

SFPD TissueID Study using PyroSequencing

- **Collaborative Effort between QIAGEN and SFPD**
 - **Presented by Sim Winitz of QIAGEN**

SFPD TissueID Study using PyroSequencing

- **Methylation Percentages known for various tissues, like semen, saliva and blood, at specific loci**
- **Assess whether Sequencing sensitivity equivalent to GlobalFiler™ sensitivity**
- **Determine methylation percentages for vaginal epithelial/semen and other mixtures**
- **Potential work with PCR multiplexes**

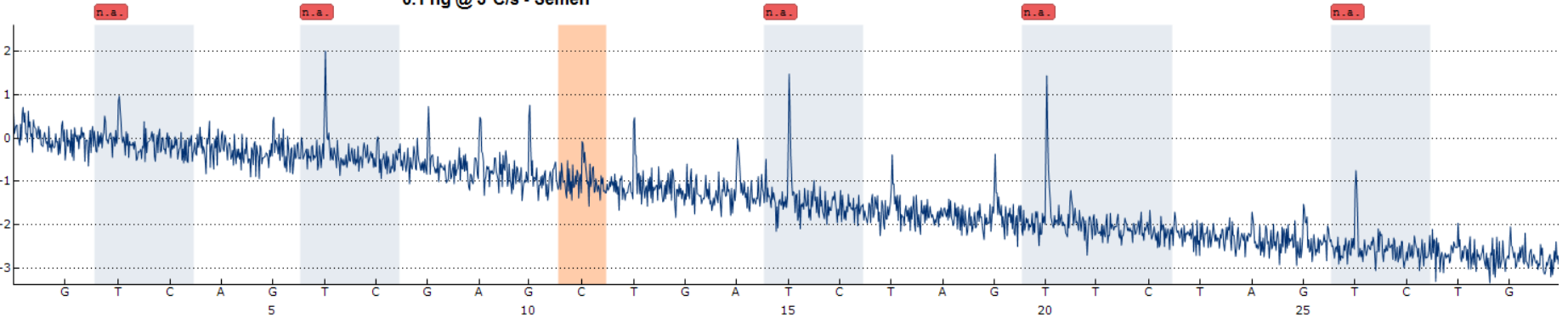
Experimental Methodology

- **Obtain sample specimens (i.e. sperm)**
- **Extract DNA using QIAGEN EZ1**
- **Quantify DNA**
- **Bisulfite Convert DNA to Track Methylation Percentage**
- **PCR region of interest**
- **Pyrosequence to determine Methylation Percentages**

