

Supporting Information

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Genotyping by Alkaline Dehybridization Using Graphically Encoded Particles

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Figure S1. Retention ratio of each side and Δ are plotted with respect to time for each hybridization case using P3/P1 particles, at increasing temperatures. Note that while the visible difference between the retention ratio curves becomes more difficult to discern at higher temperatures, the peak in Δ remains a reliable indicator.



Figure S2. The hybridization schematic (a) shows the hybridized target strands in the two homozygous experiments, :2 and :3, and the heterozygous experiment, :4. Note that the PM and MM duplexes on either side of bead :4 are a combination of the duplexes on the corresponding sides of beads :2 and :3. In (b), we see the plotted retention ratios of :2, :3 and :4 on the colon side. The heterozygous colon-side signal (:4) can be expressed as a linear combination of the two homozygous colon-side signals (:2 and :3). As shown in Eq. S2.1, we calculate the linear combination using the relative fraction of $R_{:2}$ as the fitting parameter (f₁). Plotting this fitted colon-side data over the original colon-side :4 data (c) shows an extremely good fit (R²=0.9996). A similar procedure on the label side (d; Eq. S2.2) also fits extremely well (R²=0.9998). Subtracting the fitted label side data from the fitted colon side data (Eq. S2.3) produces a delta curve (e) which closely reproduces (R²=0.9316) the $\Delta_{:4}$ calculated from the experimental data

$$R_{:4, \, label}^{fit} = f_2 R_{:2, \, label} + (1 - f_2) R_{:3, \, label} \qquad Eq (S2.2)$$



Figure S3. a) The calculated values for the [OH⁻] and pH during the first thousand seconds of a dynamic alkaline dehybridization experiment. b) The programmed injection rate of the 0.02 M NaOH solution; injection rate of water is constant at 20 μ L/min.

 Table S1. DNA sequences used in sandwich assay

DNA	Sequences
MTHFR 1 st wild- type probe	5'-/Acryd/ ATG AAA TCG GCT CCC GCA GAC/-3'
MTHFR 1 st mutated probe	5'-/Acryd/ ATG AAA TCG <u>A</u> CT CCC GCA GAC/-3'
MTHFR wide-type target	5'-/GAA GCA GGG AGC TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG AAG GTG TCT GCG GGA GCC GAT TTC AT/-3'
MTHFR mutated target	5'-/GAA GCA GGG AGC TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG AAG GTG TCT GCG GGA G <u>T</u> C GAT TTC AT/-3'
MTHFR 2 nd probe	5'-/CAA GTG CTT CAG GTC AGC CTC AAA GCT CCC TGC TTC- Alexa488/-3'
Factor II 1 st wild- type probe	5'-/Acryd/ CTC AGC GAG CCT CAA TGC TCC/-3'
Factor II 1 st mutated probe	5'-/Acryd/CTC AGC <u>A</u> AG CCT CAA TGC TCC/-3'
Factor II wide-type target	5'-/TAG TAT TAC TGG CTC TTC CTG AGC CCA GAG AGC TGC CCA TGA ATA GCA CTG GGA GCA TTG AGG CTC GCT GAG/-3'
Factor II mutated target	5'-/TAG TAT TAC TGG CTC TTC CTG AGC CCA GAG AGC TGC CCA TGA ATA GCA CTG GGA GCA TTG AGG CT <u>T</u> GCT GAG/-3'
Factor II 1 st 2 nd probe	5'-/CTC TGG GCT CAG GAA GAG CCA GTA ATA CTA/Alexa488/-3'
Factor V 1 st wild- type probe	5'-/Acryd/ TGG ACA GGC GAG GAA TAC AGG/-3'
Factor V 1 st mutated probe	5'-/Acryd/TGG ACA GGC <u>A</u> AG GAA TAC AGG/-3'
Factor V wide-type target	5'-/GAA GAA ATT CTC AGA ATT TCT GAA AGG TTA CTT CAA GGA CAA AAT ACC TGT ATT CCT CGC CTG TCC A/-3'
Factor V mutated target	5'-GAA GAA ATT CTC AGA ATT TCT GAA AGG TTA CTT CAA GGA CAA AAT ACC TGT ATT CCT <u>T</u> GC CTG TCC A/-3'
Factor V 2 nd probe	5'-/TAA CCT TTC AGA AAT TCT GAG AAT TTC TTC/Alexa488/-3'