

***Human Genetics, Supporting information to:***

**Large-scale pedigree analysis highlights rapidly mutating Y-chromosomal short tandem repeats for differentiating patrilineal relatives and predicting their degrees of consanguinity**

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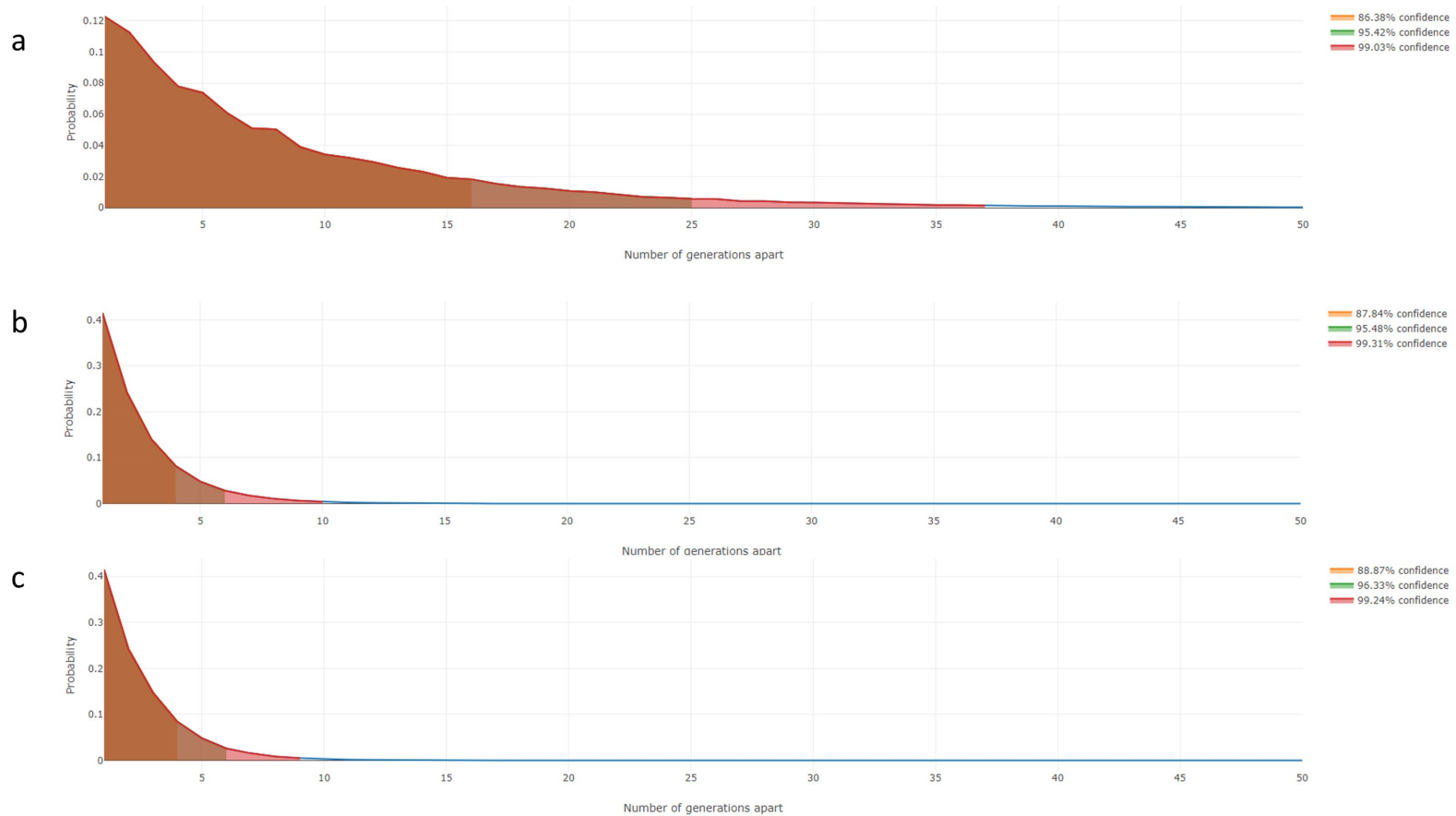
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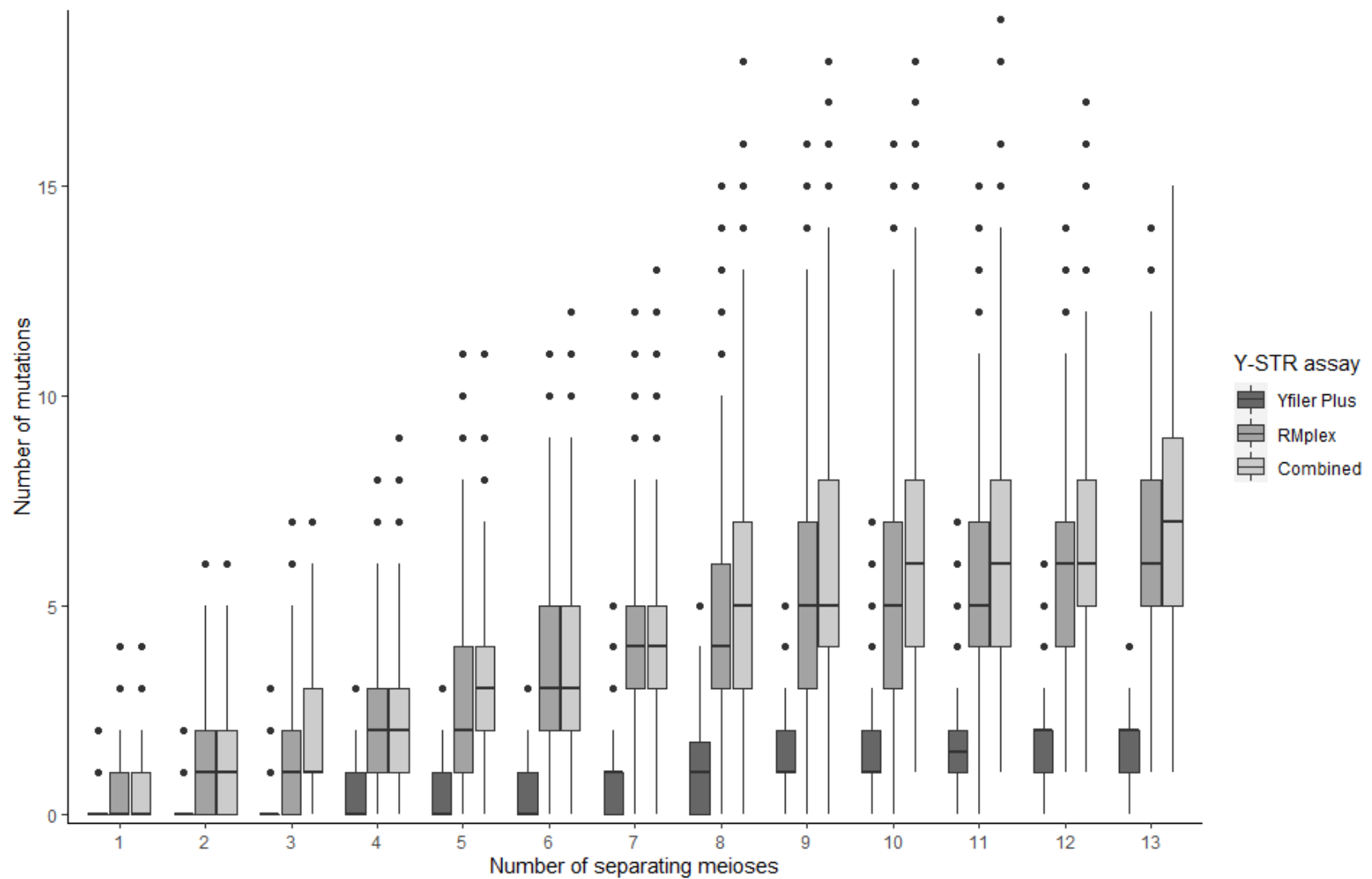
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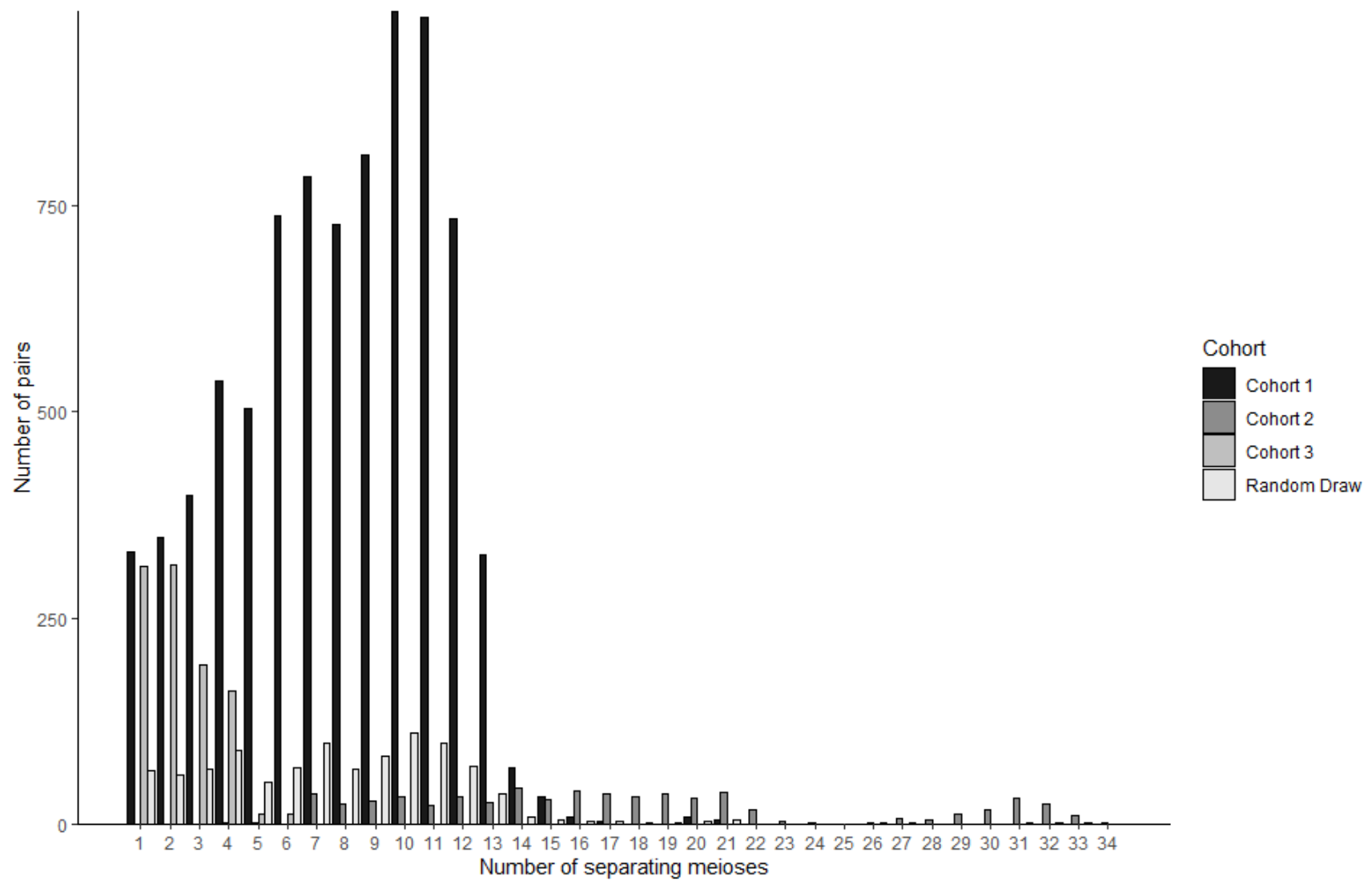
