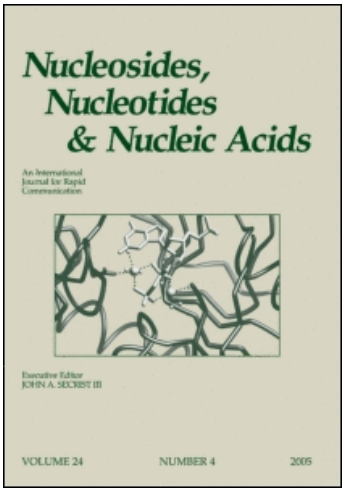

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High-Density Nucleoside Analog Probe Arrays for Enhanced Hybridization

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HIGH-DENSITY NUCLEOSIDE ANALOG PROBE ARRAYS FOR ENHANCED HYBRIDIZATION

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ABSTRACT: DNA probe arrays were synthesized with analogs of 2,6-diaminopurine and 2'-O-methyl-thymidine in place of A and T. AT-rich GeneChip® test arrays containing 14-mer or 20-mer analog probes improved hybridization to fluorescently-labeled RNA sequences under stringent conditions.

High-density oligonucleotide probe arrays allow DNA and RNA sequence interrogation through hybridization.[1-3] We have replaced adenosine and thymidine with analogs of diaminopurine (D, and D_{OMe}) and 2'-O-methyl-thymidine (T_{OMe}) to enhance RNA sequence hybridization.[4-5] The MeNPOC-phosphoramidites of 2,6-diaminopurine, 2'-O-methyl-diaminopurine and 2'-O-methyl-thymidine were synthesized[4, 6-7] and incorporated into light-directed chemically synthesized probe arrays.[8]

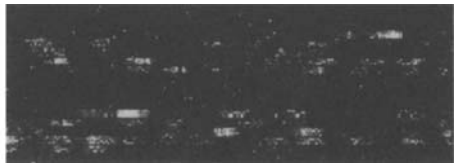
Analog probe arrays were evaluated using GeneChip® test arrays comprised of 14-mer or 20-mer probe sets that represent 1.1 kb of the AT-rich HIV protease and amino terminus of the reverse transcriptase genes.[1, 3] Arrays were hybridized to labeled cRNA fragments for 0.5 h, washed with SSPE and analyzed using scanning confocal fluorescence microscopy. GeneChip® software converts signal to sequence information.

The 20-mer D/T_{OMe} array enhanced signal intensity (I) to all sequences relative to the A/T array but decreased specificity under non-stringent conditions (TABLE 1). Sequence discrimination improved by using stringent conditions (FIG.1) or by reducing probe length (TABLE 2). The 14-mer analog arrays also maintain good discrimination under stringent conditions but conditions are not fully optimized. D and T_{OMe} may be used 20-

TABLE 1. 20-mer Probe Arrays: S and AS-RNA targets

Sense	A/T Array	D/T Array	D/T_{OMe} Array	Conditions	
				Hyb.	Wash
I_{mean}	4034	2023	6068	40 °C	20 °C, 6x
Correct Calls	97.9 %	96.6 %	82.3 %		
AS					
I_{mean}	973	1040	1809	50 °C	30 °C, 3x
Correct Calls	88.6 %	98.9 %	98.6 %		

(a). 20-mer D/T_{OMe} probe array



(b). 20-mer AT probe array

FIG.1. HIV Test Array. Analogs improve hybridization to AT-rich Fl-RNA at 50 °C.

TABLE 2. 14-mer Probe Arrays: AS-RNA Target

Array	I_{mean}	% Calls Correct	Conditions *	
			Hyb.	Wash (SSPE)
A/T	2601	94.4 %	30 °C	20 °C, 6x
A/T	555	61.2 %	50 °C	30 °C, 3x
D/T_{OMe}	2263	96.9 %	50 °C	40 °C, 3x
D_{OMe}/T_{OMe}	2521	93.9 %	50 °C	50 °C, 3x

* Assay conditions not fully optimized. AS-RNA target.

mer arrays under stringent assay conditions, or may be used in shorter probe arrays to improve discrimination, build generic or universal arrays, or reduce manufacturing cost.

REFERENCES

1. Lipshutz, R. J.; Morris, D.; Chee, M.; Hubbell, E.; Kozal, M. J.; Shah, N.; Shen, N.; Yang, R.; Fodor, S. P. A. *Biotechniques*, **1995**, *19*, 442-447.
2. Chronin, M. T.; Fucini, R. V.; Kim, S. M.; Masino, R. S.; Wespi, R. M.; Miyada, C. G. *Hum. Mutat.*, **1996**, *7*, 244-255.
3. Kozal, M. J.; Shah, N.; Shen, N.; Yang, R.; Fucini, R. V.; Merigan, T. C.; Richman, D. D.; Morris, D.; Hubbell, E.; Chee, M.; Gingeras, T. R. *Nat. Med.*,**1996**, 2, 753-759.

4. Gryaznov, D.; Schultz, R. G. *Tetrahedron Lett.*, **1994**, *35*, 2489-2492.
5. Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131-6149.
6. Gao, H.; Fathi, R; Gaffney, B. L.; Goswami, B.; Kung, P.-P.; Rhee, Y.; Jin, R.; Jones, R. A. *J. Org. Chem.* **1992**, *57*, 6954-6959.
7. McGall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. *J. Am. Chem. Soc.* **1997**, *119*, 5081-5090.
8. Pease, A. C.; Solas, D.; Sullivan, E. J.; Cronin, M. T.; Holmes, C. P.; Fodor, S. P. A.; *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 5022-5026.