

# SILICON $\mu$ PCR CHIP FOR FORENSIC STR PROFILING WITH HYBEACON

## PROBE MELTING CURVES

### AUTHORS

Senne Cornelis<sup>1,2</sup>, Olivier Tytgat<sup>1,2</sup>, Maarten Fauvart<sup>2</sup>, Yannick Gansemans<sup>1</sup>, Ann-Sophie Vander Plaetsen<sup>1</sup>, Rodrigo S Wiederkehr<sup>2</sup>, Dieter Deforce<sup>1,#,\*</sup>, Filip Van Nieuwerburgh<sup>1,#</sup>, Tim Stakenborg<sup>2,#</sup>

<sup>1</sup> Laboratory of Pharmaceutical Biotechnology, Ghent University, 9000 Gent, Belgium

<sup>2</sup> Department of Life Science Technologies, Imec, 3001 Leuven, Belgium.

# Contributed equally

\* Corresponding author at: Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Gent, Belgium.

Tel: +32 (0)9 264 80 48

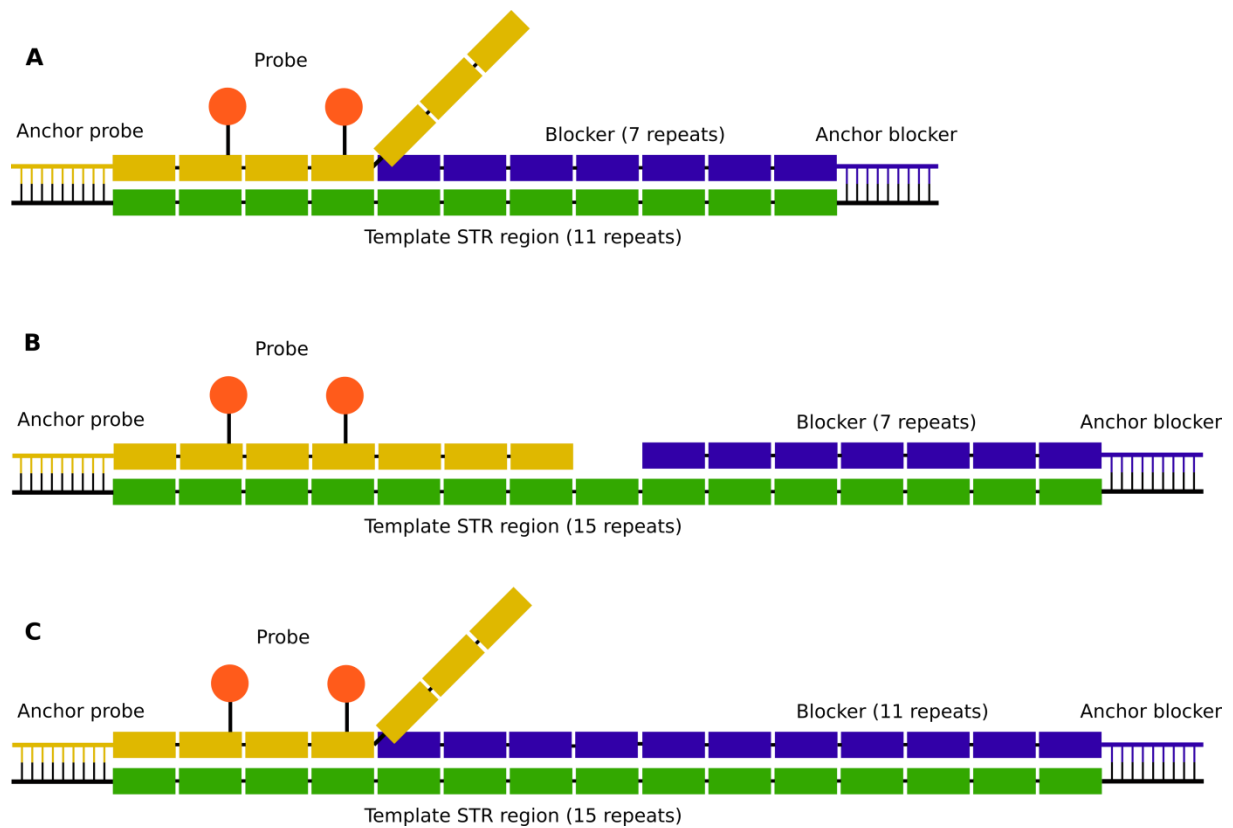
### DECLARATION OF INTEREST

None

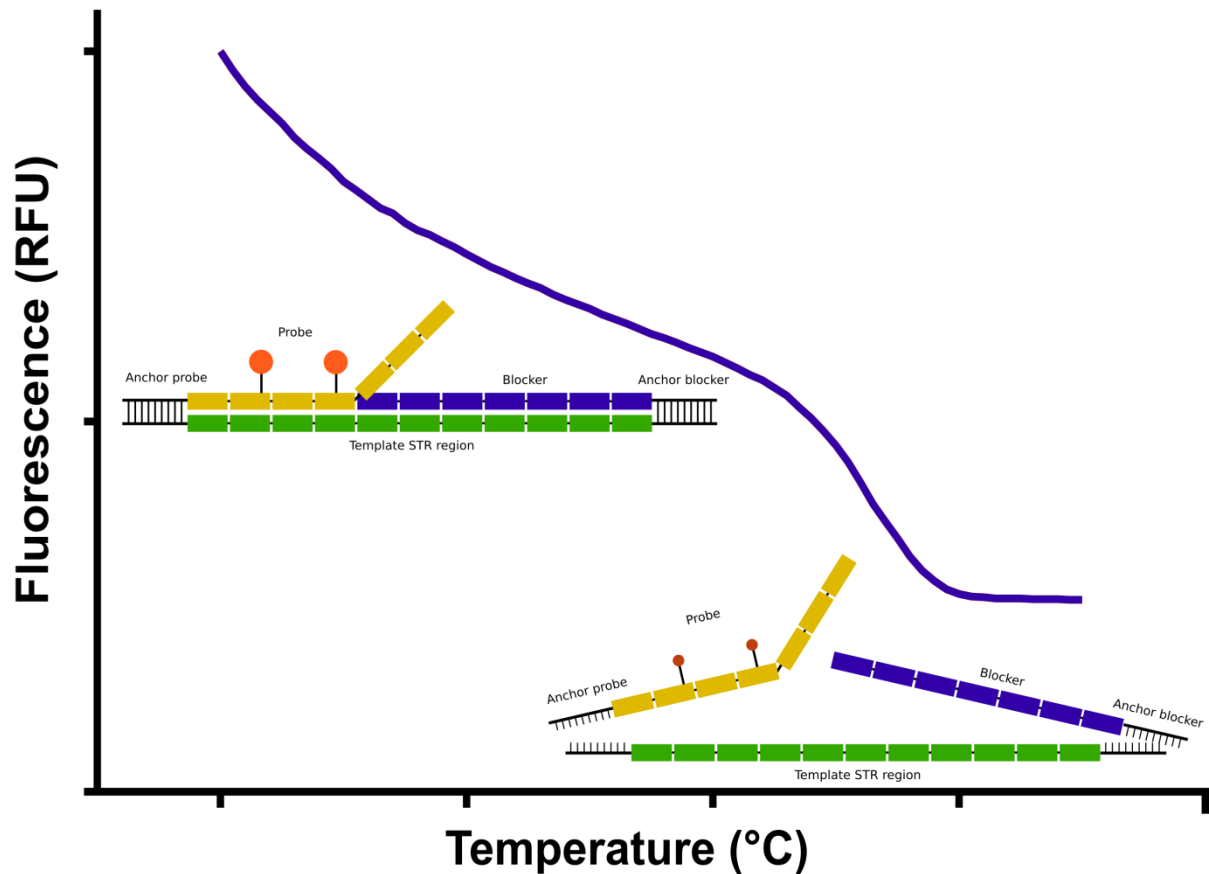
### EMAIL ADDRESSES

|                             |                                                                                            |
|-----------------------------|--------------------------------------------------------------------------------------------|