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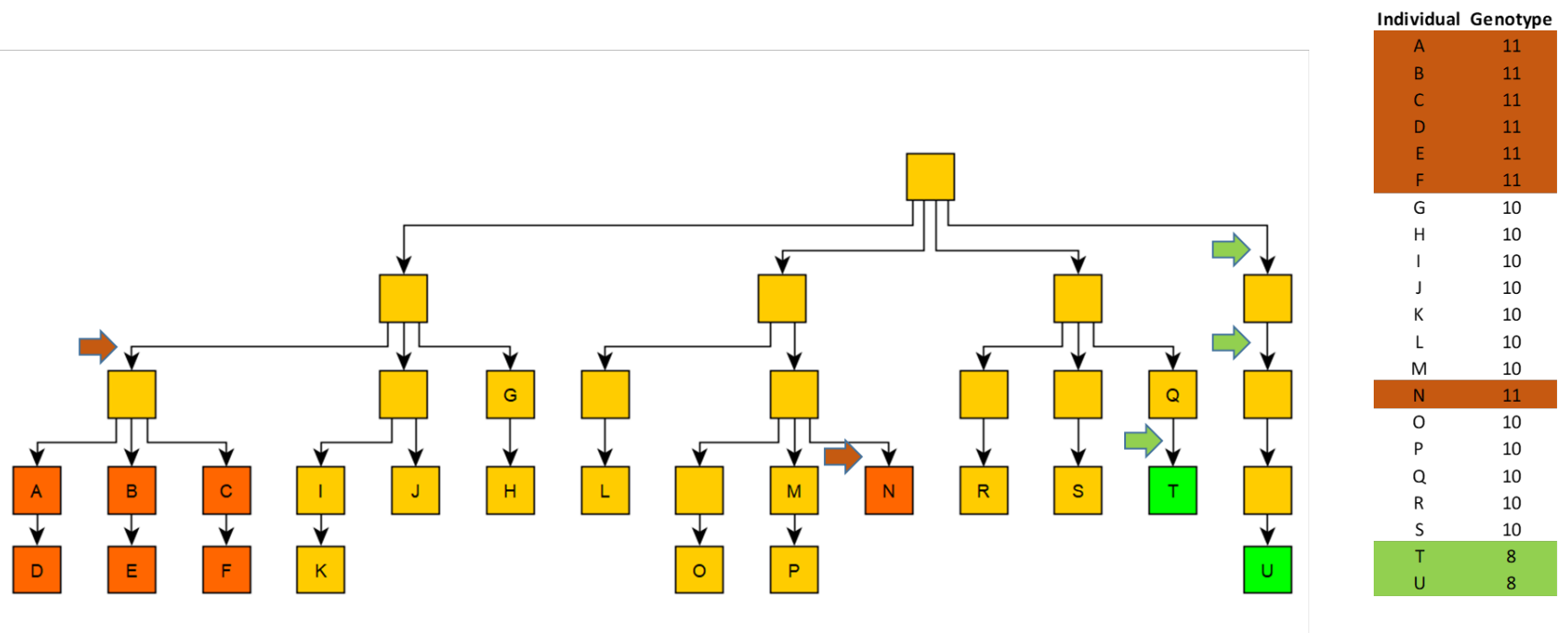
**Fig S1:** Predicted intervals with at least 85%, 95% and 99% probability with Yfiler Plus (a), RMplex (b) and the two assays combined (c) when no mutations between two individuals are observed.



