Electronic Supplementary Information

Sequence selective capture, release and analysis of DNA using a magnetic microbead-assisted toehold-mediated DNA strand displacement reaction

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Experimental Section

All ss-oligonucleotides were purchased from IDT DNA Technology, USA.

Magnetic beads immobilisation with ss-oligonucleotide capture probes

Immobilisation of the capture probes (Table S1) on the magnetic beads was performed using a BcMagQuick Oligo-DNA conjugation kit (BioClone Inc., USA), strictly following the manufacturer's recommendations.

DNA extraction and amplification

Human male and female genomic DNA was isolated from the authors' own blood samples with a QIAamp DNA blood mini kit (Qiagen, Germany), according to the recommendations of the manufacturer. PCR was performed using Q5 Hot Start Polymerase (NEB, USA) with a final primer (Table S1) concentration of 0.2 μM each. As a template 5 ng of the human male genomic DNA was used. A PCR amplification regime of 98 ^{o}C for 30 s, 30 cycles of 98 ^{o}C for 10 s, 67 ^{o}C for 10 s, 72 ^{o}C for 20 s, and a final elongation of 72 ^{o}C for 1 min was used.
Melting temperatures were calculated using an Oligo Analyser 3.1 (IDT DNA Technology, USA) under the following conditions: an oligonucleotide concentration of 0.2 μM, a Na^{+} concentration of 100 mM, a Mg^{2+} concentration of 2 mM and a deoxyribonucleotide triphosphates (dNTPs) concentration of 0.8 mM (total).

Table S1. Sequences and melting temperatures of capture probes, displacing oligonucleotides and primers.

	Sequence	Tm, ^{o}C
	Capture Probes	
AMEL-CP	5'- **GT GAT GGC** *TCT GTA AAG AAT AGT* TTT TTT TTT TTT TTT TTT TTT-Amino-3	47.5*
CSF1PO-CP	5'-**TG CCA TCG** *GAG GTA AAC TAG TCT TAA* TTT TTT TTT TTT TTT TTT TTT-Amino-3'	53.5*
	Displacing Sequences	
AMEL-Disp	5'-*ACT ATT CTT TAC AGA* **GCC ATC AC**-3'	
SCF1PO-Disp	5'-*TTA AGA CTA GTT TAC CTC* **CGA TGG CA**-3'	
	PCR primers	
AMEL-F-looped	5'-*CT ATT CTT TAC AGA* -/PEG-linker/-<u>CCC TGG GCT CTG TAA AGA ATA GTG</u>-3'	65.3 (53.2**)
AMEL-R	5'-FAM-ATC AGA GCT TAA ACT GGG AAG CT-3'	65.1
CSF1PO-F-looped	5'-*TTA AGA CTA GTT TAC CTC* - /PEG-linker/ - <u>CCG GAG GTA AAG GTG TCT TAA AGT</u>-3'	65.1 (56**)
CSF1PO -R	5'-FAM-ATT TCC TGT GTC AGA CCC TGT T-3'	66.2

*Melting temperatures for the hybridisation sequence of the capture probes were calculated using a Na^{+} concentration of 10 mM and a Mg^{2+} concentration of 11.5 mM. **Melting temperature of the intramolecular duplex. The primer's main sequence is underlined. The toehold forming sequences are in bold. Hybridisation sequences in the capture probes, modified forward primers and displacing sequences are in italics.

Hybridisation of the ss-ODN tagged ds-PCR products with magnetic bead immobilised capture probes

PCR amplification reaction mixture (25 μL) containing CSF1PO and AMEL ss-ODN tagged ds-PCR products, 4 × hybridisation buffer (4 M guanidine thiocyanate, 20 mM HEPES, pH 7.5, and 20 mM EDTA[1], 25 μL), water based suspension of CSF1PO capture probe immobilised magnetic beads (10 mg/mL, 25 μL) and water based suspension of AMEL capture probe immobilised magnetic beads (10 mg/mL, 25 μL) were mixed together and incubated for 4 h at 37 ^{o}C. After hybridisation, the magnetic beads were washed 3 times with a washing buffer (6.5×SSPE buffer pH 7.4, 0.01% Tween 20, 50 μL) and twice with water (50 μL).

