

| Supplementary Table S1: RM Y STR primer sequences, repeat structures and allelic size ranges. |                    |   |   |                         |            |
|---|--------------------|---|---|-------------------------|------------|
|   | Marker name        | Repeat motif  | Primer Sequence   | Allelic size range (bp) | Reference  |
| 1   | DYS570             | (TTTC) <sup>14-24</sup>   | Forward- FAM-AAATCCTGGCTGTGTCCTC<br>Reverse- TGGCAACCTAAGCTGAAATG           | 114-160                 | [9]        |
| 2   | DYS442             | (GATA) <sup>9-16</sup> (GACA) <sup>3</sup>  | Forward-FAM-CAATCTCATCCAAGCCACT<br>Reverse- AATTGCAAAATCACGGAACC            | 190-216                 | This study |
| 3   | DYF399S1           | (GAAA) <sup>3N</sup> <sub>7-8</sub> (GAAA) <sup>10-23</sup>   | Forward-FAMGAACCAAGGGAAATGTGGAA<br>Reverse: CTGGACAGCTTAGGATTGGAC           | 246-302                 | [9]        |
| 4   | DYS635             | (TCTA) <sub>4</sub> (TGTA) <sub>2</sub> (TCTA) <sub>2</sub> (TGTA) <sub>2</sub> (TCTA) <sub>2</sub> (TATG) <sub>0-2</sub> (TCTA) <sub>4-17</sub>  | Forward-FAM-GCAGCAAAATTCACAGTTGG<br>Reverse-GGCTGATTAGTATTCCATGGTG          | 369-408                 | This study |
| 5   | DYS464             | (CCTT) <sup>9-20</sup> N <sub>46</sub> (CCTT) <sub>3</sub> N <sub>8</sub> (CCTT) <sub>4</sub>   | Forward-VIC- TTACGAGCTTTGGGCTATGC<br>Reverse- AAGGAAGGGAGGGATGGAG           | 125-174                 | This study |
| 6   | DYS612             | (CCT) <sub>5</sub> (CTT) <sub>1</sub> (TCT) <sub>4</sub> (CCT) <sub>1</sub> (TCT) <sub>19-31</sub>  | Forward-VIC-CCCCATGCCAGTAAGAATA<br>Reverse: AAAAGGGAAGTGGGGAAGG             | 196-226                 | [9]        |
| 7   | DYF387S1           | (AAAG) <sub>3</sub> (GTAG) <sub>1</sub> (GAAG) <sub>4</sub> N <sub>16</sub> (GAAG) <sub>9</sub> (AAAG) <sub>13</sub>  | Forward-VIC-GGGTGACAGAGCTAGATTCC<br>Reverse: TGGTGCCACAGTGTGAGAAG           | 250-286                 | [9]        |
| 8   | DYS449             | (TTCT) <sub>13-19</sub> N <sub>22</sub> (TTCT) <sub>3</sub> N <sub>12</sub> (TTCT) <sub>13-19</sub>   | Forward-VIC-TTTAGGCTAGAGATTCTTGGAGTC<br>Reverse: TCTAGGTTGGACAACAAGAGTAAGAC | 317-357                 | [9]        |
| 9   | DYS385a<br>DYS385b | (AAGG) <sub>4</sub> N <sub>14</sub> (AAAG) <sub>3</sub> N <sub>12</sub> (AAAG) <sub>3</sub> N <sub>29</sub> (AAGG) <sub>6-7</sub> (GAAA) <sub>7-23</sub><br>(AAGG) <sub>4</sub> N <sub>14</sub> (AAAG) <sub>3</sub> N <sub>12</sub> (AAAG) <sub>3</sub> N <sub>29</sub><br>(AAGG) <sub>6-7</sub> (GAAA) <sub>7-23</sub> | Forward-VICCATGCGTGGTGACAGAGCTA<br>Reverse- ATGCCCCGCTACTCAATGTTC           | 395-442                 | This study |
| 10  | DYS576             | (AAAG) <sub>13-22</sub>   | Forward-NEDTTGGGCTGAGGAGTTCAATC<br>Reverse-TTCCTGGAGATGAAGGAGGA             | 163-207                 | [9]        |
| 11  | DYS626             | (GAAA) <sub>14-23</sub> N <sub>24</sub> (GAAA) <sub>3</sub> N <sub>6</sub> (GAAA) <sub>5</sub> (AAA) <sub>1</sub> (GAAA) <sub>2-3</sub> (GAAG) <sub>1</sub> (GAAA)  | Forward-NED-TGGGTGACAGAGTGCAAGAC<br>Reverse- GAAGAATTTTGGGACATGTTTG         | 235-267                 | [9]        |
| 12  | DYS518             | GAAA[2]GGAA[1]GAAA[15-16]GGGA[1]GAAA[4]GGAA[1]GA[1]GAAA[11-14]GGAA[1]GA[1]GGGA[2]AGAA[1]GAAA[1]AGAA[2]GGAA[3]   | Forward-NEDATCCCACTACTCATGAGGCT<br>Reverse- CACATCTCAGCTCTTACCATGG          | 352-404                 | This study |
| 13  | DYS549             | (GATA) <sup>9-15</sup>  | Forward-NED-CCAATGAACCCCAACCAAG<br>Reverse-TGTGTGCATAGAGGTGTTTCTAGA         | 407-423                 | This study |
| 14  | DYS389 II          | (TCTG) <sub>5</sub> (TCTA) <sub>12</sub> N <sub>28</sub> (TCTG) <sub>3</sub> (TCTA) <sub>10</sub>   | Forward-PET-CTGCATTTTGGTACCCCATATA<br>Reverse- TGAGTGGGAGAAATGGATGA         | 189-229                 | This study |