**Fig S2:** Boxplots showing the accumulation of mutations with increasing numbers of meioses.



**Fig S3:** Distribution of number of separating meioses for all pairs of males in the different cohorts and for the 1000 randomly drawn pairs for the prediction model comparisons.



**Fig S4:** Example of a pedigree spanning 35 meioses, where a total of five mutations were estimated based on the observed genotype variations.

|         |    |         |    |    |
|---------|----|---------|----|----|
| a.      |    | Indv. B |    |    |
|         |    | 9       | 11 | 16 |
| Indv. A | 10 | -1      | +1 | +6 |
|         | 11 | -2      | 0  | +5 |
|         | 16 | -7      | -5 | 0  |

|         |    |         |    |    |
|---------|----|---------|----|----|
| b.      |    | Indv. B |    |    |
|         |    | 9.2     | 11 | 16 |
| Indv. A | 10 | -1      | +1 | +6 |
|         | 12 | -3      | -1 | +4 |
|         | 16 | -7      | -5 | 0  |

|         |      |         |    |    |
|---------|------|---------|----|----|
| c.      |      | Indv. B |    |    |
|         |      | 9.2     | 11 | 16 |
| Indv. A | 10   | -1      | +1 | +6 |
|         | 11.2 | -2      | -1 | +5 |
|         | 16   | -7      | -5 | 0  |

|         |    |         |    |
|---------|----|---------|----|
| d.      |    | Indv. B |    |
|         |    | 11      | 14 |
| Indv. A | 10 | +1      | +4 |
|         | 11 | 0       | +3 |
|         | 16 | -5      | -2 |

**Fig S5:** Various scenarios that could be encountered when dealing with multi-copy loci, the solutions that our approach preferred in these cases are highlighted.