Sequence selective release of the hybridised ds-PCR products

Sequence selective release of the captured hybridised ds-PCR products from the magnetic

beads (0.1 mg/mL water based suspension, 10 µL) was achieved by incubation of the beads in a solution (20 µL) consisting of a displacing sequence (1 µM final concentration, Table S1) and TEM buffer (final concentration of 10 mM Tris pH 8.0, 1 mM EDTA, 12.5 mM $MgCl_2$) at 30 ^{o}C.

Capillary electrophoresis

Capillary electrophoresis (CE) analysis was carried out on a ABI-3130XL Genetic Analyzer (Life Technologies, USA), using a GeneScan 500 LIZ size standard (Life Technologies, USA).

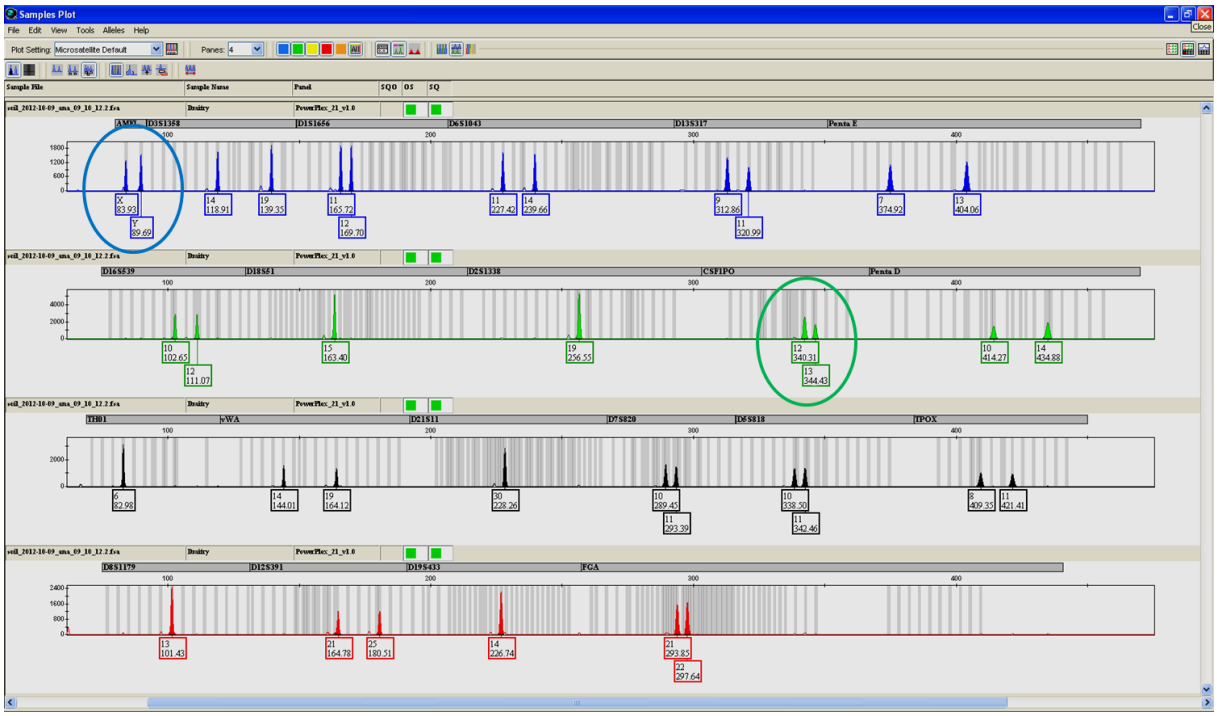

Fig. S1. PowerPlex® 21 System (Promega) genetic profile of the human male genomic DNA used in the research. AMEL and CSF1PO loci profiles are shown in blue and green circles, respectively.