|    |          |  |  |         |            |
|----|----------|--|--|---------|------------|
| 15 | DYF393S1 | (AAG) <sub>4</sub> (AA) <sub>1</sub> (AAG) <sub>16-30</sub> (CAG) <sub>1-2</sub>   | Forward-PET-CAGACTGGCAACCAAAAGG<br>Reverse- GATGTCTGCGTGTGTGTGTG       | 232-273 | This study |
| 16 | DYF404S1 | (TTTC) <sub>10-20</sub> N <sub>42</sub> (TTTC) <sub>3</sub>  | Forward-PET-GGCAGGACACATTTAAACAA<br>Reverse- GCCAGGTATTCTGGTTGAGG      | 290-318 | [9]        |
| 17 | DYS627   | (AGAA) <sub>3</sub> N <sub>16</sub> (AGAG) <sub>3</sub> (AAAG) <sub>12-24</sub> N <sub>81</sub> (AAGG) <sub>3</sub>  | Forward-PET-GCACAGATAATGCCACTGC<br>Reverse-CCATGTGGATAATGAGCAAA        | 333-377 | [9]        |
| 18 | DYS526b  | (CCCT) <sub>3</sub> N <sub>20</sub> (CTTT) <sub>11-17</sub> (CCTT) <sub>6-10</sub> N <sub>113</sub> (CCTT) <sub>10-17</sub>  | Forward-PET-CAGCAAACACCTTAGCTAATCA<br>Reverse-AGTAAACGTTTGGGTTACTCG    | 400-446 | [9]        |
| 19 | DYS547   | (CCTT) <sub>9-13</sub> T(CTTC) <sub>4-5</sub> N <sub>56</sub> (TTTC) <sub>10-22</sub> N <sub>10</sub> (CCTT) <sub>4</sub> (TCTC) <sub>1</sub> (TTTC) <sub>9-16</sub> N <sub>14</sub> (TTTC) <sub>3</sub> | Forward-PET-TGACCTCTAGTTCCAATTCTATCCA<br>Reverse- CAGCCTGAGTGACAGAGCAT | 450-485 | [9]        |

Supplementary Table S2: Pedigrees and sample information along with no. of generations, meiosis, pairwise meiosis and their mutations events.

| Pedigree no. | No. of Individuals | No. of generations | Meioses | Pairwise Meioses | Mutational events RM Y-STRs |
|--------------|--------------------|--------------------|---------|------------------|-----------------------------|
| 1            | 4                  | 2                  | 2       | 6                | 2                           |
| 2            | 3                  | 2                  | 2       | 3                | 0                           |
| 3            | 2                  | 2                  | 1       | 1                | 1                           |
| 4            | 3                  | 3                  | 2       | 4                | 2                           |
| 5            | 2                  | 2                  | 1       | 1                | 1                           |
| 6            | 2                  | 1                  | 1       | 1                | 1                           |
| 7            | 2                  | 2                  | 1       | 1                | 0                           |
| 8            | 2                  | 3                  | 2       | 1                | 1                           |
| 11           | 2                  | 3                  | 2       | 1                | 1                           |
| 12           | 2                  | 2                  | 1       | 1                | 1                           |
| 14           | 2                  | 3                  | 2       | 1                | 0                           |
| 10           | 3                  | 3                  | 2       | 4                | 2                           |
| 16           | 3                  | 3                  | 2       | 4                | 1                           |
| 17           | 2                  | 2                  | 1       | 1                | 1                           |
| 18           | 3                  | 3                  | 2       | 4                | 3                           |
| 20           | 2                  | 1                  | 1       | 1                | 0                           |
| 21           | 2                  | 1                  | 1       | 1                | 0                           |
| 22           | 2                  | 2                  | 1       | 1                | 1                           |
| 23           | 2                  | 1                  | 1       | 1                | 2                           |
| 24           | 2                  | 2                  | 1       | 1                | 0                           |
| 25           | 3                  | 3                  | 2       | 4                | 2                           |
| 26           | 3                  | 3                  | 2       | 4                | 3                           |
| 27           | 3                  | 3                  | 2       | 4                | 1                           |