PCR Ramp Rate Comparisons

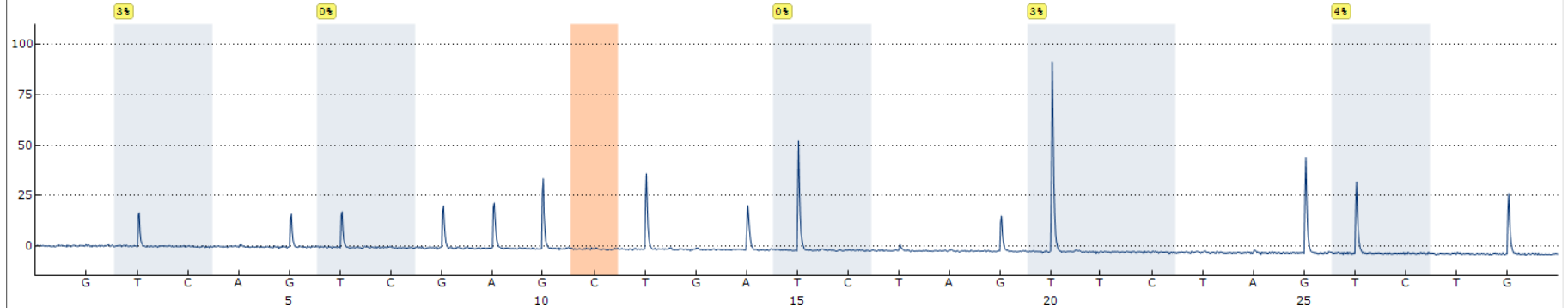
C5 YGYGAGGTTATTYGTGTTTTYGGGTYGGT

0.1 ng @ 3°C/s - Semen



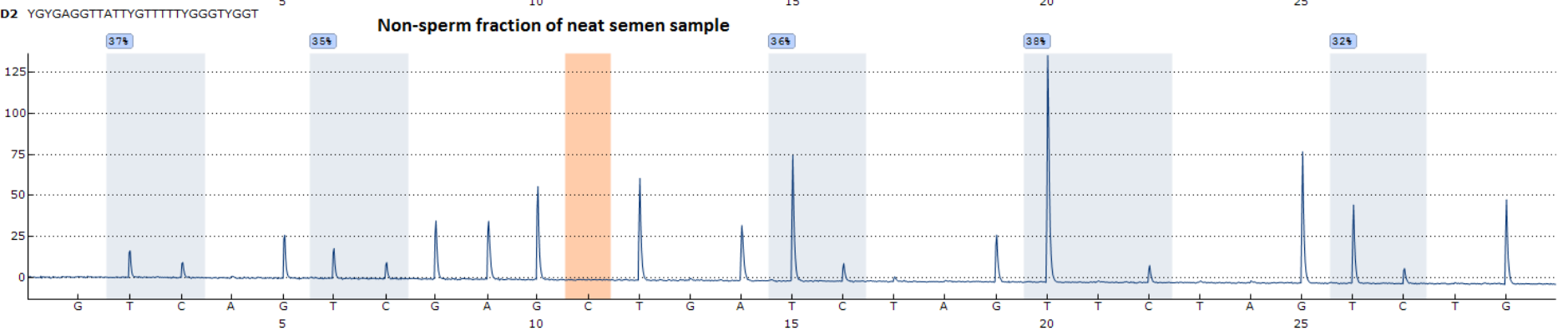
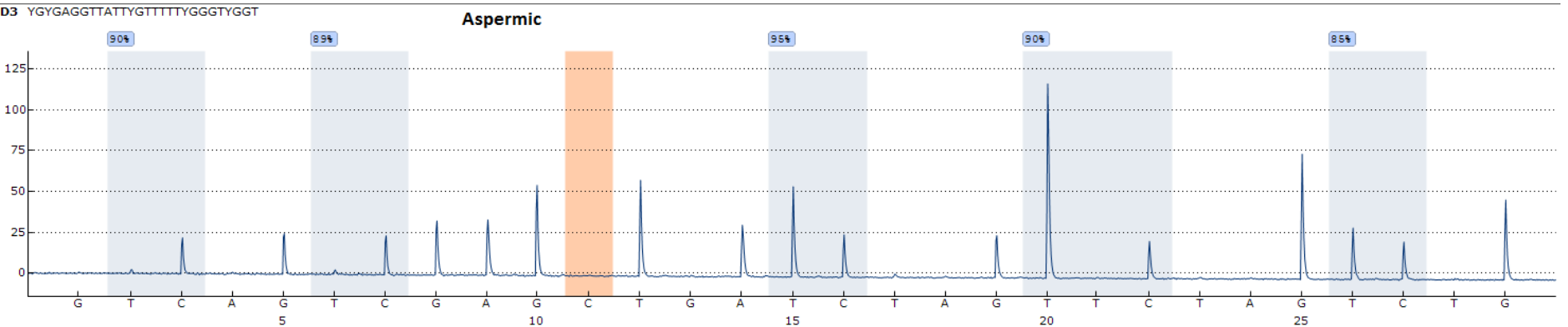
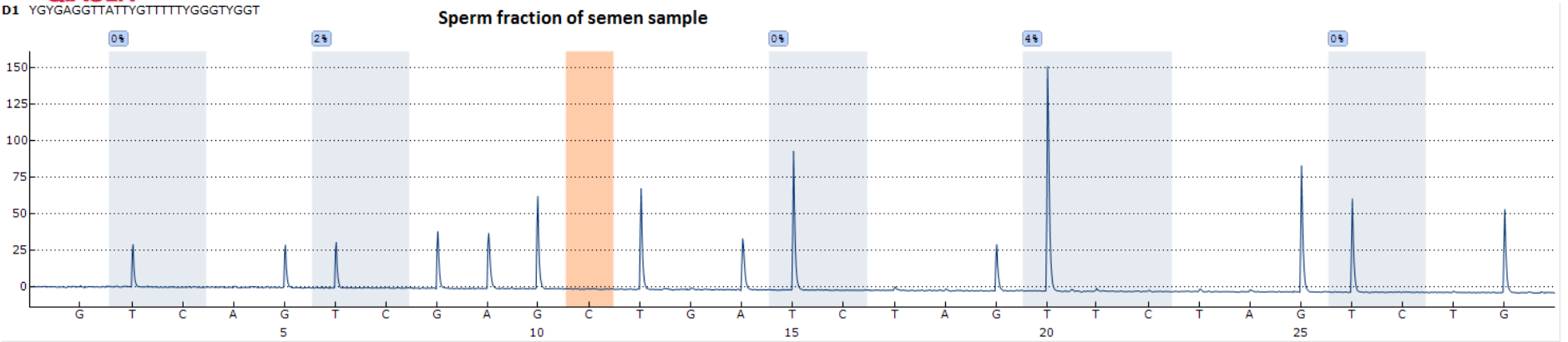
A5 YGYGAGGTTATTYGTGTTTTYGGGTYGGT