| Senne Cornelis:             | <a href="mailto:Senne.Cornelis@UGent.be">Senne.Cornelis@UGent.be</a> ,                     |
| Olivier Tytgat:             | <a href="mailto:Oitytgat.Tytgat@UGent.be">Oitytgat.Tytgat@UGent.be</a> ,                   |
| Maarten Fauvart:            | <a href="mailto:Maarten.Fauvart@imec.be">Maarten.Fauvart@imec.be</a> ,                     |
| Yannick Gansemans:          | <a href="mailto:Yannick.Gansemans@UGent.be">Yannick.Gansemans@UGent.be</a> ,               |
| Ann-Sophie Vander Plaetsen: | <a href="mailto:AnnSophie.VanderPlaetsen@UGent.be">AnnSophie.VanderPlaetsen@UGent.be</a> , |
| Rodrigo Sergio Wiederkehr:  | <a href="mailto:Rodrigo.Sergio.Wiederkehr@imec.be">Rodrigo.Sergio.Wiederkehr@imec.be</a> , |
| Dieter Deforce:             | <a href="mailto:Dieter.Deforce@UGent.be">Dieter.Deforce@UGent.be</a> ,                     |
| Tim Stakenborg:             | <a href="mailto:Tim.Stakenborg@imec.be">Tim.Stakenborg@imec.be</a> ,                       |
| Filip Van Nieuwerburgh:     | <a href="mailto:Filip.VanNieuwerburgh@UGent.be">Filip.VanNieuwerburgh@UGent.be</a>         |