|    |    |   |    |     |    |
|----|----|---|----|-----|----|
| 28 | 2  | 1 | 1  | 1   | 0  |
| 29 | 3  | 3 | 2  | 4   | 0  |
| 30 | 2  | 3 | 1  | 1   | 1  |
| 31 | 2  | 2 | 1  | 1   | 0  |
| 32 | 2  | 2 | 1  | 1   | 0  |
| 33 | 2  | 3 | 1  | 1   | 1  |
| 34 | 2  | 1 | 1  | 1   | 0  |
| 35 | 3  | 3 | 2  | 4   | 0  |
| 36 | 2  | 1 | 1  | 1   | 0  |
| 37 | 2  | 2 | 1  | 1   | 0  |
| 38 | 2  | 1 | 1  | 1   | 1  |
| 39 | 5  | 3 | 4  | 20  | 1  |
| 40 | 2  | 1 | 1  | 1   | 0  |
| 41 | 2  | 2 | 1  | 1   | 0  |
| 42 | 2  | 1 | 1  | 1   | 1  |
| 43 | 2  | 1 | 1  | 1   | 0  |
| 44 | 5  | 3 | 4  | 20  | 1  |
| 45 | 2  | 2 | 1  | 1   | 0  |
| 46 | 2  | 2 | 1  | 1   | 2  |
| 47 | 4  | 3 | 3  | 6   | 4  |
| 48 | 3  | 2 | 2  | 4   | 0  |
| 49 | 2  | 2 | 1  | 1   | 0  |
| 50 | 2  | 2 | 1  | 1   | 0  |
| 51 | 2  | 2 | 1  | 1   | 0  |
| 52 | 2  | 2 | 1  | 1   | 0  |
| 53 | 4  | 3 | 3  | 10  | 2  |
| 54 | 5  | 4 | 4  | 22  | 3  |
| 55 | 6  | 4 | 5  | 32  | 2  |
| 56 | 2  | 2 | 1  | 1   | 0  |
| 57 | 8  | 3 | 7  | 72  | 6  |
| 58 | 17 | 4 | 16 | 276 | 15 |
| 59 | 9  | 3 | 8  | 90  | 2  |
| 60 | 2  | 2 | 1  | 1   | 2  |
| 61 | 4  | 3 | 3  | 10  | 1  |
| 62 | 16 | 4 | 14 | 335 | 14 |
| 63 | 4  | 3 | 3  | 10  | 3  |
| 64 | 4  | 2 | 4  | 12  | 4  |
| 65 | 2  | 2 | 1  | 1   | 0  |
| 66 | 2  | 2 | 1  | 1   | 0  |
| 67 | 4  | 2 | 3  | 9   | 0  |
| 68 | 2  | 2 | 1  | 1   | 0  |