0.1 ng @ 1°C/s - Semen





Confirmation of sperm as ZC3H12D target



Methylation Percentages of Semen Dilutions using Semen Primer

| Well | Assay | SampleID | Pos. 1 | Pos. 2 | Pos. 3 | Pos. 4 | Pos. 5 |
|------|---------|-------------|---------|---------|---------|---------|---------|
| | | | Meth. % | Meth. % | Meth. % | Meth. % | Meth. % |
| C1 | ZC3H12D | Semen C 0.5 | 3.5 | 3.11 | 2.79 | 5.62 | 3.26 |
| C6 | ZC3H12D | Semen C 0.5 | 3.61 | 2.34 | 3.62 | 6.13 | 4.66 |
| D1 | ZC3H12D | Semen C 0.5 | 4.16 | 4.38 | 3.63 | 5.23 | 4.89 |
| C2 | ZC3H12D | Semen C 0.4 | 4.68 | 1.92 | 4.39 | 3.88 | 5.56 |
| C7 | ZC3H12D | Semen C 0.4 | 3.68 | 4 | 5.06 | 5.44 | 4 |
| D2 | ZC3H12D | Semen C 0.4 | 5.15 | 2.04 | 3.75 | 6.23 | 3.58 |
| C3 | ZC3H12D | Semen C 0.3 | 9 | 3.14 | 5.34 | 7.76 | 5.46 |
| C8 | ZC3H12D | Semen C 0.3 | 4.35 | 2.9 | 4.06 | 8.58 | 6.42 |
| D3 | ZC3H12D | Semen C 0.3 | 4.45 | 4.32 | 5.61 | 7.14 | 5.12 |
| C4 | ZC3H12D | Semen C 0.2 | 3.59 | 2.31 | 3.03 | 7.44 | 4.31 |
| C9 | ZC3H12D | Semen C 0.2 | 4.78 | 4.05 | 4.75 | 8.61 | 7 |
| D4 | ZC3H12D | Semen C 0.2 | 3.52 | 5.88 | 5.3 | 6.72 | 6.65 |
| C5 | ZC3H12D | Semen C 0.1 | 10.1 | 8.18 | 9.42 | 10.75 | 12.31 |
| C10 | ZC3H12D | Semen C 0.1 | 5.96 | 6.62 | 7.61 | 9.74 | 7.18 |
| D5 | ZC3H12D | Semen C 0.1 | - | - | - | - | - |