**Table S1:** Combined mutation rate estimates as derived from all pedigreed included within the three analyzed cohorts.

| Marker    | Total meioses | Mutations | Mutation rate<br>( $\times 10^{-3}$ ) | 95% confidence range<br>( $\times 10^{-3}$ ) | Reference value [1]<br>( $\times 10^{-3}$ ) | Fisher's exact<br>p-value |
|-----------|---------------|-----------|---------------------------------------|----------------------------------------------|---------------------------------------------|---------------------------|
| DYF399S1  | 4354          | 305       | 70.1                                  | 62.6 - 78.0                                  | 62.8                                        | 0.125                     |
| DYF1001   | 4354          | 233       | 53.5                                  | 47.0 - 60.6                                  | 48.0                                        | 0.372                     |
| DYF1000   | 4354          | 211       | 48.5                                  | 42.3 - 55.3                                  | 35.9                                        | <b>0.018</b>              |
| DYF403S1a | 4354          | 168       | 38.6                                  | 33.1 - 44.7                                  | 27.3                                        | <b>0.001</b>              |
| DYS724    | 4354          | 167       | 38.4                                  | 32.8 - 44.5                                  | 48.0                                        | 0.074                     |
| DYS711    | 4354          | 131       | 30.1                                  | 25.2 - 35.6                                  | 26.6                                        | 0.479                     |
| DYS712    | 4354          | 113       | 26.0                                  | 21.4 - 31.1                                  | 31.1                                        | 0.221                     |
| DYS612    | 4354          | 99        | 22.7                                  | 18.5 - 27.6                                  | 16.3                                        | <b>0.012</b>              |
| DYS1012   | 4354          | 97        | 22.3                                  | 18.1 - 27.1                                  | 15.8                                        | 0.091                     |
| DYR88     | 4354          | 94        | 21.6                                  | 17.5 - 26.4                                  | 26.3                                        | 0.253                     |
| DYS1010   | 4354          | 84        | 19.3                                  | 15.4 - 23.8                                  | 14.0                                        | 0.133                     |
| DYF1002   | 4354          | 78        | 17.9                                  | 14.2 - 22.3                                  | 16.8                                        | 0.841                     |
| DYS518    | 4354          | 71        | 16.3                                  | 12.8 - 20.5                                  | 13.3                                        | 0.152                     |
| DYF404S1  | 4354          | 61        | 14.0                                  | 10.7 - 18.0                                  | 12.5                                        | 0.509                     |
| DYS547    | 4354          | 60        | 13.8                                  | 10.5 - 17.7                                  | 14.7                                        | 0.751                     |
| DYS627    | 4354          | 59        | 13.6                                  | 10.3 - 17.4                                  | 14.5                                        | 0.709                     |
| DYS1007   | 4354          | 59        | 13.6                                  | 10.3 - 17.4                                  | 17.2                                        | 0.274                     |
| DYS526b   | 4354          | 57        | 13.1                                  | 9.9 - 16.9                                   | 12.3                                        | 0.735                     |
| DYS713    | 4354          | 53        | 12.2                                  | 9.1 - 15.9                                   | 13.9                                        | 0.555                     |
| DYS626    | 4354          | 50        | 11.5                                  | 8.5 - 15.1                                   | 8.6                                         | 0.122                     |
| DYS449    | 4354          | 45        | 10.3                                  | 7.5 - 13.8                                   | 11.2                                        | 0.673                     |
| DYS570    | 4354          | 44        | 10.1                                  | 7.4 - 13.5                                   | 8.3                                         | 0.295                     |
| DYF393S1  | 4354          | 42        | 9.6                                   | 7.0 - 13                                     | 7.1                                         | 0.332                     |
| DYS1003   | 4354          | 41        | 9.4                                   | 6.8 - 12.8                                   | 12.6                                        | 0.245                     |
| DYS576    | 4354          | 40        | 9.2                                   | 6.6 - 12.5                                   | 12.7                                        | 0.072                     |
| DYF387S1  | 4354          | 37        | 8.5                                   | 6.0 - 11.7                                   | 10.2                                        | 0.363                     |
| DYS458    | 1860          | 15        | 8.1                                   | 4.5 - 13.3                                   | 8.5                                         | 1.000                     |
| DYF403S1b | 4354          | 30        | 6.9                                   | 4.7 - 9.8                                    | 9.1                                         | 0.243                     |
| DYS1005   | 4354          | 26        | 6.0                                   | 3.9 - 8.7                                    | 9.8                                         | 0.118                     |
| DYS460    | 1860          | 11        | 5.9                                   | 3.0 - 10.6                                   | 4.3                                         | 0.343                     |
| DYS1013   | 4354          | 21        | 4.8                                   | 3.0 - 7.4                                    | 10.8                                        | <b>0.009</b>              |
| DYS385    | 1860          | 8         | 4.3                                   | 1.9 - 8.5                                    | 7.5                                         | 0.137                     |
| DYS390    | 1860          | 8         | 4.3                                   | 1.9 - 8.5                                    | 2.7                                         | 0.245                     |
| DYS391    | 1860          | 7         | 3.8                                   | 1.5 - 7.7                                    | 2.5                                         | 0.328                     |
| DYS389II  | 1860          | 7         | 3.8                                   | 1.5 - 7.7                                    | 5.5                                         | 0.487                     |
| DYS439    | 1860          | 7         | 3.8                                   | 1.5 - 7.7                                    | 4.8                                         | 0.713                     |
| DYS442    | 4354          | 15        | 3.4                                   | 1.9 - 5.7                                    | 7.4                                         | <b>0.047</b>              |
| DYS448    | 1860          | 6         | 3.2                                   | 1.2 - 7.0                                    | 0.8                                         | <b>0.016</b>              |
| DYS533    | 1860          | 6         | 3.2                                   | 1.2 - 7.0                                    | 3.5                                         | 1.000                     |
| DYS635    | 1860          | 5         | 2.7                                   | 0.9 - 6.3                                    | 3.8                                         | 0.541                     |
| YGATAH4   | 1860          | 4         | 2.2                                   | 0.6 - 5.5                                    | 1.9                                         | 0.777                     |
| DYS389I   | 1860          | 4         | 2.2                                   | 0.6 - 5.5                                    | 2.4                                         | 1.000                     |
| DYS456    | 1860          | 3         | 1.6                                   | 0.3 - 4.7                                    | 4.4                                         | 0.108                     |
| DYS481    | 1860          | 3         | 1.6                                   | 0.3 - 4.7                                    | 4.7                                         | 0.070                     |
| DYS19     | 1860          | 3         | 1.6                                   | 0.3 - 4.7                                    | 2.0                                         | 1.000                     |
| DYS438    | 1860          | 2         | 1.1                                   | 0.1 - 3.9                                    | 0.3                                         | 0.142                     |
| DYS393    | 1860          | 1         | 0.5                                   | 0.0 - 3.0                                    | 1.7                                         | 0.346                     |
| DYS437    | 1860          | 1         | 0.5                                   | 0.0 - 3.0                                    | 1.2                                         | 0.710                     |
| DYS392    | 1860          | 0         | 0.0                                   | 0.0 - 2.0                                    | 0.8                                         | 0.621                     |