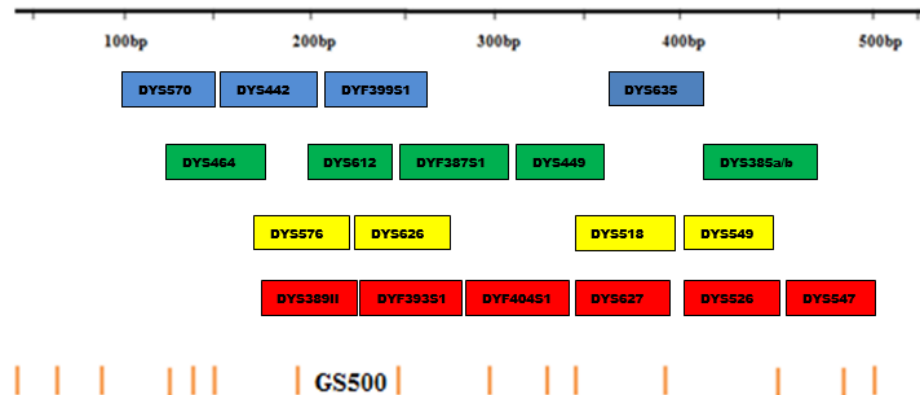
|       |     |    |     |      |     |
|-------|-----|----|-----|------|-----|
| 69    | 2   | 2  | 1   | 1    | 1   |
| 70    | 4   | 2  | 3   | 9    | 4   |
| 71    | 4   | 3  | 3   | 10   | 2   |
| 72    | 3   | 2  | 2   | 4    | 0   |
| 73    | 2   | 2  | 1   | 1    | 0   |
| 74    | 2   | 2  | 1   | 1    | 0   |
| 75    | 2   | 2  | 1   | 1    | 1   |
| 76    | 4   | 3  | 3   | 10   | 5   |
| 77    | 4   | 3  | 3   | 10   | 6   |
| 78    | 5   | 3  | 4   | 18   | 1   |
| 79    | 5   | 3  | 4   | 18   | 0   |
| 80    | 6   | 3  | 5   | 30   | 1   |
| 81    | 2   | 2  | 1   | 1    | 0   |
| 82    | 2   | 2  | 1   | 1    | 0   |
| 83    | 3   | 3  | 2   | 6    | 4   |
| 84    | 2   | 2  | 1   | 1    | 0   |
| 85    | 3   | 2  | 3   | 4    | 2   |
| 86    | 3   | 2  | 2   | 4    | 1   |
| 87    | 2   | 2  | 1   | 1    | 1   |
| 88    | 3   | 2  | 2   | 4    | 2   |
| 89    | 2   | 2  | 1   | 1    | 0   |
| 90    | 4   | 3  | 3   | 10   | 1   |
| 91    | 2   | 2  | 1   | 1    | 0   |
| 92    | 3   | 2  | 2   | 4    | 0   |
| 93    | 2   | 2  | 1   | 1    | 0   |
| 94    | 2   | 1  | 1   | 1    | 0   |
| 95    | 2   | 1  | 1   | 1    | 0   |
| 96    | 2   | 1  | 1   | 1    | 0   |
| 97    | 4   | 3  | 3   | 10   | 0   |
| 98    | 4   | 3  | 3   | 10   | 0   |
| 99    | 2   | 1  | 1   | 1    | 1   |
| 100   | 2   | 1  | 1   | 1    | 0   |
| Total | 302 | NA | 209 | 1188 | 126 |