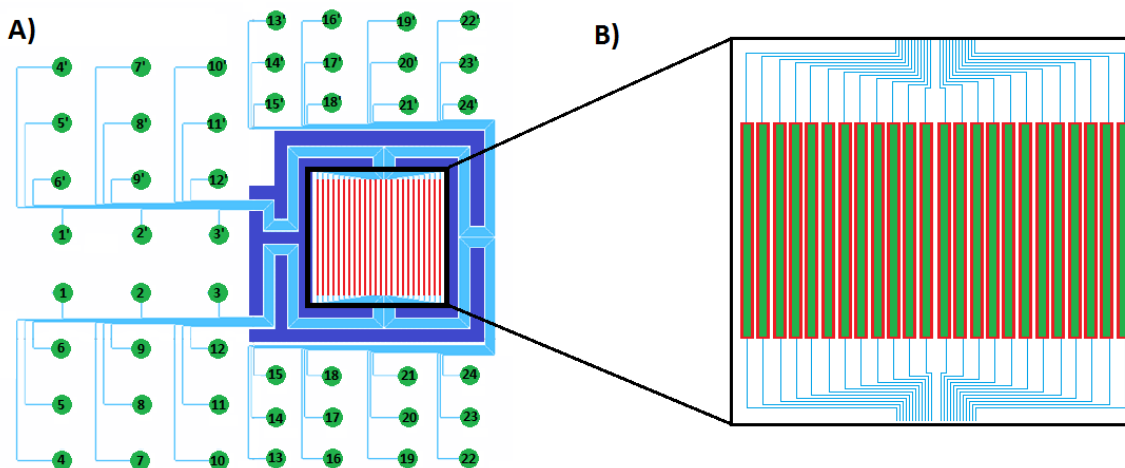
## SUPPLEMENTARY INFORMATION



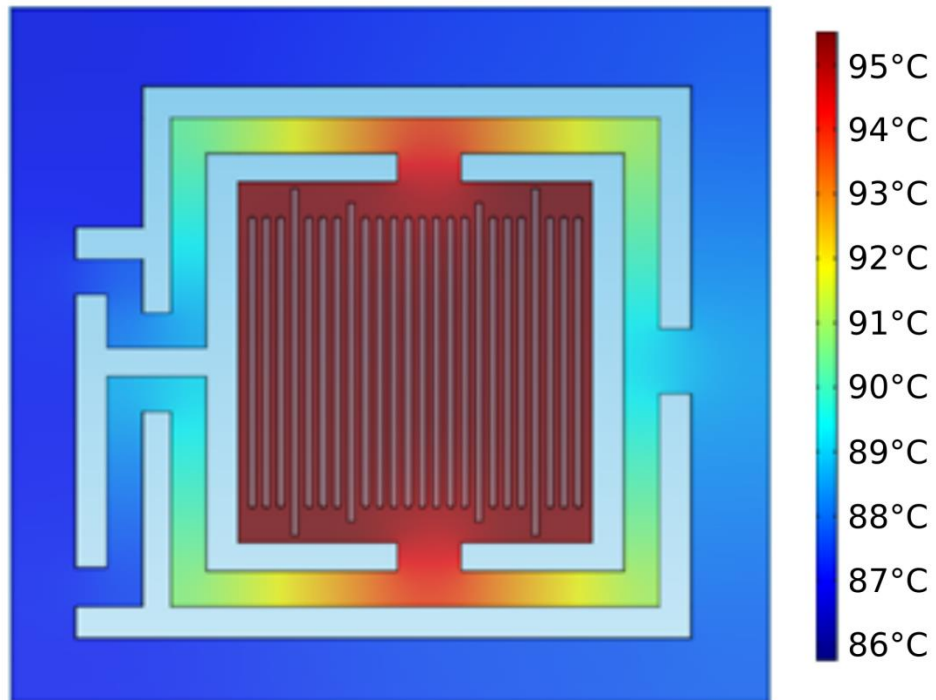
**Supplementary Figure 1:** Schematic overview of the HyBeacon system with a fluorescent HyBeacon probe and non-fluorescent blocker oligonucleotide. Probe, blocker and target sequence repeats are represented by yellow, blue and green boxes, respectively. Both probe and blocker oligonucleotides possess anchor regions that hybridize to non-repetitive flanking sequences to prevent slippage [19]. **A)** STR target region comprising of 11 repeat units partially blocked by a 7 repeat blocker and bound to the probe. **B)** STR region comprising of 15 repeat units in combination with a 7 repeat blocker results in full probe hybridization which makes discrimination with a 14 repeat STR region unfeasible. **C)** A blocker of 11 repeat blocks a larger fraction of the 15 repeat target sequence which allows partial probe hybridization and identification of the 15 STR region.



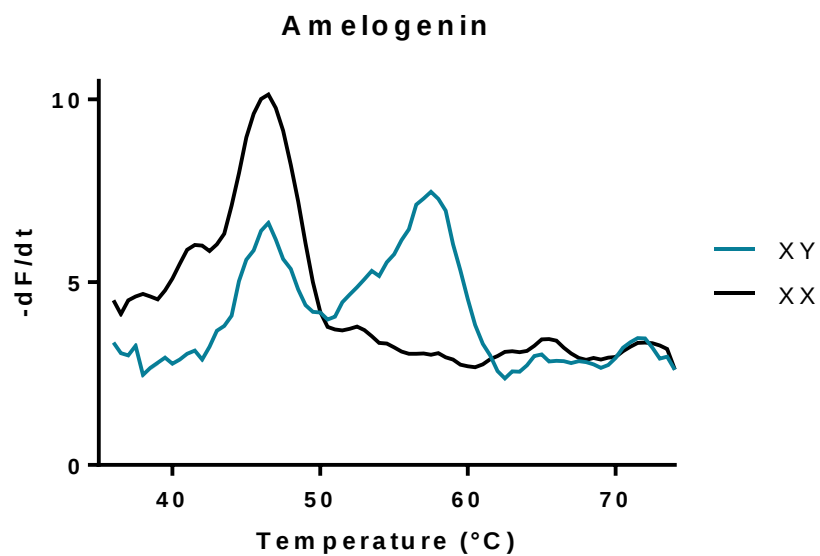
**Supplementary Figure 2:** Schematic overview of the working mechanism of a fluorescent HyBeacon probe and blocker oligonucleotide. Probe, blocker and target sequence repeats are represented by yellow, blue and green boxes, respectively. Both probe and blocker oligonucleotides possess anchor regions that hybridize to non-repetitive flanking sequences to prevent slippage [19]. In hybridized state no quenching of the probes' fluorophores is experienced and high fluorescence intensity is observed. Upon heating denaturation of the probe is favored resulting in a quenching effect which reduces the fluorescence intensity.



**Supplementary Figure 3:** **A)** Schematic overview of the 24 reaction cavity chip with, inlet and outlet holes (green), microfluidic channels (light blue) from and to the reaction cavities (red) and the air trenches etched in silicon (dark blue). The inlet and outlet holes are numbered from 1-24 according to the position of reaction chambers from left to right. **B)** A close-up of the 24 parallel reaction cavities and the microfluidic inlet and outlet channels leading to them.



**Supplementary Figure 4:** Numerical modeling was performed to estimate the thermal characteristics of the novel chip design accommodating 24 PCR cavities with a total heated zone of 5 mm by 5 mm. 93.05 % of the heated zone was within  $\pm 0.3^\circ\text{C}$  of the set temperature.