[1] the reference values are the consensus mutation rate estimates from: Franz Neuhuber, et al.,  
Improving the differentiation of closely related males by RMplex analysis of 30 Y-STRs with high mutation rates,  
*Forensic Sci Int Genet.* 2022 May;58:102682

**Table S2:** Observed differentiation rates using RMplex in three different cohorts and all three cohorts combined.

| Meioses | Cohort 1        |                    |                     | Cohort 2        |                    |                     | Cohort 3        |                    |                     | Overall         |                    |                     |
|---------|-----------------|--------------------|---------------------|-----------------|--------------------|---------------------|-----------------|--------------------|---------------------|-----------------|--------------------|---------------------|
|         | Number of pairs | Differentiated (%) | Mean mutations (SD) | Number of pairs | Differentiated (%) | Mean mutations (SD) | Number of pairs | Differentiated (%) | Mean mutations (SD) | Number of pairs | Differentiated (%) | Mean mutations (SD) |
| 1       | 324             | 144 (44.4)         | 0.6 (0.8)           | 0               |                    |                     | 313             | 132 (42.2)         | 0.6 (1.0)           | 637             | 276 (43.3)         | 0.6 (0.9)           |
| 2       | 332             | 227 (68.4)         | 1.2 (1.2)           | 0               |                    |                     | 315             | 200 (63.5)         | 1.1 (1.3)           | 647             | 427 (66.0)         | 1.2 (1.2)           |
| 3       | 385             | 287 (74.5)         | 1.6 (1.5)           | 0               |                    |                     | 193             | 154 (79.8)         | 1.5 (1.2)           | 578             | 441 (76.3)         | 1.6 (1.4)           |
| 4       | 524             | 431 (82.3)         | 1.9 (1.5)           | 1               | 1 (100)            | 2 (0)               | 161             | 141 (87.6)         | 1.8 (1.2)           | 686             | 573 (83.5)         | 2.1 (2.3)           |
| 5       | 489             | 456 (93.3)         | 2.8 (1.8)           | 1               | 1 (100)            | 7 (0)               | 13              | 13 (100)           | 1.6 (1.1)           | 503             | 470 (93.4)         | 2.8 (2.1)           |
| 6       | 710             | 681 (95.9)         | 3.4 (2.0)           | 0               |                    |                     | 12              | 12 (100)           | 1.2 (0.4)           | 722             | 693 (96.0)         | 3.6 (2.9)           |
| 7       | 762             | 749 (98.3)         | 4.0 (2.2)           | 23              | 22 (95.7)          | 4.8 (2.1)           |                 |                    |                     | 785             | 771 (98.2)         | 4.1 (2.6)           |
| 8       | 686             | 676 (98.5)         | 4.6 (2.5)           | 15              | 15 (100)           | 5.2 (3.0)           |                 |                    |                     | 701             | 691 (98.6)         | 4.9 (3.3)           |
| 9       | 764             | 757 (99.1)         | 5.1 (2.8)           | 16              | 16 (100)           | 5.5 (3.1)           |                 |                    |                     | 780             | 773 (99.1)         | 5.3 (3.5)           |
| 10      | 940             | 936 (99.6)         | 5.3 (2.6)           | 16              | 16 (100)           | 12.4 (17.2)         |                 |                    |                     | 956             | 952 (99.6)         | 5.7 (4.1)           |
| 11      | 954             | 952 (99.8)         | 5.4 (2.4)           | 12              | 12 (100)           | 7.4 (3.4)           |                 |                    |                     | 966             | 964 (99.8)         | 5.6 (3.0)           |
| 12      | 707             | 707 (100)          | 5.8 (2.3)           | 23              | 23 (100)           | 7.7 (2.2)           |                 |                    |                     | 730             | 730 (100)          | 6.0 (2.8)           |
| 13      | 316             | 316 (100)          | 6.3 (2.3)           | 18              | 18 (100)           | 8.3 (2.6)           |                 |                    |                     | 334             | 334 (100)          | 6.6 (2.8)           |
| 14      | 67              | 67 (100)           | 6.6 (2.3)           | 16              | 16 (100)           | 8.3 (2.9)           |                 |                    |                     | 83              | 83 (100)           | 6.9 (2.5)           |
| 15      | 34              | 34 (100)           | 7.9 (1.4)           | 12              | 12 (100)           | 7.9 (3.4)           |                 |                    |                     | 46              | 46 (100)           | 7.9 (2.1)           |