Supplementary Table S3: Copy number variants observed at duplicated loci

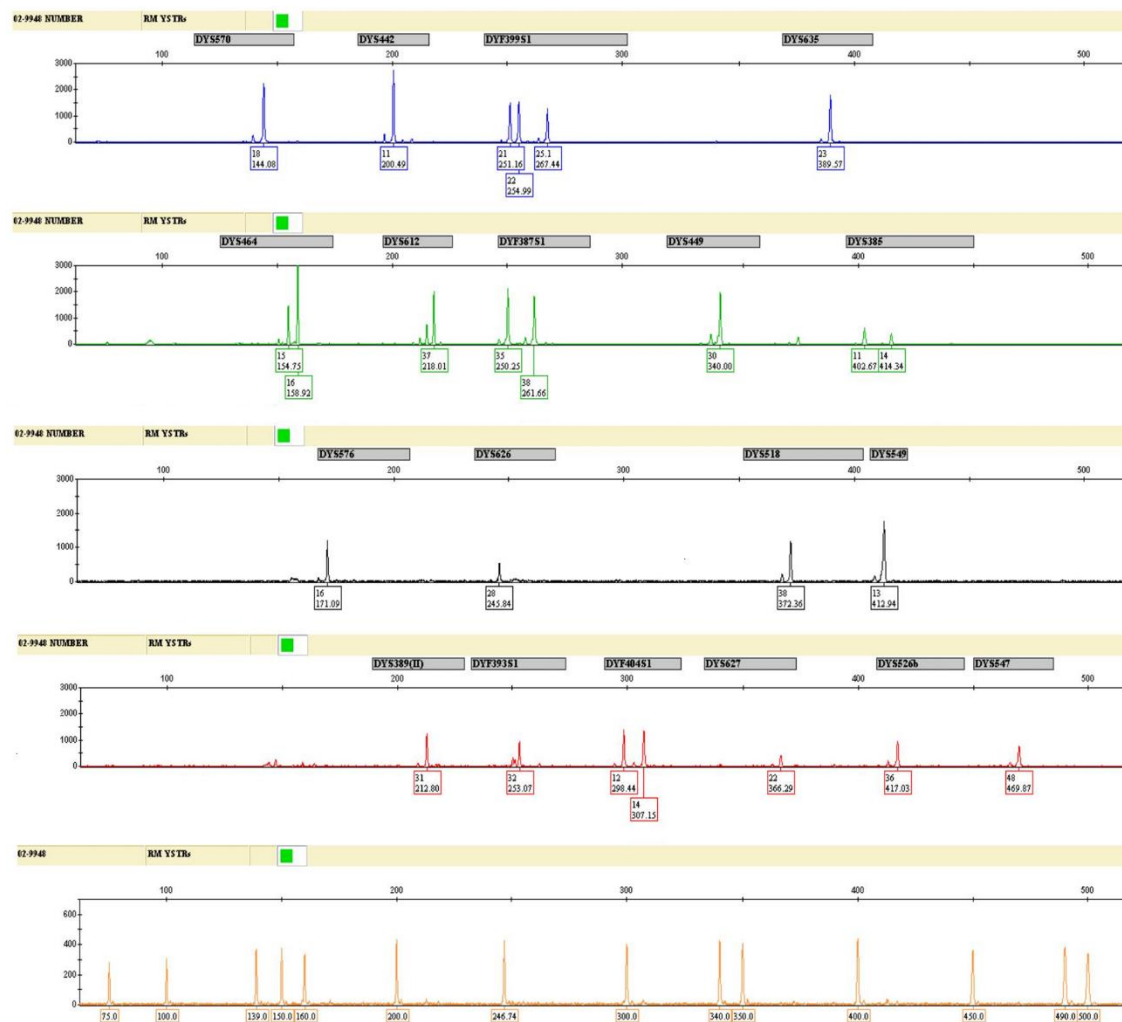
| Locus    | Genotype                | CNV type      | No. of individuals showed the genotype | 9948 Genotype |
|----------|-------------------------|---------------|--|---------------|
| DYS464   | 12,13,14,15,2,16        | Pentaplicate  | 3                                      | 15,16         |
| DYS464   | 14,15,16,17,19          | Pentaplicate  | 5                                      | 15,16         |
| DYS464   | 12,14,15,16,20          | Pentaplicate  | 3                                      | 15,16         |
| DYS464   | 14,15,17,18,19,20       | Hexaplicate   | 2                                      | 15,16         |
| DYS464   | 10,3,12,14,15,16        | Pentaplicate  | 2                                      | 15,16         |
| DYS464   | 11,13,15,15,2,16        | Pentaplicate  | 3                                      | 15,16         |
| DYS464   | 13,14,15,15,3,16,<br>17 | Hexaplicate   | 2                                      | 15,16         |
| DYS464   | 13,14,16,17,19          | Pentaplicate  | 3                                      | 15,16         |
| DYS464   | 12,14,15,16,17          | Pentaplicate  | 9                                      | 15,16         |
| DYS464   | 12,13,14,15,16          | Pentaplicate  | 2                                      | 15,16         |
| DYS464   | 10,2,14,15,16,17        | Pentaplicate  | 1                                      | 15,16         |
| DYS464   | 10,2,14,15,2,16         | Quadruplicate | 1                                      | 15,16         |
| DYF387S1 | 36,37,39,40             | Quadruplicate | 13                                     | 35,38         |
| DYF387S1 | 36,37,41,42             | Quadruplicate | 11                                     | 35,38         |
| DYF387S1 | 37,38,39,40             | Quadruplicate | 19                                     | 35,38         |
| DYF387S1 | 37,38,39,41,42          | Quadruplicate | 2                                      | 35,38         |
| DYF387S1 | 37,38,39,40,41          | Quadruplicate | 2                                      | 35,38         |
| DYF387S1 | 37,38,40,41             | Quadruplicate | 6                                      | 35,38         |
| DYF387S1 | 37,38,41,42             | Quadruplicate | 2                                      | 35,38         |
| DYF387S1 | 38,39,42,43             | Quadruplicate | 2                                      | 35,38         |
| DYF387S1 | 38,40,42,43             | Quadruplicate | 1                                      | 35,38         |
| DYF387S1 | 36,37,38,39             | Quadruplicate | 16                                     | 35,38         |
| DYF387S1 | 35,36,38,39             | Quadruplicate | 4                                      | 35,38         |
| DYF387S1 | 35,36,39,40             | Quadruplicate | 6                                      | 35,38         |
| DYF387S1 | 37,38,38,1,39           | Quadruplicate | 2                                      | 35,38         |

