Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Rapid typing of STRs in the human genome by HyBeacon melting

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Supporting Information

Table S1 Oligonucleotide sequences in paper and supporting information.

5 = fluorescein dT, **P =** phosphate, **Y** = 5' trimethoxystilbene, HEG = hexaethylene glycol

Oligo ID	Sequence
D16FL1	TATCTATC**5**ATCTATC**5**ATCTATC**5**ATCTATCGCCGC**P**
PRIM1	GATCCCAAGCTCTTCCTCTT
PRIM2	ACGTTTGTGTGTGCATCTGTAAGCATGTATC
D16BS4	GCGGC$(TATC)_4$CACCTGTCTGTCTGTCTGTA-HEG-GATCCCAAGCTCTTCCTCTT
D16BS4b	GCGGC$(TATC)_4$CACCTGTCTGTCTGTCTGTA**P**
D16BS5	GCGGC$(TATC)_5$CACCTGTCTGTCTGTCTGTA-HEG-GATCCCAAGCTCTTCCTCTT
D16BS5b	GCGGC$(TATC)_5$CACCTGTCTGTCTGTCTGTA**P**
HYBTH01	**Y**TGGFGAATGAAFGAATGAATGAATGAATGA**P**
TH01F	GGCTTCCGAGTGCAGGTCA
TH01R	GGTGATTCCCATTGGCCTG
TH01UBL2.1	GAATGAATGAGGGAAATAAGGGAGGAAC-HEG-GGTGATTCCCATTGGCCTG
THO1BL2.1	GAATGAATGAGGGAAATAAGGGAGGAAC**P**

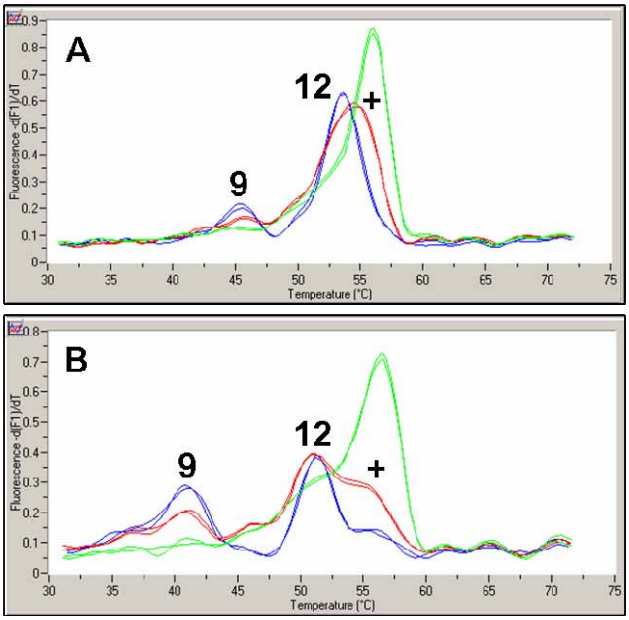

Figure S1 Comparison of unimolecular and bimolecular blocking strategies using the D16FL1 (75nM) probe to analyze an extracted DNA samples possessing 9 and 12 repeat D16S539 alleles. Blue is 500nM unimolecular, red is the bimolecular system at 500nM of blocker and green is a bimolecular at 250nM blocker. **A)** Analysis performed with unimolecular (D16BS4) and bimolecular (D16BS4b) blockers possessing four repeats of d(TATC). **B)** Analysis performed with unimolecular (D16BS5) and bimolecular (D16BS5b) blockers possessing five repeats of d(TATC). Assays using 500nM (~6:1; blocker:probe) of unimolecular blocker (blue) generate clear 9 and 12 repeat melting peaks. However, when using 500nM of the bimolecular blockers (red) the height of the 9 repeat peak is reduced. With the bimolecular blocker full-length probe hybridization to inappropriate target repeats occurs, yielding broad peaks or generating additional peak data (+). Using 250nM (~3:1; blocker:probe) of bimolecular blocker (green) is not sufficient to prevent probe hybridisation to inappropriate target repeats, such that 9 and 12 repeat target alleles are not detected.