PyroSequencing Results and STR Results for Semen Dilutions

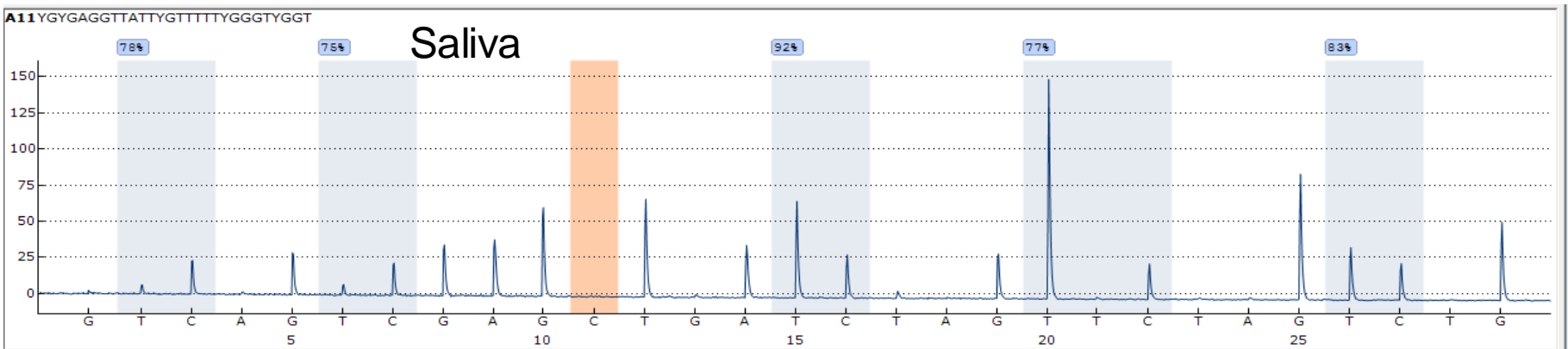
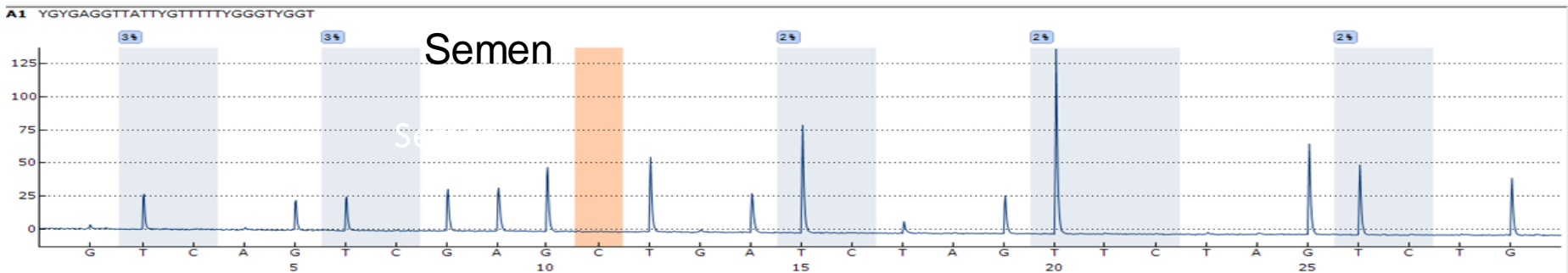
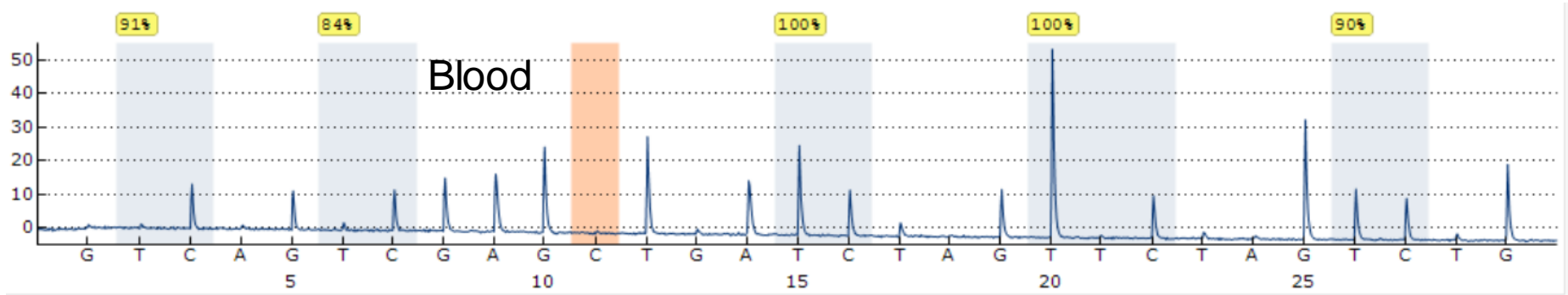
| Assay | SampleID | Meth. % | Meth. % | Meth. % | Meth. % | Meth. % | Total Vol Input (ul) | Quant (ng/ul) | Total Input DNA (ng) | No. of alleles | Full profile (Y/N) |
|----------------------------|--------------|---------|---------|---------|---------|---------|----------------------|---------------|----------------------|----------------|--------------------|
| Semen B_C dilutions | | | | | | | | | | | |
| 030618 | | | | | | | | | | | |
| ZC3H12D | Semen B NS | 44.15 | 42.02 | 45.23 | 50 | 41.6 | 0.6 | 1.68020 | 1.00812 | 40 | Y |
| ZC3H12D | Semen B SP | 6.84 | 4.79 | 6.03 | 6.5 | 7.54 | 0.5 | 2.32090 | 1.16045 | 40 | Y |
| ZC3H12D | Semen B 0.5 | 15.09 | 13.52 | 15.24 | 19.51 | 13.29 | 15 | 0.03260 | 0.48900 | 40 | Y |
| ZC3H12D | Semen B 0.5 | 5.82 | 3.81 | 3.52 | 6.4 | 10.85 | 15 | 0.03290 | 0.49350 | 40 | Y |
| ZC3H12D | Semen B 0.1 | 5.41 | 4.41 | 2.71 | 7.17 | 5.13 | 15 | 0.00860 | 0.12900 | 40 | Y |
| ZC3H12D | Semen B 0.1 | 30.26 | 25.51 | 30.5 | 9.12 | 31.28 | 15 | 0.00710 | 0.10650 | 40 | Y |
| ZC3H12D | Semen B 0.1 | 74.49 | 68.54 | 79.52 | 86.82 | 73.94 | 15 | 0.00620 | 0.09300 | 40 | Y |