|          |                     |               |     |       |
|----------|---------------------|---------------|-----|-------|
| DYF387S1 | 35,36,40,41         | Quadruplicate | 2   | 35,38 |
| DYF387S1 | 37,38,38.2,39.2     | Quadruplicate | 3   | 35,38 |
| DYF387S1 | 36,37,37.2,39,41,42 | Hexaplicate   | 2   | 35,38 |
| DYF387S1 | 35,36,37,38         | Quadruplicate | 6   | 35,38 |
| DYF387S1 | 38,39,40,41         | Quadruplicate | 4   | 35,38 |
| DYF387S1 | 34,35,38,39         | Quadruplicate | 3   | 35,38 |
| DYF387S1 | 37,38,39            | Triplicate    | 50  | 35,38 |
| DYF387S1 | 36,38,39            | Triplicate    | 1   | 35,38 |
| DYF387S1 | 36,37,38            | Triplicate    | 2   | 35,38 |
| DYF404   | 13.3,15,16          | Triplicate    | 1   | 12,14 |
| Total    |                     |               | 196 |       |

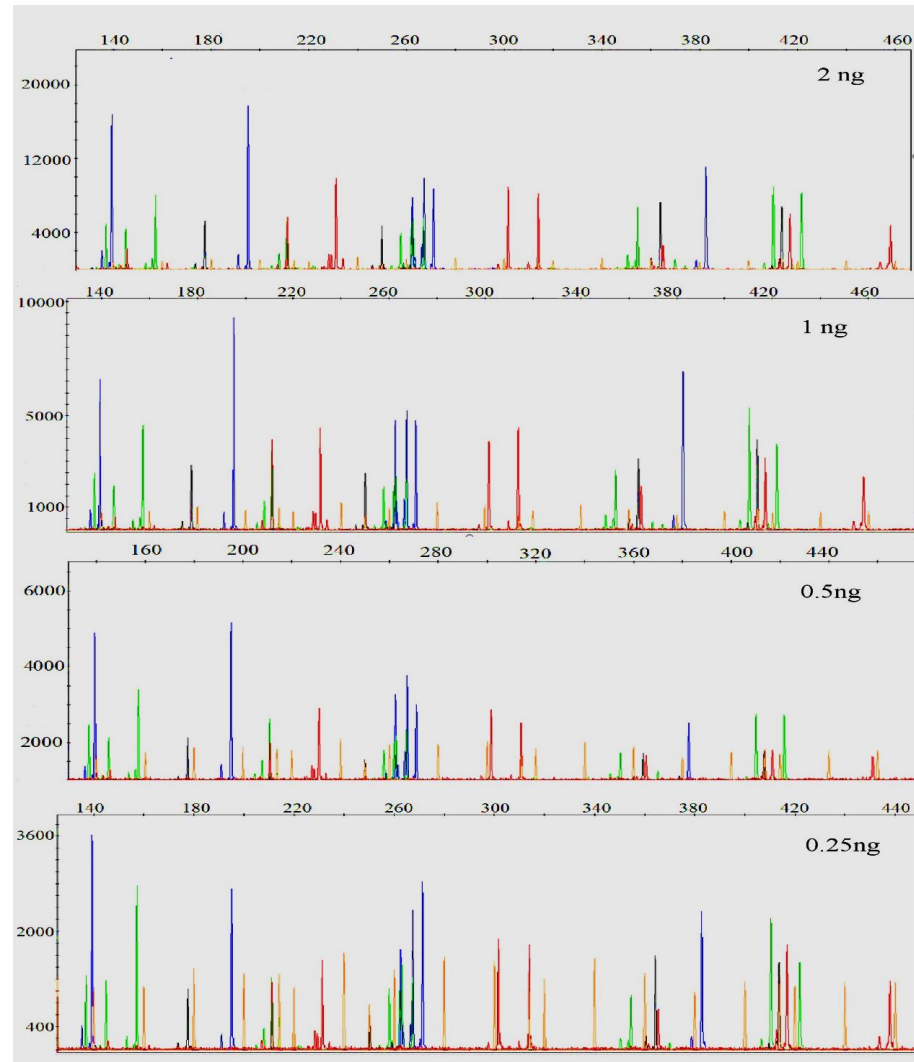
| Supplementary Table S4: The mutation rate found in present and previous studies for 19 Y STRs |                         |                 |                            |            |                 |                 |
|---|-------------------------|-----------------|----------------------------|------------|-----------------|-----------------|
|   | Faqeeha (Present study) | Neuhuber (2022) | Ralf (2021)- cell line DNA | Adnan 2016 | Ballantyne 2010 | Ballantyne 2014 |
| DYF399S1  | 197/536                 | 41/530          | 2/93 pairs                 | 27/428     | 139/1794        | 51/911          |
| DYS449  | 10/536                  | 11/530          | 0/93 pairs                 | 2/428      | 19/1617         | 11/911          |
| DYS612  | 14/536                  | 8/530           | 1/93 pairs                 | 14/428     | 25/1767         | 19/911          |
| DYS526b   | 34/536                  | 7/528           | 0/93 pairs                 | 6/428      | 20/1651         | 12/911          |
| DYS570  | 15/536                  | 6/530           | 0/93 pairs                 | 4/428      | 17/1426         | 9/911           |
| DYS385  | 0/536                   | 6/530           | 0/93 pairs                 |            |                 |                 |
| DYF387S1  | 4/536                   | 6/530           | 1/93 pairs                 | 10/428     | 28/1804         | 4/911           |
| DYS576  | 18/536                  | 5/530           | 0/93 pairs                 | 6/428      | 24/1727         | 9/911           |
| DYF404S1  | 20/536                  | 3/530           | 1/93 pairs                 | 5/428      | 21/1739         | 12/911          |
| DYS547  | 51/536                  | 3/529           | 0/93 pairs                 | 8/428      | 39/1679         | 12/911          |
| DYS626  | 55/536                  | 2/529           | 1/93 pairs                 | 3/428      | 20/1689         | 8/911           |
| DYS627  | 5/536                   | 2/530           | 0/93 pairs                 | 3/428      | 21/1766         | 19/911          |
| DYS518  | 10/536                  | 2/530           | 2/93 pairs                 | 14/428     | 28/1556         | 18/911          |
| DYF393S1  | 6/536                   | 2/530           | 1/93 pairs                 |            |                 |                 |
| DYS442  | 4/536                   | 1/528           | 0/93 pairs                 |            |                 |                 |
| DYS389II  | 6/536                   | 0/530           | 0/93 pairs                 |            |                 |                 |
| DYS464  | 37/536                  |                 |                            |            |                 |                 |
| DYS635  | 6/536                   | 0/530           |                            |            | 6/1732          |                 |
| DYS549  | 0/536                   |                 |                            |            |                 |                 |