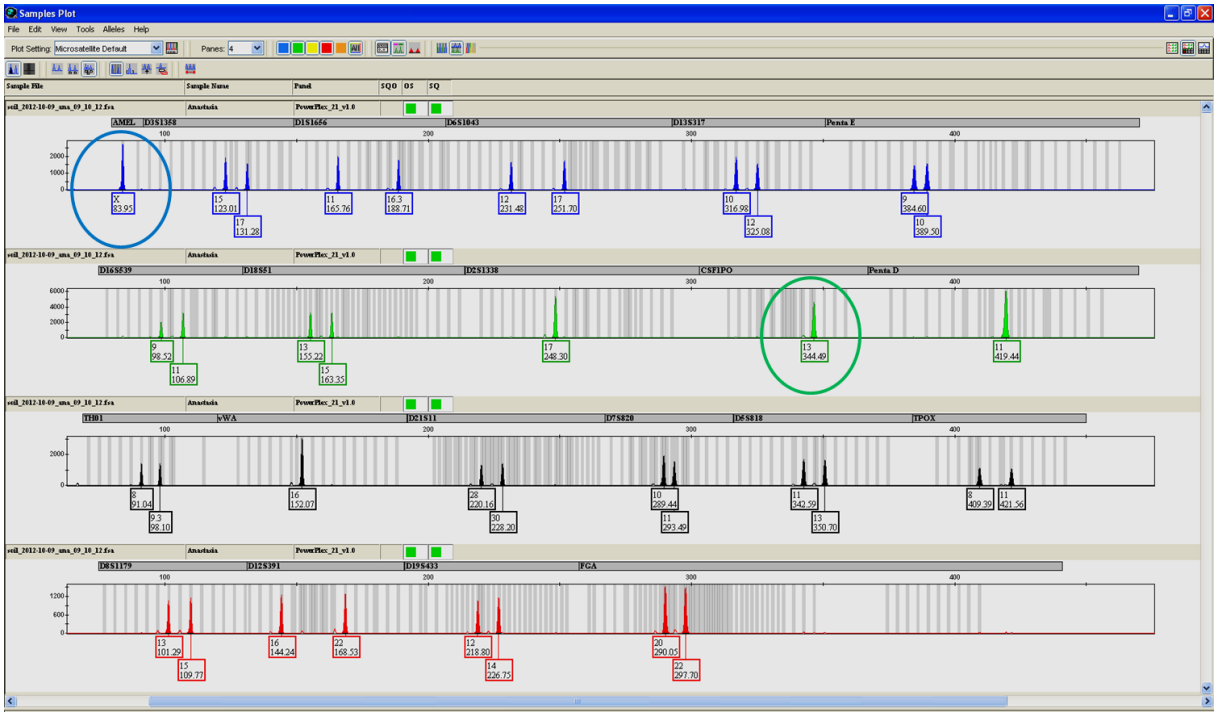

Fig. S2. PowerPlex® 21 System (Promega) genetic profile of the human female genomic DNA used in the research. AMEL and CSF1PO loci profiles are shown in blue and green circles, respectively.

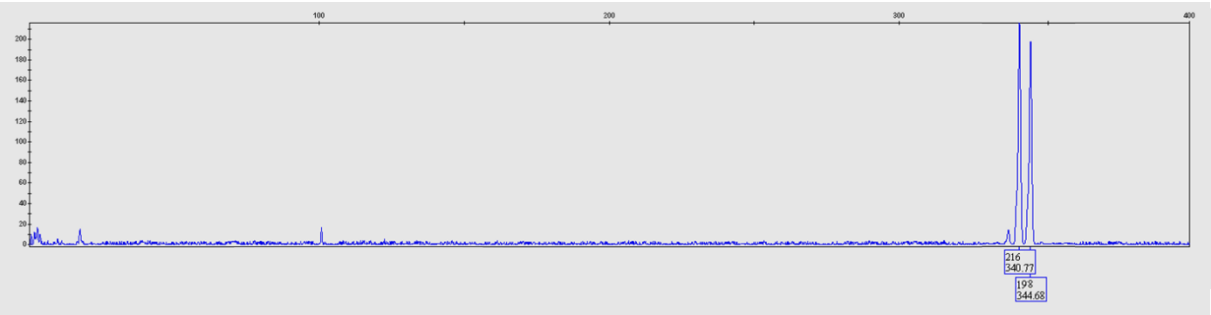

Fig. S3. CE electropherogram of the displacement solution containing a CSF1PO displacing sequence (1 µM) and 1×TEM buffer (10 mM Tris pH 8.0, 1 mM EDTA, 12.5 mM $MgCl_2$). Sequence specific release from the bead mixture washed after hybridisation with 1×TEM was performed for 6 h at 30 ^{o}C. The electropherogram shows two peaks corresponding to the CSF1PO PCR amplification products with intensities of 216 and 198 RFU.