| 16      | 8               | 8 (100)            | 8.6 (1.3)           | 25              | 25 (100)           | 10.1 (2.7)          |                 |                    |                     | 33              | 33 (100)           | 9.7 (2.5)           |
| 17      | 4               | 4 (100)            | 9.3 (0.8)           | 23              | 23 (100)           | 10.5 (5.0)          |                 |                    |                     | 27              | 27 (100)           | 10.3 (4.6)          |
| 18      |                 |                    |                     | 22              | 22 (100)           | 8.8 (3.1)           |                 |                    |                     | 22              | 22 (100)           | 8.8 (3.1)           |
| 19      |                 |                    |                     | 20              | 20 (100)           | 8.9 (3.2)           |                 |                    |                     | 20              | 20 (100)           | 8.9 (3.2)           |
| 20      | 9               | 9 (100)            | 14 (1.2)            | 16              | 16 (100)           | 8.1 (3.6)           |                 |                    |                     | 25              | 25 (100)           | 10.2 (4.1)          |
| 21      | 5               | 5 (100)            | 14.2 (1.5)          | 24              | 24 (100)           | 11.4 (4.4)          |                 |                    |                     | 29              | 29 (100)           | 11.9 (4.2)          |
| 22      |                 |                    |                     | 10              | 10 (100)           | 14.7 (3.7)          |                 |                    |                     | 10              | 10 (100)           | 14.7 (3.7)          |
| 23      |                 |                    |                     | 3               | 3 (100)            | 15.7 (4.0)          |                 |                    |                     | 3               | 3 (100)            | 15.7 (4.0)          |
| 24      |                 |                    |                     | 1               | 1 (100)            | 8 (0)               |                 |                    |                     | 1               | 1 (100)            | 8 (0)               |
| 25      |                 |                    |                     |                 |                    |                     |                 |                    |                     |                 |                    |                     |
| 26      |                 |                    |                     | 1               | 1 (100)            | 14 (0)              |                 |                    |                     | 1               | 1 (100)            | 14 (0)              |
| 27      |                 |                    |                     | 6               | 6 (100)            | 15.2 (3.7)          |                 |                    |                     | 6               | 6 (100)            | 15.2 (3.7)          |
| 28      |                 |                    |                     | 3               | 3 (100)            | 14.7 (2.6)          |                 |                    |                     | 3               | 3 (100)            | 14.7 (2.6)          |
| 29      |                 |                    |                     | 2               | 2 (100)            | 19.5 (4.5)          |                 |                    |                     | 2               | 2 (100)            | 19.5 (4.5)          |
| 30      |                 |                    |                     | 6               | 6 (100)            | 13.7 (2.8)          |                 |                    |                     | 6               | 6 (100)            | 13.7 (2.8)          |
| 31      |                 |                    |                     | 14              | 14 (100)           | 13.8 (2.9)          |                 |                    |                     | 14              | 14 (100)           | 13.8 (2.9)          |
| 32      |                 |                    |                     | 15              | 15 (100)           | 13.1 (1.6)          |                 |                    |                     | 15              | 15 (100)           | 13.1 (1.6)          |
| 33      |                 |                    |                     | 7               | 7 (100)            | 13.9 (1.8)          |                 |                    |                     | 7               | 7 (100)            | 13.9 (1.8)          |
| 34      |                 |                    |                     | 1               | 1 (100)            | 16 (0)              |                 |                    |                     | 1               | 1 (100)            | 16 (0)              |