| ZC3H12D | Semen B 0.05 | 91.37 | 81.26 | 97.6 | 12.23 | 92.15 | 15 | 0.00350 | 0.05250 | 40 | Y |
| ZC3H12D | Semen B 0.05 | 65.3 | 60.38 | 65.99 | 74.9 | 63.73 | 15 | 0.00290 | 0.04350 | 39 | N |
| ZC3H12D | Semen B 0.05 | 89.3 | 86.04 | 99.21 | 100 | 95.03 | 15 | 0.00280 | 0.04200 | 39 | N |
| ZC3H12D | Semen C NS | 2.97 | 3.29 | 3.72 | 5.15 | 3.67 | 0.1 | 10.15240 | 1.01524 | 42 | Y |
| ZC3H12D | Semen C SP | 3.34 | 2.73 | 3.75 | 4.3 | 4.88 | 0.12 | 8.68340 | 1.04201 | 42 | Y |
| ZC3H12D | Semen C 0.5 | 3.05 | 2.49 | 3.73 | 4.13 | 3.69 | 15 | 0.03070 | 0.46050 | 42 | Y |
| ZC3H12D | Semen C 0.5 | 3.72 | 2.75 | 5.35 | 4.18 | 5 | 15 | 0.02820 | 0.42300 | 42 | Y |
| ZC3H12D | Semen C 0.1 | 4.21 | 2.53 | 2.91 | 4.02 | 4.25 | 15 | 0.00520 | 0.07800 | 35 | N |
| ZC3H12D | Semen C 0.1 | 4.22 | 2.49 | 3.88 | 4.48 | 5.13 | 15 | 0.00570 | 0.08550 | 37 | N |
| ZC3H12D | Semen C 0.1 | 2.7 | 3.57 | 52.57 | 6.03 | 4.24 | 15 | 0.00570 | 0.08550 | 35 | N |
| ZC3H12D | Semen C 0.05 | 6.02 | 4.67 | 5.72 | 10.01 | 10.41 | 15 | 0.00330 | 0.04950 | 32 | N |
| ZC3H12D | Semen C 0.05 | 3.46 | 3.74 | 3.68 | 5.41 | 5.41 | 15 | 0.00220 | 0.03300 | 27 | N |
| ZC3H12D | Semen C 0.05 | 11.66 | 5.28 | 14.5 | 13.26 | 25.03 | 15 | 0.00220 | 0.03300 | 32 | N |
| ZC3H12D | NTC | - | - | - | - | - | NA | NA | NA | | |

