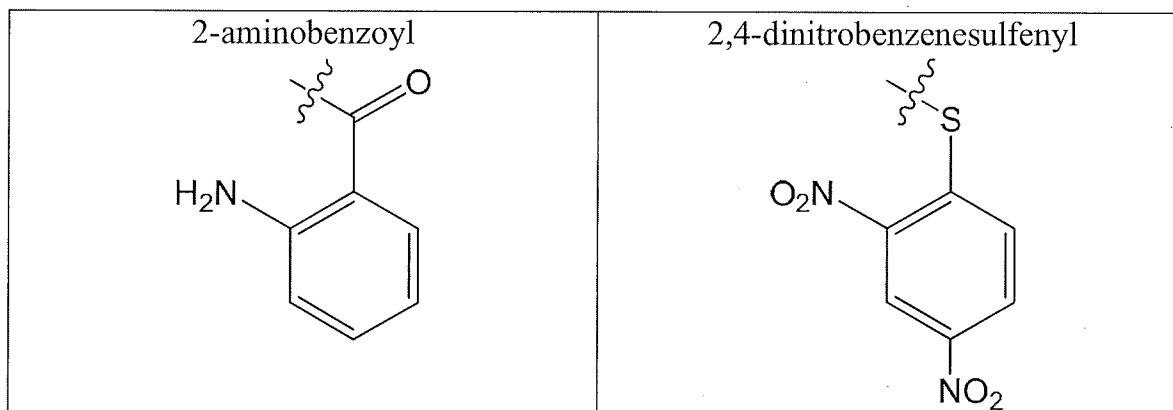
**IV. Canard et al., Gene, 148:1-6 (1994) ("Canard," Ex. 1028)**

17. I agree with Dr. Branchaud that a 2,4-dinitrobenzenesulfenyl moiety would be considered a relatively bulky 3'-protecting group. *See* Second declaration of Dr. Bruce Branchaud ¶ 61 (Ex. 1021). But a person of ordinary skill in the art would not have expected a polymerase to accurately incorporate a nucleotide bearing a 2,4-dinitrobenzenesulfenyl 3'-OH protecting group based on

Canard (Ex. 1028). For example, Canard stated that “high concentrations of 3’-RTa-dNTPs were needed to reach high incorporation levels” for its relatively bulky 3’-group. Ex. 1028 at 3. The “high concentrations” of nucleotide used by Canard were 1 mM and 2 mM. *Id.* at Fig. 3A. However, polymerases lose the ability to accurately replicate a DNA strand at high nucleotide concentrations because the selectivity of polymerases is hindered at high nucleotide concentrations. *See, e.g.*, Fersht, Proc. Natl. Acad. Sci. USA, 76:4946-4950 at 4947 (1979) (Ex. 2053); Fersht et al., Proc. Natl. Acad. Sci. USA, 78:4251-4255 at 4252-53 (1981) (Ex. 2054). Furthermore, polymerases are prone to insert or delete nucleotide residues during synthesis at high nucleotide concentrations. *See, e.g.*, Bebenek et al., Proc. Natl. Acad. Sci. USA, 87:4946-4950 at 4947 (1990) (Ex. 2055); Bebenek et al., J. Biol. Chem., 267:3589-3596 at 3591 (1992) (Ex. 2056). Thus, it is not surprising that Canard taught that “these modified nt are not very efficient chain terminators, a result awaiting precise determination of their  $K_m$ ,  $V_{max}$  and  $k_{cat}$  (work in progress).” Ex. 1028 at 3. A person of ordinary skill in the art seeking to develop sequencing-by-synthesis methods would recognize the need to use a nucleotide with a 3’-protecting group that could be incorporated by a polymerase with high fidelity at moderate to low concentrations. Therefore, a person of skill in the art would have been dissuaded from using a bulky 2,4-dinitrobenzenesulfonyl 3’-protecting group for sequencing-by-synthesis based on the disclosure of Canard.



18. Furthermore, it was known in 2002 that polymerases would not incorporate a nucleotide bearing a 2-aminobenzoyl 3'-OH protecting group. Metzker at 4263,<sup>2</sup> Table 2 (Ex. 2011). The 2-aminobenzoyl protecting group is similar in size and structure to a 2,4-dinitrobenzenesulfonyl protecting group. Therefore, a person of skill in the art would have expected that a nucleotide with a 2,4-dinitrobenzenesulfonyl 3'-OH protecting group would not have been incorporated by a polymerase in 2002.