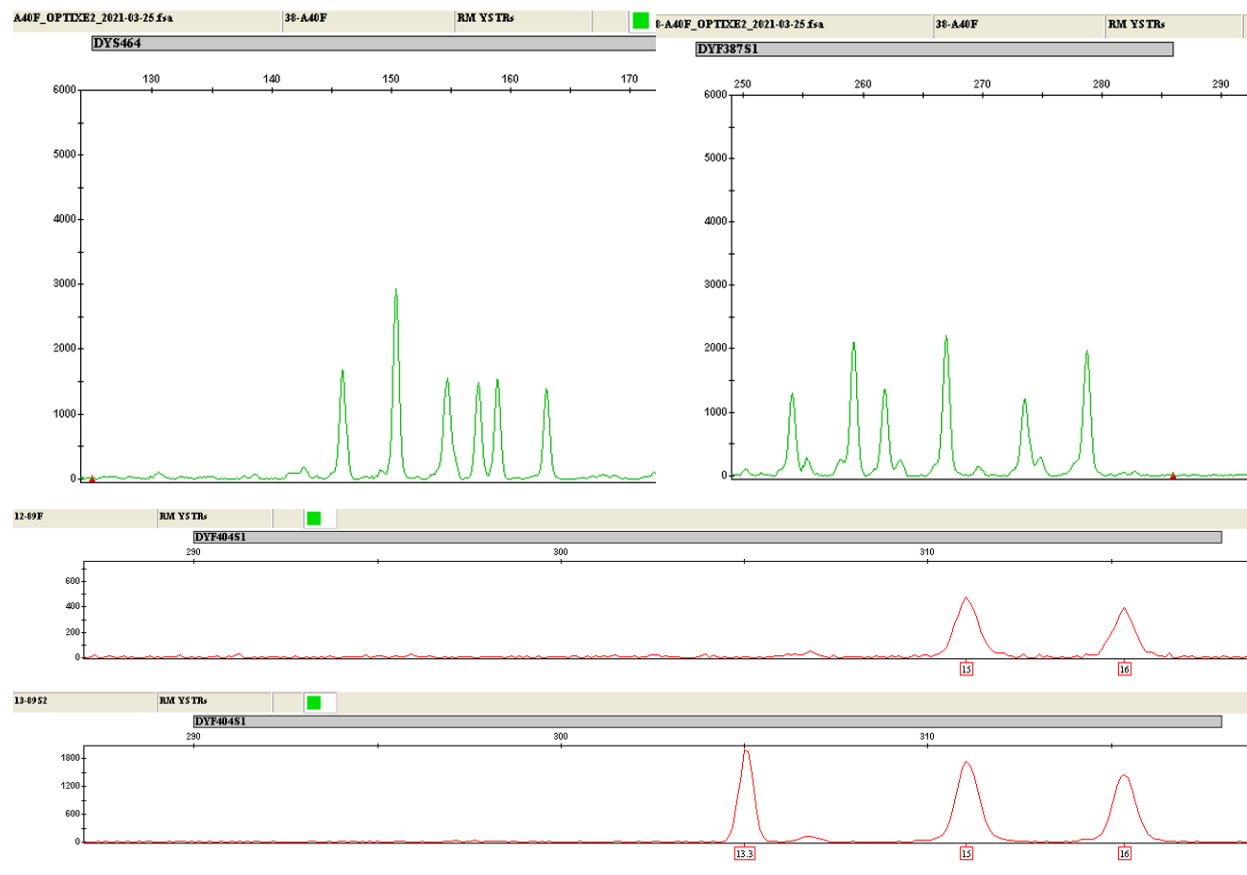
Supplementary Figure S1: Schematic representation of florescent dye colors with marker product allelic size ranges for the 19 RM Y STR markers incorporated in the multiplex.