**Supplementary Figure 5:** Melting peaks generated using the amelogenin assay of a male (XY) and female (XX) sample.

| Name (D18S51)  | Sequence                                        | Concentration |
|----------------|-------------------------------------------------|---------------|
| Forward primer | TGCCACTGCACTTCACTCTGA                           | 1 $\mu$ M     |
| Reverse primer | GTGTGGAGATGTCTTACAATAACAGTTG                    | 0.1 $\mu$ M   |
| HyBeacon probe | TTCTTTCTTTCTTTCTTTCTTTCTT <b>F</b> CTTTCFGAGACP | 0.150 $\mu$ M |
| D18 Blocker 7  | CTTTCCTCTCTCTTT(TTCT) <sub>7</sub> P            | 0.225 $\mu$ M |
| D18 Blocker 10 | CTTTCCTCTCTCTTT(TTCT) <sub>10</sub> P           | 0.225 $\mu$ M |
| D18 Blocker 14 | CTTTCCTCTCTCTTT(TTCT) <sub>14</sub> P           | 0.225 $\mu$ M |

**Supplementary Table 1:** Sequences of Probes, Primers and Blockers of the D18S51 locus assay and their respective concentrations. F: Internal fluorescein dT, P: 3' Phosphate cap.

| Name (D8S1179) | Sequence                                                 | Concentration |
|----------------|----------------------------------------------------------|---------------|
| Forward primer | CGGCCTGGCAACTTATATGT                                     | 0.1 $\mu$ M   |
| Reverse primer | GCCTTAATTTATTACCTATCCTGTAGA                              | 1 $\mu$ M     |
| HyBeacon probe | TCTATCTATCTATCTATCTATCTAT <b>F</b> ATCTA <b>F</b> TCCCCP | 0.075 $\mu$ M |
| D8 Blocker 5   | GTATTTCACTGTGTACATTCGTA(TCTA) <sub>5</sub> P             | 0.375 $\mu$ M |
| D8 Blocker 8   | GTGTACATTCGTA(TCTA) <sub>8</sub> P                       | 0.375 $\mu$ M |
| D8 Blocker 11  | GTGTACATTCGTA(TCTA) <sub>11</sub> P                      | 0.375 $\mu$ M |

**Supplementary Table 2:** Sequences of Probes, Primers and Blockers of the D8S1179 locus assay and their respective concentrations. F: Internal fluorescein dT, P: 3' Phosphate cap.

| Name (TH01)      | Sequence                                          | Concentration |
|------------------|---------------------------------------------------|---------------|
| Forward primer   | GGCTTCCGAGTGCAGGTCA                               | 0.1 $\mu$ M   |
| Reverse primer   | GGTGATTCCCATTGGCCTG                               | 1 $\mu$ M     |
| HyBeacon probe   | TGG <b>F</b> GAATGAA <b>F</b> GAATGAATGAATGAATGAP | 0.075 $\mu$ M |
| TH01 Blocker 3.3 | ATGAATGAATGAATGAGGGAAATAAGGGP                     | 0.375 $\mu$ M |
| TH01 Blocker 2.1 | GAATGAATGAGGGAAATAAGGGAGGAACP                     | 0.375 $\mu$ M |

**Supplementary Table 3:** Sequences of Probes, Primers and Blockers of the TH01 locus assay and their respective concentrations. F: Internal fluorescein dT, P: 3' Phosphate cap.

| Name (D16S539)                | Sequence                                                              | Concentration |
|-------------------------------|-----------------------------------------------------------------------|---------------|
| Forward primer /<br>Blocker 4 | GCGGC(TATC) <sub>4</sub> CACCTGTCTGTCTGTCTGTA-HEG-GATCCCAAGCTCTTCTCTT | 1 $\mu$ M     |
| Forward primer /<br>Blocker 6 | GCGGC(TATC) <sub>6</sub> CACCTGTCTGTCTGTCTGTA-HEG-GATCCCAAGCTCTTCTCTT | 1 $\mu$ M     |
| Reverse primer                | ACGTTTGTGTGTGCATCTGTAAGCATGTATC                                       | 0.1 $\mu$ M   |
| HyBeacon probe                | TATCTAT <b>F</b> CTAT <b>F</b> ATCTAT <b>F</b> ATCTATCGCCGCP          | 0.075 $\mu$ M |