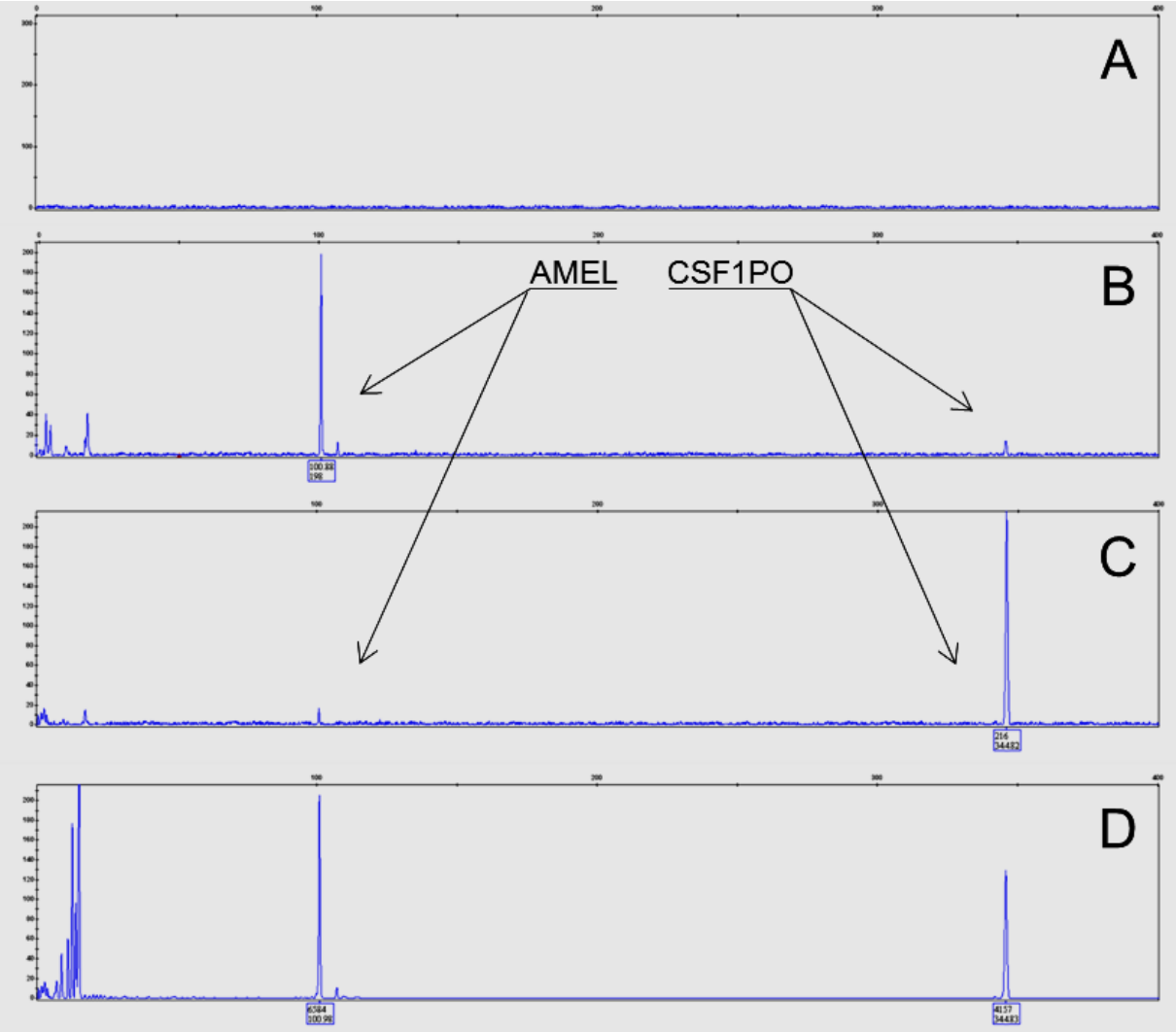

Fig. S4. CE electropherograms of the sequence selective release of ds-PCR products (generated from female DNA) dehybridised from magnetic beads using a toehold strand displacement reaction in the presence of (A) no displacing sequence, (B) an AMEL displacing sequence, (C) a CSF1PO displacing sequence and (D) both AMEL and CSF1PO displacing sequences

References

1. V. A Vasiliskov, A. V Chudinov, V. R. Chechetkin, S. A Surzhikov, A. S. Zasedatelev, and V. M. Mikhailovich, *Journal of Biomolecular Structure & Dynamics*, 2009, **27**, 347.