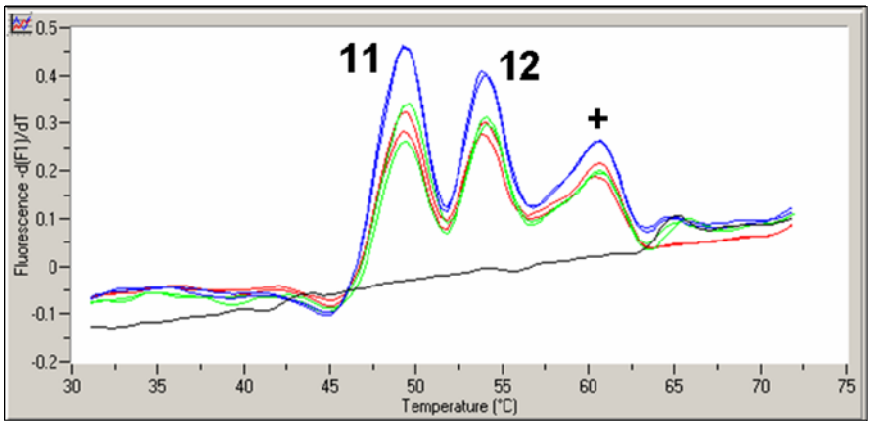

Figure S2 Analysis of the D16S539 locus using the D16FL4 probe and D16BL7 blocker (unimolecular approach without mini-duplex between primer-probe and blocker). Purified DNA (blue), unpurified saliva (red) and an unpurified buccal swab (green) yield melting peaks of comparable height and quality, efficiently detecting 11 and 12 repeat alleles. An additional higher Tm peak (+) was generated through full-length probe hybridization to unblocked target repeats. This artifact peak can be greatly reduced by incorporation a short region of complementarity between the primer-probe and the blocker (mini-duplex). A no template control is included for comparison (black).

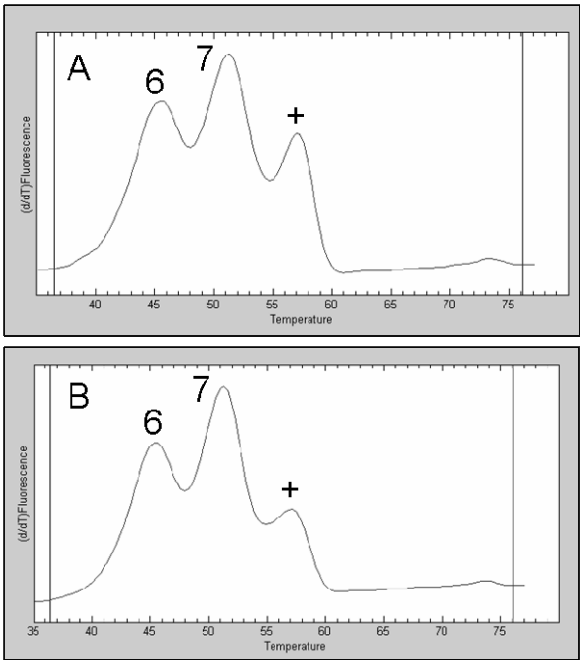

Figure S3 Comparison of unimolecular (TH01UBL2.1) and bimolecular (TH01BL2.1) blocking strategies for the analysis of the TH01 locus using the HYBTH01 probe. Amplification and melting curve analysis of target sequences was performed with a 384-well Tetrad thermal cycler (MJ Research) and a LightTyper® instrument as described previously (Forensic Science International in Press). **A)** Analysis of a purified DNA sample using the bimolecular blocker TH01BL2.1 generates clear melting peaks with 6 and 7 repeat alleles. Full length probe hybridization to unblocked target repeats generates an additional melting peak (+). **B)** The unimolecular TH01UBL2.1 oligonucleotide increases the blocking efficiency and clearly reduces the height of the + melting peak, even though this unimolecular example does not have a mini-duplex between the primer-probe and the blocker.

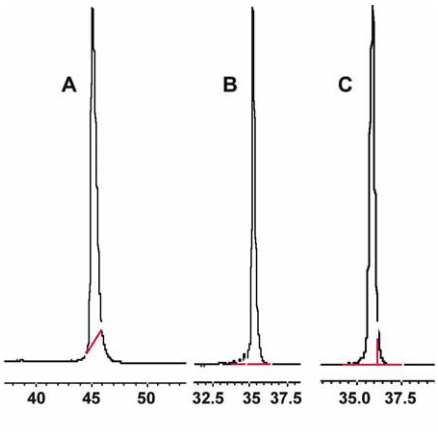

Figure S4 Capillary electrophoresis of representative oligonucleotides. A is the 74-mer PCR primer-blocker D16BS7, B is 37-mer fluorescent HyBeacon® probe D16FL1 containing 3 x FAM dT, and C is 45-mer bimolecular blocker D16BS5b.