PyroSequencing Results and STR Results for Mixtures

| | Semen C:Vag | Pos. 1 | Pos. 2 | Pos. 3 | Pos. 4 | Pos. 5 | | | | | |
|---------|-------------|---------|---------|---------|---------|---------|----------------------|---------------|-----------------------|------------------------|-------------------------|
| Assay | SampleID | Meth. % | Meth. % | Meth. % | Meth. % | Meth. % | Total Vol Input (ul) | Quant (ng/ul) | Target Input DNA (ng) | No. of foreign alleles | Full male profile (Y/N) |
| ZC3H12D | Mix 1 9:1 | 31.38 | 20.43 | 31.43 | 24.57 | 30.85 | NA | 0.05590 | 1 | 32 | Y |
| ZC3H12D | Mix 1 9:1 | 21.14 | 20.32 | 22.2 | 25.83 | 21.75 | NA | 0.06600 | 1 | 32 | Y |
| ZC3H12D | Mix 1 4:1 | 25.62 | 23.4 | 28.3 | 27.46 | 23.68 | NA | 0.07830 | 1 | 32 | Y |
| ZC3H12D | Mix 1 4:1 | 17.72 | 17.39 | 24.79 | 20.01 | 22.79 | NA | 0.08930 | 1 | 32 | Y |
| ZC3H12D | Mix 1 2:1 | 23.83 | 34.93 | 41.21 | 39.33 | 39.17 | NA | 0.07260 | 1 | 32 | Y |
| ZC3H12D | Mix 1 2:1 | 21.56 | 21.01 | 32.78 | 33.67 | 31.42 | NA | 0.07650 | 1 | 32 | Y |
| ZC3H12D | Mix 1 1:1 | 20.59 | 25.08 | 39.72 | 28.38 | 37.86 | NA | 0.05000 | 1 | 32 | Y |
| ZC3H12D | Mix 1 1:1 | 33.07 | 30.69 | 36.02 | 36.65 | 35.31 | NA | 0.04620 | 1 | 32 | Y |
| ZC3H12D | Mix 1 1:2 | 46.16 | 36.57 | 64.69 | 49.07 | 52.73 | NA | 0.05160 | 1 | 31 | N |
| ZC3H12D | Mix 1 1:2 | 39.4 | 53.76 | 55.52 | 48.92 | 60.72 | NA | 0.05380 | 1 | 32 | Y |
| ZC3H12D | Mix 1 1:4 | 61.86 | 54.91 | 66.23 | 57.78 | 61.94 | NA | 0.07920 | 1 | 28 | N |
| ZC3H12D | Mix 1 1:4 | 40.93 | 23.9 | 49.02 | 36.19 | 37.13 | NA | 0.05110 | 1 | 31 | N |
| ZC3H12D | Mix 1 1:9 | 54.32 | 55.34 | 58.85 | 50.69 | 57.49 | NA | 0.06760 | 1 | 27 | N |
| ZC3H12D | Mix 1 1:9 | 89.26 | 86.44 | 89.31 | 84.76 | 94.25 | NA | 0.05880 | 1 | 29 | N |
| ZC3H12D | NTC | - | - | - | - | - | NA | NA | NA | | |



Typical Pyrograms at .1 ng input DNA using Sperm Marker, ZC3H12D



Questions?

- **Thanks to Amy Lee and Peter St. Andre of SFPD for providing the Data**