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Title:

Denaturing strategies for detection of double stranded PCR products on GMR magnetic sensors

Authors & affiliations:

Giovanni Rizzi[1], Jung-Rok Lee[2], Per Guldberg[3],Martin Dufva[1], Shan X. Wang[2],Mikkel F. Hansen[1]

1. *DTU Nanotech, Technical University of Denmark, Kgs. Lyngby, Denmark.*
2. *Geballe Laboratory for Advanced Materials, Stanford University, Stanford CA.*
3. *The Danish Cancer Society, Copenhagen, Denmark.*

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Arrays of GMR magnetic field sensors have been demonstrated for the detection of proteins[1] and DNA[2]. The readout is based on the detection of the target-mediated binding of magnetic nanoparticle (MNP) labels to the sensor surface. The assay is insensitive to the sample matrix as there is virtually no detectable magnetic response from biological samples.

Here, we employ the GMR array platform to detect PCR products from melanoma cell lines, with the final goal of profiling mutations of diagnostic relevance[3]. The sensor surface is functionalized with ssDNA probes. The forward PCR primers are biotinylated to facilitate binding to streptavidin coated MNPs. The dsDNA product has to be denatured to enable target binding to the sensor surface. We aim to obtain the highest binding signal, as specificity can be increased by optimizing the stringency condition during washing[4].

In this work, we tested two approaches for the denaturation of PCR products and magnetic labelling:
(1) heat denaturation followed by shock cooling, on-chip hybridization and on-chip labelling with MNPs (**Fig.1a**).
(2) labelling of dsDNA PCR products with MNPs, immobilization of MNPs in a magnetic separation column, denaturation in 6M urea in DI water at 75°C, release of MNPs with ssDNA labels, on-chip detection (**Fig.1b**).

Figure 1c and **Fig.1d** show the GMR signal from experiments using heat denaturation and magnetic column separation, respectively. In **Fig.1c** the binding is faster because we measure the biotin-streptavidin binding, whereas the binding in **Fig.1d** is limited by the DNA hybridisation and diffusion of beads with targets. Both methods offer high signals with small deviations. The denaturation in magnetic column results in slightly higher signal due to the complete removal of reverse complement. Both techniques are viable for detection of PCR products on GMR sensors, the choice will be driven by a trade-off between assay time and signal intensity.

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1-Gaster, Richard S., *et al.*, 2009, *Nature medicine* **15**(11) 1327-1332.
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3-Dahl, Christina, *et al.*, 2013, *Molecular Cancer Research* **11**(10) 1166-1178.
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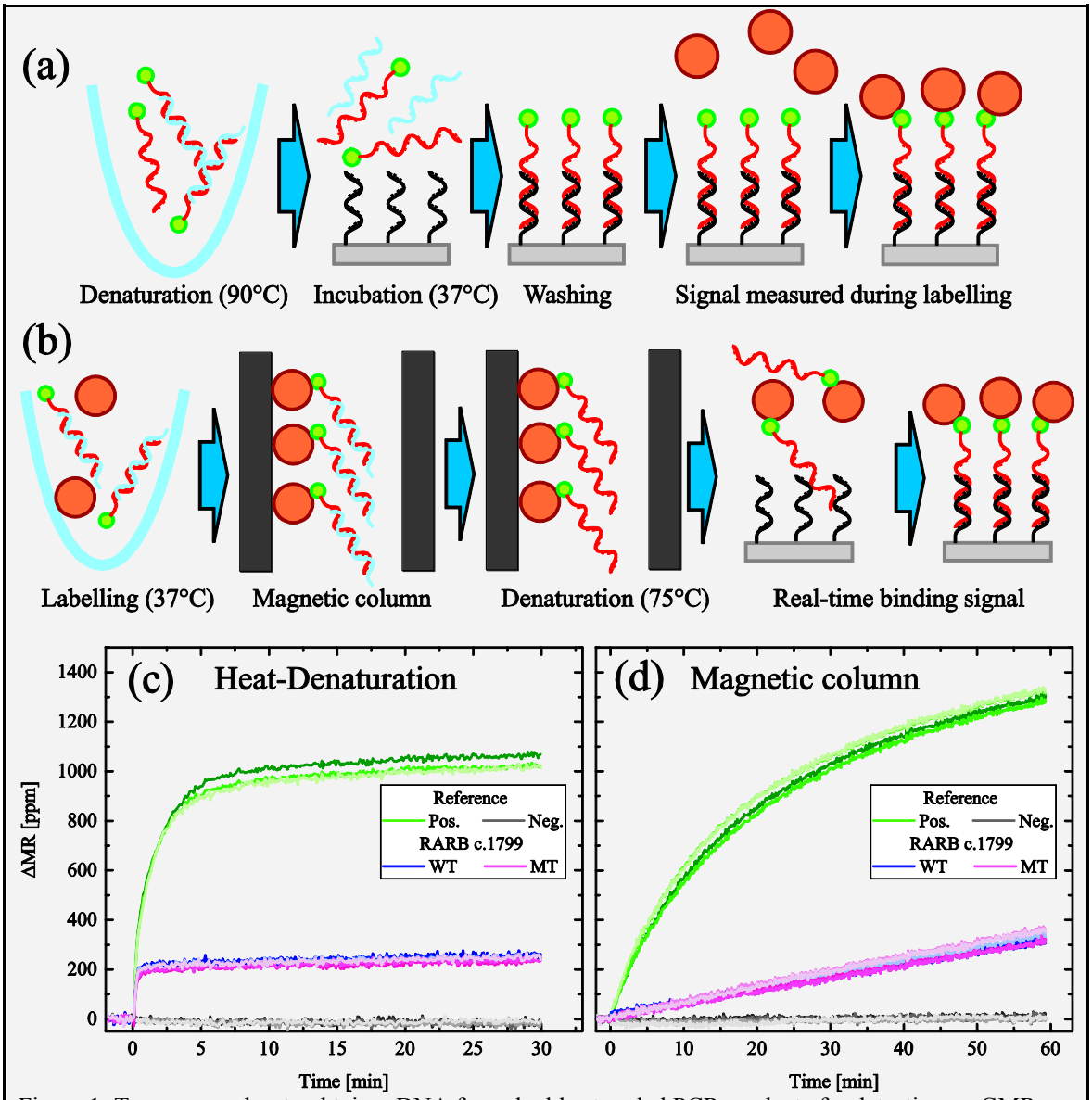

Figure 1: Two approaches to obtain ssDNA from double stranded PCR products for detection on GMR sensors. The forward primers are biotinylated to bind streptavidin-coated magnetic nanoparticles (MNPs). **(a)** Heat denaturation. The sample is heated to 90°C and cooled abruptly to inhibit re-hybridisation. The sample is then incubated on the sensor surface at 37°C for 60 min. After washing, the chip is mounted in the system and the signal is monitored during labelling with MNPs. **(b)** Separation in magnetic column. The sample is mixed with MNPs for labelling (30min) and then immobilized in a magnetic column. The reverse strand is denatured with 6M urea in DI buffer at 75°C. The labelled forward strand is re-suspended in 2xSSC buffer and injected over the sensors. The hybridization is monitored in real-time. **(c)** Signal measured during labelling after heat denaturation. **(d)** Signal measured during hybridization after separation in magnetic column. Four sensors were functionalized with each probe.