**Supplementary Table 4:** Sequences of Probes, Primers and Blockers of the D16S539 locus assay and their respective concentrations. F: Internal fluorescein dT, P: 3' Phosphate cap, HEG: Hexaethylene glycol spacer.

| Name (Amelogenin) | Sequence                           | Concentration |
|-------------------|------------------------------------|---------------|
| Forward primer    | CCCTGGGCTCTGTAAAGAA                | 1 $\mu$ M     |
| Reverse primer    | ATCAGAGCTTAAACTGGGAAGCTG           | 0.1 $\mu$ M   |
| HyBeacon probe    | TCAAGTGG <b>F</b> CCCAATTTTACAGTTP | 0.075 $\mu$ M |

**Supplementary Table 5:** Sequences of Probes, Primers and Blockers of the Amelogenine locus assay and their respective concentrations. F: Internal fluorescein dT, P: 3' Phosphate cap

|            | 9947    | 9948    | 2800    |
|------------|---------|---------|---------|
| D8S1179    | 13 : 13 | 12 : 13 | 14 : 15 |
| D16S539    | 11 : 12 | 11 : 11 | 9 : 13  |
| D18S51     | 15 : 19 | 15 : 18 | 16 : 18 |
| TH01       | 8 : 9.3 | 6 : 9.3 | 6 : 9.3 |
| Amelogenin | X : X   | X : Y   | X : Y   |

**Supplementary Table 7.6:** STR profiles based on the examined loci of three reference samples (9947, 9948 and 2800)

| D18S51     | Allele | Tm 1<br>(°C) | Tm 2<br>(°C) | Tm 3<br>(°C) | Average Tm<br>(°C) | SD   |
|------------|--------|--------------|--------------|--------------|--------------------|------|
| Blocker 7  | 11     | 48,70        | 47,77        | 48,19        | 48,22              | 0,47 |
|            | 12     | 54,20        | 53,01        | 54,47        | 53,89              | 0,78 |
|            | 13     | 58,37        | 57,87        | 58,53        | 58,26              | 0,34 |
|            | 14     | 61,20        | 62,24        | 61,03        | 61,49              | 0,66 |
|            | 14+    | 62,75        | 63,10        | 62,79        | 62,88              | 0,19 |
| Blocker 10 | 14     | 47,36        | 47,97        | 48,29        | 47,87              | 0,47 |
|            | 15     | 54,14        | 54,33        | 54,80        | 54,42              | 0,34 |
|            | 16     | 57,52        | 58,46        | 58,67        | 58,22              | 0,61 |
|            | 17     | 61,23        | 61,25        | 62,30        | 61,59              | 0,61 |
|            | 17+    | 62,43        | 62,72        | 63,35        | 62,83              | 0,47 |
| Blocker 14 | 18     | 49,22        | 48,62        | 48,55        | 48,80              | 0,37 |
|            | 19     | 55,62        | 54,62        | 54,51        | 54,92              | 0,61 |
|            | 20     | 59,34        | 58,37        | 58,45        | 58,72              | 0,54 |
|            | 21+    | 64,27        | 63,00        | 62,98        | 63,42              | 0,74 |

**Supplementary Table 7:** Melting temperatures of different alleles of the D18S51 locus using respectively the BL7, BL10 and BL14 blocker

| D8S1179    | Allele | Tm 1<br>(°C) | Tm 2<br>(°C) | Tm 3<br>(°C) | Average Tm<br>(°C) | SD   |
|------------|--------|--------------|--------------|--------------|--------------------|------|
| Blocker 5  | 8      | 41,32        | 40,81        | 39,91        | 40,68              | 0,71 |
|            | 9      | 48,12        | 47,50        | 47,66        | 47,76              | 0,32 |
|            | 10     | 51,84        | 52,65        | 52,10        | 52,20              | 0,41 |
|            | 11     | 55,78        | 55,85        | 54,86        | 55,50              | 0,55 |
|            | 11+    | 60,43        | 60,26        | 59,10        | 59,93              | 0,72 |
| Blocker 8  | 11     | 39,56        | 38,44        | 39,83        | 39,28              | 0,74 |
|            | 12     | 46,63        | 48,27        | 47,18        | 47,36              | 0,83 |
|            | 13     | 51,61        | 51,00        | 50,93        | 51,18              | 0,37 |
|            | 14     | 54,00        | 55,72        | 54,94        | 54,89              | 0,86 |
|            | 14+    | 57,43        | 56,50        | 57,65        | 57,19              | 0,61 |
| Blocker 11 | 14     | 39,14        | 39,60        | 39,86        | 39,53              | 0,36 |
|            | 15     | 46,28        | 46,43        | 46,66        | 46,46              | 0,19 |
|            | 16     | 50,41        | 50,93        | 51,26        | 50,87              | 0,43 |