**Table S3:** Direct comparison of Yfiler Plus, RMplex and both assays combined on differentiation of males separated by 1-13 meioses in Cohort 1

| Assay       | Meioses | Pairs | Differentiated | Differentiation<br>rate (%) | Clopper-Pearson CI<br>lower bound | Clopper-Pearson CI<br>upper bound |
|-------------|---------|-------|----------------|-----------------------------|-----------------------------------|-----------------------------------|
| Yfiler Plus | 1       | 324   | 32             | 9.88                        | 6.85                              | 13.66                             |
| Yfiler Plus | 2       | 332   | 73             | 21.99                       | 17.65                             | 26.83                             |
| Yfiler Plus | 3       | 385   | 88             | 22.86                       | 18.76                             | 27.38                             |
| Yfiler Plus | 4       | 524   | 144            | 27.48                       | 23.7                              | 31.52                             |
| Yfiler Plus | 5       | 489   | 174            | 35.58                       | 31.34                             | 40.01                             |
| Yfiler Plus | 6       | 710   | 299            | 42.11                       | 38.45                             | 45.84                             |
| Yfiler Plus | 7       | 762   | 391            | 51.31                       | 47.7                              | 54.92                             |
| Yfiler Plus | 8       | 686   | 463            | 67.49                       | 63.85                             | 70.99                             |
| Yfiler Plus | 9       | 764   | 604            | 79.06                       | 76                                | 81.89                             |
| Yfiler Plus | 10      | 940   | 761            | 80.96                       | 78.3                              | 83.42                             |
| Yfiler Plus | 11      | 954   | 800            | 83.86                       | 81.37                             | 86.14                             |
| Yfiler Plus | 12      | 707   | 633            | 89.53                       | 87.04                             | 91.69                             |
| Yfiler Plus | 13      | 316   | 284            | 89.87                       | 86.01                             | 92.97                             |
| RMplex      | 1       | 324   | 144            | 44.44                       | 38.95                             | 50.04                             |
| RMplex      | 2       | 332   | 227            | 68.37                       | 63.07                             | 73.34                             |
| RMplex      | 3       | 385   | 287            | 74.55                       | 69.89                             | 78.82                             |
| RMplex      | 4       | 524   | 431            | 82.25                       | 78.71                             | 85.43                             |
| RMplex      | 5       | 489   | 456            | 93.25                       | 90.65                             | 95.31                             |
| RMplex      | 6       | 710   | 681            | 95.92                       | 94.19                             | 97.25                             |
| RMplex      | 7       | 762   | 749            | 98.29                       | 97.1                              | 99.09                             |
| RMplex      | 8       | 686   | 676            | 98.54                       | 97.34                             | 99.3                              |
| RMplex      | 9       | 764   | 757            | 99.08                       | 98.12                             | 99.63                             |
| RMplex      | 10      | 940   | 936            | 99.57                       | 98.91                             | 99.88                             |
| RMplex      | 11      | 954   | 952            | 99.79                       | 99.24                             | 99.97                             |
| RMplex      | 12      | 707   | 707            | 100                         | 99.48                             | 100                               |
| RMplex      | 13      | 316   | 316            | 100                         | 98.84                             | 100                               |
| Combined    | 1       | 324   | 147            | 45.37                       | 39.86                             | 50.97                             |
| Combined    | 2       | 332   | 239            | 71.99                       | 66.82                             | 76.75                             |
| Combined    | 3       | 385   | 295            | 76.62                       | 72.07                             | 80.76                             |
| Combined    | 4       | 524   | 436            | 83.21                       | 79.72                             | 86.31                             |
| Combined    | 5       | 489   | 461            | 94.27                       | 91.83                             | 96.16                             |
| Combined    | 6       | 710   | 685            | 96.48                       | 94.85                             | 97.71                             |
| Combined    | 7       | 762   | 751            | 98.56                       | 97.43                             | 99.28                             |
| Combined    | 8       | 686   | 682            | 99.42                       | 98.51                             | 99.84                             |
| Combined    | 9       | 764   | 759            | 99.35                       | 98.48                             | 99.79                             |
| Combined    | 10      | 940   | 940            | 100                         | 99.61                             | 100                               |
| Combined    | 11      | 954   | 954            | 100                         | 99.61                             | 100                               |
| Combined    | 12      | 707   | 707            | 100                         | 99.48                             | 100                               |
| Combined    | 13      | 316   | 316            | 100                         | 98.84                             | 100                               |