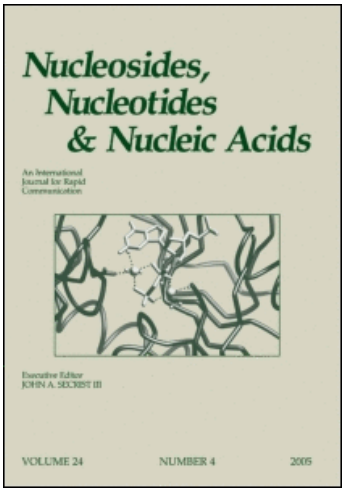


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Detection of Nucleic Acids by Cycling Probe Technology on Magnetic Particles: High Sensitivity and Ease of Separation

Ram Bhatt[a]; Brad Scott[a]; Scott Whitney[a]; Robert N. Bryan[b]; Lynn Cloney[b]; Alexandre Lebedev[a]
[a] ID Biomedical, Inc, San Diego, CA, USA [b] ID Biomedical Corp., Burnaby, BC, Canada

To cite this Article Bhatt, Ram , Scott, Brad , Whitney, Scott , Bryan, Robert N. , Cloney, Lynn and Lebedev, Alexandre(1999) 'Detection of Nucleic Acids by Cycling Probe Technology on Magnetic Particles: High Sensitivity and Ease of Separation', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1297 — 1299
To link to this Article: DOI: 10.1080/07328319908044696
URL: http://dx.doi.org/10.1080/07328319908044696

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1297–1299 (1999)

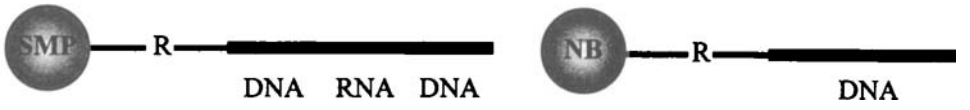
DETECTION OF NUCLEIC ACIDS BY CYCLING PROBE TECHNOLOGY ON MAGNETIC PARTICLES: HIGH SENSITIVITY AND EASE OF SEPARATION

Ram Bhatt*, Brad Scott, Scott Whitney, Robert N. Bryan[Φ], Lynn Cloney[Φ]
and Alexandre Lebedev
ID Biomedical, Inc., 11588 Sorrento Valley Road #16, San Diego, CA, 92121, USA and
[Φ]ID Biomedical Corp., 8855 Northbrook Ct., Burnaby, BC, V5J 5J1, Canada

ABSTRACT

Cycling Probe Technology (CPT) is a signal amplification system that allows detection of nucleic acid target sequences without target amplification. CPT employs a sequence specific chimeric probe, typically DNA-RNA-DNA, which hybridizes to a complementary target DNA sequence and becomes a substrate for RNase H. Cleavage occurs at the RNA internucleotide linkages and results in dissociation of the probe from the target, thereby making it available for the next probe molecule. This communication describes the use of oligonucleotides attached to solid supports for target capture and release followed by solution and solid phase cycling. Through the attachment of chimeric probes to Sera-Mag™ magnetic particles (SMP) a simple and effective method of separating the cleaved probe from non-cycled probe has been developed. By capturing the target DNA on particles and separating it from the extraneous non-specific DNA we are able to dramatically reduce background and thus discriminate between samples of Methicillin Resistant (MRSA) and Methicillin Sensitive (MSSA) Staphylococcus Aureus. We conjugated oligonucleotide probes to SMPs (~1 um) and Nylon beads (NB) which

were coated with ID Biomedical's proprietary coating materials (R, patent pending). The general structure of the constructs is shown below:



SMP = Sera-Mag™ Magnetic Particles NB = Nylon Bead

TABLE 1: Solution Cycling of 29-mer probe with Crude Cell Lysate of MRSA and MSSA after Target Capture and Release

Target/Control	Amount	%Probe Cut*	Signal/C2	Signal/C4
C1	-	2.3	-	-
C2	-	4.0	-	-
MSSA	0.25 ng	0.8	0.2	-
MSSA	2.5 ng	1.2	0.3	-
MSSA	25 ng	3.1	0.8	-
MSSA	250 ng	4.4	1.1	-
MRSA	0.25 ng	2.9	0.7	3.5
MRSA	2.5 ng	16.7	4.2	13.9
MRSA	25 ng	55.4	11.3	17.8
MRSA	250 ng	80.1	20.0	18.2

* Average of duplicates
C1 = Hydrolysis of Probe in Reaction Buffer at 58 °C for 30 min.
C2 = Hydrolysis of Probe with RNase H in Reaction Buffer at 58 °C for 30 min.

We have found that magnetic particles can serve as excellent supports for subattomole detection of target DNA upon cycling with chimeric probes. In addition, by employing target capture to isolate the desired DNA from non-specific sequences we can improve detection of a crude lysate sample of MRSA to 0.25 ng. By contrast, direct solution cycling of chimeric probes does not appreciably discriminate between MRSA and MSSA under numerous experimental conditions using 1 μg of input genomic DNA. The data

TABLE 2: Cleavage of Chimeric Probe on Sera-Mag Particles with Crude Cell Lysates of MRSA and MSSA

Target/Control	Amount	%Probe Cut	Signal/C2	Signal/C4
C1	-	0.1	-	-
C2	-	0.2	-	-
MRSA	200 ng	6.9	34.5	7.6
MRSA	20 ng	1.7	8.5	4.2
MSSA	200 ng	0.9	4.5	-
MSSA	20 ng	0.4	2	-

* Average of duplicates/triplicates

demonstrates that improvements in sensitivity and specificity can be achieved by capturing the target on solid supports, employing a stringent wash, and releasing the target into solution prior to cycling. Following the capture and release protocol, signal to background values of 13.9 and 3.5 were observed for MRSA/MSSA in solution cycling at an input mass of 2.5 ng and 0.25 ng respectively (TABLE 1). When chimeric probes covalently bound to magnetic particles are used for cycling after capture and release of crude lysate DNA, a signal to background (MRSA/MSSA) of 7.6 and 4.2 were obtained at 200 and 20 ng of input DNA respectively (TABLE 2).

Our results indicate that oligonucleotide probes attached to solid supports can provide a rapid way to isolate a desired target and that CPT can be readily performed on magnetic particles for the identification of MRSA in crude cell lysates.