19. Furthermore, Ju states that the 3'-protecting group should be "small" because the amino acid residues in the polymerase active site are very crowded in the area surrounding the 3'-position of a nucleotide. *See, e.g.*, Ju at col. 2, ll. 31-

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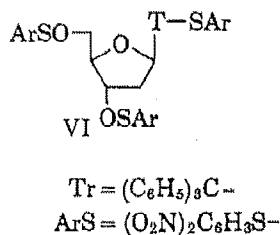
<sup>2</sup> Metzker mistakenly reported that a different nucleotide analog (bearing a 2-nitrobenzyl 3'-protecting group) could be incorporated by a polymerase. By 1999, it was recognized that the potential incorporation of a nucleotide with a 2-nitrobenzyl group was "perplexing" (Welch at 955 (Ex. 2045)), and it was later determined in 2007 that the nucleotide with a 2-nitrobenzyl group was not incorporated by a polymerase (Wu at 6339-6340 (Ex. 2047)).

59, col. 5, ll. 31-39 (Ex. 1002). A 2,4-dinitrobenzenesulfenyl protecting group would not qualify as the type of “small” protecting group that would fit within the active site of a polymerase. *Id.* at col. 8, ll. 9-12 (providing methoxymethyl and allyl protecting groups as exemplary “small” 3'-OH protecting groups).

20. For at least the reasons discussed above, a skilled artisan would not have expected that a polymerase would incorporate a nucleotide having a 2,4-dinitrobenzylsulfenyl 3'-OH protecting group in 2002 for sequencing-by-synthesis.

V. Letsinger et al., J. Org. Chem., 29:2615-2618 (1964) (“Letsinger,” Ex. 1036)

21. Letsinger reports an 80% cleavage efficiency for the removal of three 2,4-dinitrobenzenesulfenyl esters from tris-2,4-dinitrobenzyenesulfenylthymidine. Letsinger at 2618 (Ex. 1036). As shown below, tris-2,4-dinitrobenzyenesulfenylthymidine has one ester attached to a primary hydroxyl group at the 5'-position, another ester attached to a secondary hydroxyl group at the 3'-position, and a third ester attached to the thymine base.



*Id.* at 2616, Chart I.

22. Dr. Branchaud stated that “Letsinger’s cleavage efficiency for removing a single 2,4-dinitrobenzenesulfonyl group from the 3’ oxygen is likely closer to 93% ( $0.93^3 \approx 80\%$ ).” *See* Second declaration of Dr. Bruce Branchaud ¶ 55 (Ex. 1021). Dr. Branchaud’s calculation assumes that the three different esters cleave with the same yield. A person of ordinary skill in the art would not expect the three different types of esters to cleave with the same yield. It is likely that the ester attached to the 3’-OH group cleaves with a lower yield than the other two esters. For example, the 2,4-dinitrobenzenesulfonyl group attached to the thymine base is more labile than the group attached to the 3’-OH. Letsinger at 2616 (showing conversion of VII to IV in Chart I) (Ex. 1036). Moreover, a secondary ester would be expected to cleave with a lower yield than a primary ester. *See, e.g.,* Greene and Wuts, *Protective Groups in Organic Synthesis*, 3rd ed., Chapter 1 at 4 (1999) (Ex. 2057). Therefore, the cleavage efficiency of the 2,4-dinitrobenzenesulfonyl group from the 3’ oxygen is not “likely closer to 93%.”

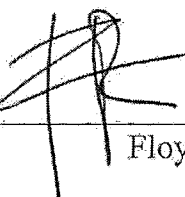
23. Furthermore, protecting groups for use in sequencing-by-synthesis applications should be removable under aqueous conditions. *See, e.g.,* Metzker et al., *Nucleic Acids Research*, 22:4259-4267 (1994) (Ex. 2011) at 4259 (“The complete [sequencing] scheme demands nucleotide analogs that are tolerated by polymerases, spectroscopically distinct for each base, stable during the polymerization phase, and deprotected efficiently under mild conditions in aqueous

solution. These stringent requirements are formidable obstacles for the design and synthesis of the requisite analogs.”); Welch et al., *Nucleosides & Nucleotides*, 18:197-201 (1999) (Ex. 2012) at 197-198 (“A major challenge in development of [the Base Addition Sequencing Scheme], however, is that the 3’-blocking group must be removed in an aqueous medium using reagents/conditions that do not denature or modify double strand DNA.”).

24. Letsinger did not obtain an 80% yield cleaving 2,4-dinitrobenzenesulfenyl esters using an *aqueous* solvent; Letsinger obtained an 80% yield using *pyridine* as a solvent. Letsinger at 2618 (Ex. 1036). Letsinger obtained a 63% yield by cleaving two 2,4-dinitrobenzenesulfenyl esters (where one of the 2,4-dinitrobenzenesulfenyl esters protected the 3’-OH group of a nucleotide) in a mixture of water and methanol. *Id.* If a person of ordinary skill in the art had considered Letsinger’s 2,4-dinitrobenzenesulfenyl ester as a protecting group for sequencing-by-synthesis, the person would have been dissuaded from doing so by the low yield obtained in water/methanol because the water/methanol solvent system is more likely to be compatible with sequencing-by-synthesis than pyridine.

25. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Dated: 3/21/14

By:  \_\_\_\_\_  
Floyd Romesberg, Ph.D

17467978