**Supplementary Table 8:** Melting temperatures of different alleles of the D8S1179 locus using respectively the BI5, BI8 and BI11 blocker

| <b>TH01</b>           | <b>Allele</b> | <b>Tm 1<br/>(°C)</b> | <b>Tm 2<br/>(°C)</b> | <b>Tm 3<br/>(°C)</b> | <b>Average Tm<br/>(°C)</b> | <b>SD</b> |
|-----------------------|---------------|----------------------|----------------------|----------------------|----------------------------|-----------|
| Blocker<br><b>2.1</b> | 6             | 46,57                | 46,36                | 46,27                | 46,40                      | 0,15      |
|                       | 7             | 52,44                | 52,25                | 52,60                | 52,27                      | 0,24      |
|                       | 8+            | 58,63                | 58,46                | 58,42                | 58,50                      | 0,11      |
| Blocker<br><b>3.3</b> | 8             | 50,76                | 51,46                | 51,84                | 51,35                      | 0,55      |
|                       | 9             | 54,86                | 55,7                 | 55,83                | 55,46                      | 0,53      |
|                       | 9.3           | 58,45                | 58,74                | 58,94                | 58,71                      | 0,25      |
|                       | 10            | 58,16                | 58,52                | 58,82                | 58,50                      | 0,33      |

**Supplementary Table 9:** Melting temperatures of different alleles of the DTH01 locus using respectively the BI2.1 and BI3.3 blocker

| <b>D16S539</b>   | <b>Allele</b> | <b>Tm 1<br/>(°C)</b> | <b>Tm 2<br/>(°C)</b> | <b>Tm 3<br/>(°C)</b> | <b>Average Tm<br/>(°C)</b> | <b>SD</b> |
|------------------|---------------|----------------------|----------------------|----------------------|----------------------------|-----------|
| Blocker <b>4</b> | 8             | 43,23                | 43,84                | 42,3                 | 43,12                      | 0,78      |
|                  | 9             | 48,44                | 48,65                | 48,12                | 48,40                      | 0,27      |
|                  | 10            | 52,81                | 53,12                | 52,59                | 52,84                      | 0,27      |
|                  | 11            | 55,85                | 56,04                | 55,63                | 55,84                      | 0,21      |
|                  | 11+           | 57,41                | 57,6                 | 57,29                | 57,43                      | 0,16      |
| Blocker <b>6</b> | 11            | 49,08                | 49,29                | 49,28                | 49,22                      | 0,12      |
|                  | 12            | 52,75                | 53,09                | 52,94                | 52,93                      | 0,17      |
|                  | 13            | 56,16                | 56,32                | 56,48                | 56,32                      | 0,16      |
|                  | 14            | 57,59                | 57,74                | 58,03                | 57,79                      | 0,22      |

**Supplementary Table 10:** Melting temperatures of different alleles of the D16S59 locus using respectively the BI4 and BI6 blocker

| <b>Amelogenin</b> | <b>Allele</b> | <b>Tm 1<br/>(°C)</b> | <b>Tm 2<br/>(°C)</b> | <b>Tm 3<br/>(°C)</b> | <b>Average Tm<br/>(°C)</b> | <b>SD</b> |
|-------------------|---------------|----------------------|----------------------|----------------------|----------------------------|-----------|
|                   | X (♀)         | 50,58                | 51,73                | 51,08                | 51,13                      | 0,58      |
|                   | X (♂)         | 49,56                | 51,21                | 50,76                | 50,51                      | 0,85      |
|                   | Y             | 60,86                | 61,14                | 61,37                | 61,12                      | 0,26      |

**Supplementary Table 11:** Melting temperatures of different alleles (XX- XY) of the amelogenin locus.