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Title:

DNA mutation and methylation analysis for cancer diagnostics using GMR biosensors

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Genetic mutations are useful biomarkers for cancer to assess genetic risk, classify cancer subtype, and to predict and monitor treatment efficacy. Epigenetic modifications are gaining interest since they can complement the mutation information. The combined genetic and epigenetic data have higher diagnostic and prognostic value than the two approaches applied separately for several types of cancer. Here, we present a method to simultaneously genotype mutation and methylation events on giant magnetoresistive (GMR) biosensor arrays. We investigated five mutation sites in *BRAF* and *NRAS* genes and the methylation status of *RARB* and *KIT* promoters. We analysed genetic material from seven human melanoma cell lines from the European Searchable Tumour Line Database to compare our method with traditional techniques.^{1,2}

To genotype mutation, we used a hybridization assay followed by melting curve measurement.³ The GMR biosensor chips (Figure 1a) consisted of 64 single GMR sensors. The surface of each sensor was functionalized with ssDNA probes complementary to the wild type (WT) or mutant type (MT) target sequences. Biotinylated PCR products were labelled with magnetic nanoparticles. Upon hybridization to the surface probes, the nanoparticles were tethered to the sensor surface and their magnetic dipole field quantified by the GMR sensors.⁴ After hybridization, melting curves were measured by ramping up the chip temperature (Figure 1b). The difference in melting temperature between the probes ΔT_m is indicative of the mutation status of the cell line (Figure 2). The threshold values for ΔT_m in Figure 3 were used for genotyping and the results were found to agree with previous studies.^{1,2}

To investigate methylation status, we employed bisulphite conversion to translate a methylation event in a single point mutation. The measured ΔT_m values (Figure 4) were found to correlate linearly with methylation density measurements by bisulphite pyrosequencing. GMR biosensors allowed for simultaneous mutation genotyping and quantitative methylation analysis.

(1) Dahl, C. *et al.* **Mol. Cancer Res.** 2013, **11**, 1166–1178.

(2) Dahl, C. *et al.* **J. Invest. Dermatol.** 2015, **135**, 516–524.

(3) Rizzi, G. *et al.* **ACS Nano.** 2017, **11**, 8864–8870.

(4) Rizzi, G. *et al.* **Biosens. Bioelectron.** 2017, **93**, 155–160.

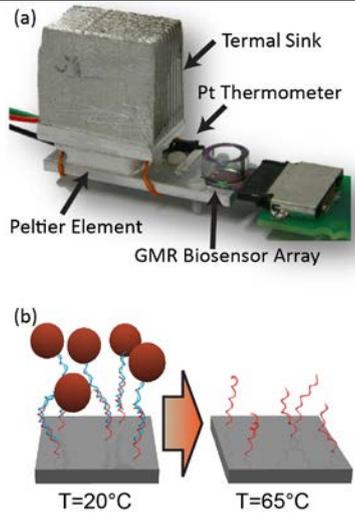


Figure 1: (a) GMR biosensor array mounted in the temperature-controlled chip holder. (b) Schematic of the melting curve assay: ss-DNA PCR products are bound to an allele-specific DNA probe tethered to the surface of the GMR sensor. The target is labelled with streptavidin coated magnetic nanoparticles using biotinylated primers. Upon heating, the DNA hybrids denature causing a decrease of the magnetic signal.

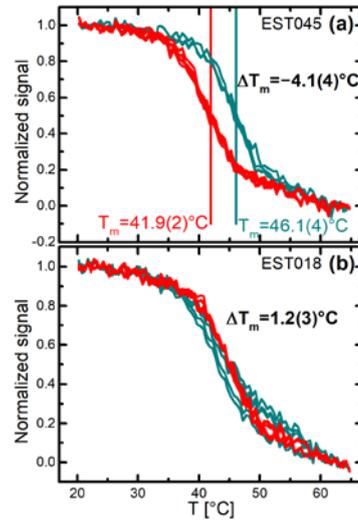


Figure 2: Melting curves measured for WT (blue) and MT (red) probes targeting *BRAF* c.1799T>A single point mutation. Curves measured for (a) wild type EST045 and (b) heterozygous mutant EST018 cell lines. Melting temperature corresponds to halved signal.

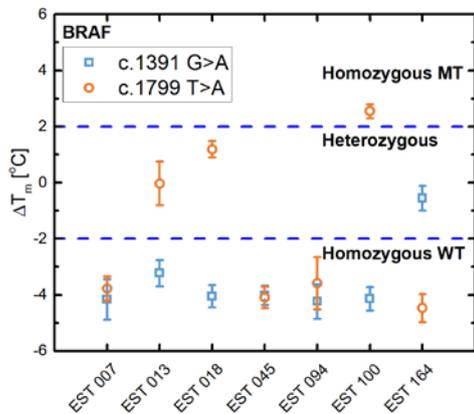


Figure 3: Genotyping of *BRAF* mutations in EST cell lines. ΔT_m was measured from melting curves. Probes for *BRAF* c.1391 G>A and *BRAF* c.1799 T>A mutations were used. Horizontal lines correspond to genotyping threshold values and divide sample in heterozygous WT, homozygous and heterozygous MT. Genotyping results were consistent with traditional methods.

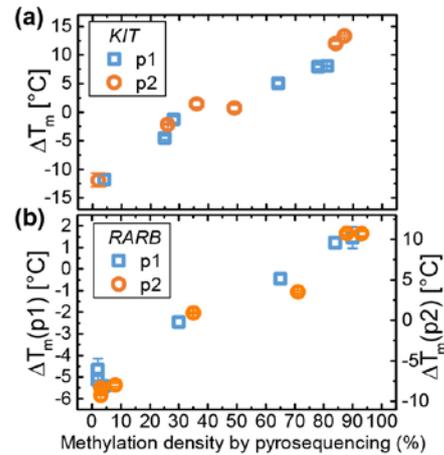


Figure 4: Melting temperature difference measured with methylation DNA probes vs methylation density measured by bisulphite pyrosequencing.³ Methylation level was measured for two locations in both *KIT* (a) and *RARB* (b) promoter regions. ΔT_m correlates linearly with the methylation density.³