Supplementary Figure S2: Electropherogram of control DNA 9948 analyzed with the 19 RM Y STR multiplex at varying primer concentration (0.31  $\mu$ M - 0.65  $\mu$ M).



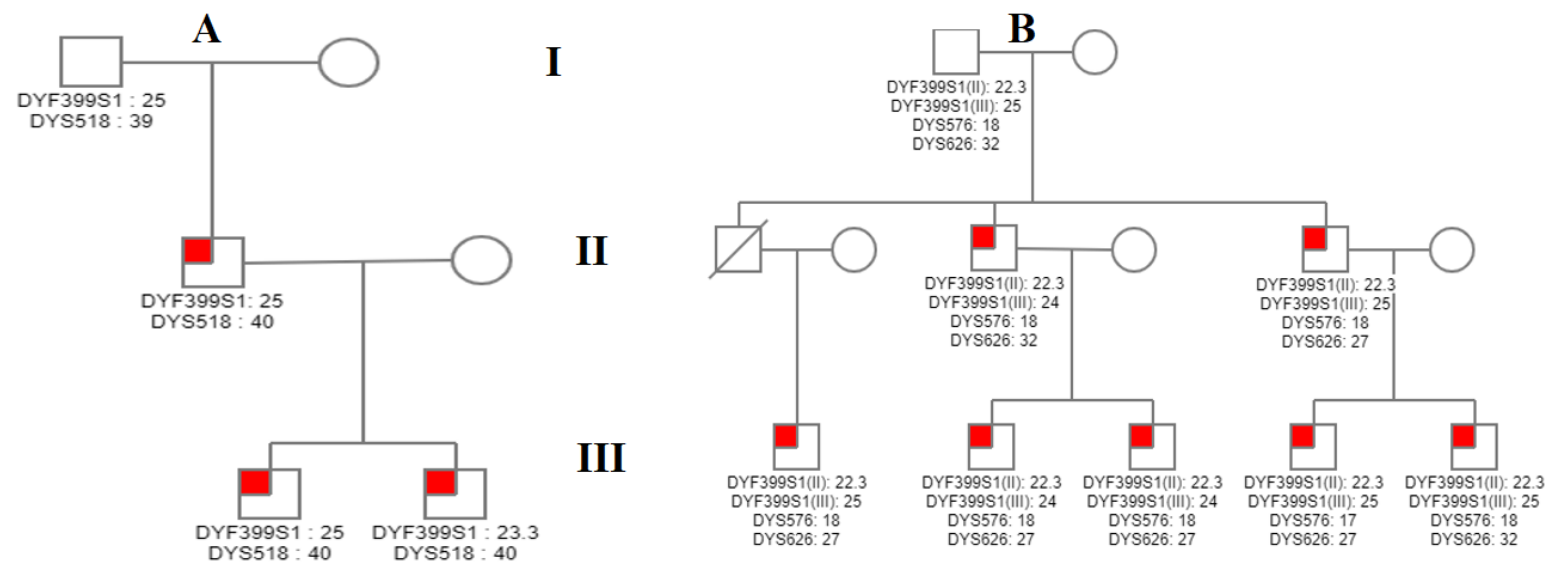
Supplementary Figure S3: Serial dilutions of 2800M control DNA display a gradual decrease in RFU with decrease in concentration.



A

B

Supplementary Figure S4: (A) Hexaplicate alleles observed at DYS464 and DYF387S1 (B) Triplicate allele observed at DYF404S1



Supplementary Figure S5: (A) Paternal lineages of family ID 53 and (B) family ID 57 has been illustrated as representing mutation at one or more loci with red symbols while subtext showing the alleles.