



Evaluation of next generation sequencing platforms for forensic casework

Systematic sensitivity testing of Illumina's ForenSeq™ DNA Signature Prep Kit

August 3, 2016
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Illumina's ForenSeq DNA Signature Prep Kit

| Features: | # of Markers: |
|-------------------------------|---------------|
| Global STRs | 27 |
| Y STRs | 24 |
| X STRs | 7 |
| Identity SNPs | 94 |
| Phenotypic SNPs | 22 |
| Biogeographical Ancestry SNPs | 56 |

Total:
Kit A: 152
Kit B: 228

vs. Identifiler: 16 STRs
PowerPlex Fusion: 24 STRs



Systematic sensitivity testing of Illumina's ForenSeq™ Kit

Nine experimental runs A-I (32 samples)

- Concordance of STRs
- Sensitivity and Flow cell capacity



Systematic sensitivity testing of Illumina's ForenSeq™ Kit

Concordance for STRs by using PowerPlex Fusion: 9 male and 7 female samples

| Run | Comments | # of samples | Experiment: samples: DNA input | Primer Mix |
|-----|----------------------------|--------------|--|--|
| A | Concordance: M1-3, F1-3 | 32 | Concordance and Sensitivity: 3M and 3F: 800pg, 400pg, 200pg, 100pg, 50pg | Kit A (Lot#3206C085), not expired |
| B | Repeat of A | 32 | Concordance and Sensitivity: 3M and 3F: 800pg, 400pg, 200pg, 100pg, 50pg | Kit A (Lot#3206C085), not expired |
| C | Same samples as in A and B | 32 | Concordance and Sensitivity: 3M and 3F: 800pg, 400pg, 200pg, 100pg, 50pg | Kit B (Lot#3206C086), not expired |
| F | Concordance: M4-6, F4-6 | 32 | Concordance: 3M and 3F: ~1000pg | Kit A (Lot#20140818), not expired |
| H | Concordance: M7,8, F7 | 32 | Concordance: 2M and 1F: ~500pg | Kit A (Lot#20140818) expired for four months |
| I | Concordance: M9 | 32 | Concordance: 1M: 1000pg | Kit B (Lot#20140815) expired for nine months |



Systematic sensitivity testing of Illumina's ForenSeq™ Kit

Concordance for STRs by using PowerPlex Fusion: 9 male and 7 female samples

- **Concordance** was verified for all 16 samples for the common 24 STR loci using PowerPlex Fusion
- PentaD showed drop-outs (runs H and I, expired kits)

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Concordance for STRs by using PowerPlex Fusion: 9 male and 7 female samples

- **Consistent outcomes** was verified for remaining loci (5 a-, 23Y-, and 7 X-STRs)
- Expired kits (runs H and I): drop-outs at DXS10103, DXS10135, DYF387S1, DYS385a-b, DYS389II, DYS390, and DYS448

Three inconsistencies:

Run A: F3 at 800pg DNA 16 reads at DYS505 flagged "many alleles"

Run B: F3 at 400pg DNA 60 reads at DYS576 flagged "many alleles"

Run A: F1 at 100pg DNA: DXS10103: 15 (12 reads) 18 (13 reads), while at other concentrations and runs 16, 18 was obtained



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Sensitivity

| Run | Comments | # of samples | Experiment: samples: DNA input | Primer Mix |
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| B | Repeat of run A | 32 | Sensitivity: 3M and 3F: 800pg, 400pg, 200pg, 100pg, 50pg | Kit A (Lot#3206C085), not expired |
| C | Same samples as in runs A and B | 32 | Sensitivity: 3M and 3F: 800pg, 400pg, 200pg, 100pg, 50pg | Kit B (Lot#3206C086), not expired |
| D | | 32 | Sensitivity: 1M and 1F: 100pg, 50pg, 26 samples: 1000pg | Kit A (Lot#3206C085), not expired |
| E | Repeat of D | 32 | Sensitivity: 1M and 1F: 100pg, 50pg, 26 samples: 1000pg | Kit A (Lot#3206C085), not expired |
| G | Confirmation of sensitivity | 32 | Sensitivity: 3M and 3F: ~1000pg, ~500pg, ~200pg, ~100pg, ~50pg | Kit A (Lot#20140818) expired for one month |



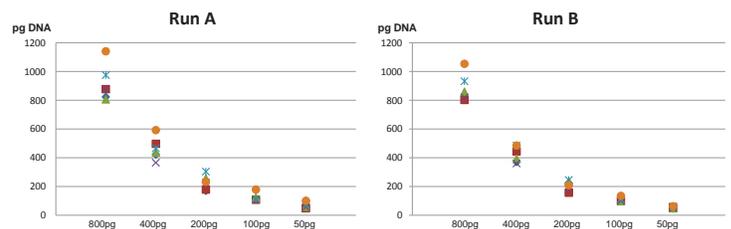
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Sensitivity

Concentration of dilutions measured using Quantifiler® Trio
Six samples per DNA input: 800pg, 400pg, 200pg, 100pg, 50pg
Duplicate measurements, average shown
Newly diluted

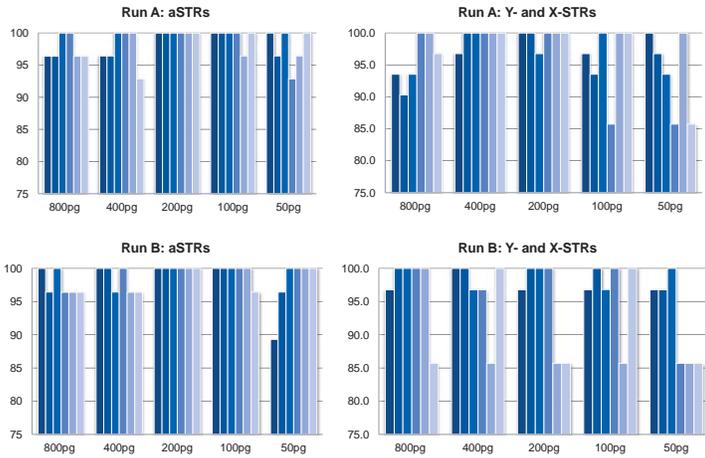


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Sensitivity: correct outcome of six samples at various dilutions in two runs



Sensitivity

- Correct outcomes for all DNA inputs, including 50pg
- At higher DNA input: allele drop in – **editable**
 - D7S820 adding of T at end of sequence:
GATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGATTG
ATAGTTTT
 - Typed
 - < 4% of true allele
 - 800pg DNA input: Run A and B: 4 of 6 samples
 - 400pg DNA input: Run A: 3 of 6 samples; Run B: 2 of 6 samples



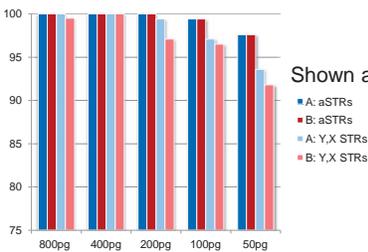
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Sensitivity

- Correct outcomes for all DNA inputs, including 50pg
- At higher DNA input: allele drop in (insertion of 1nt at D7S820; < 4% of true allele) - **editable**
- At lower DNA input: allele drop out: D1S1656 and DXS10103, and less frequent at Amelogenin and vWA



Shown average of 6 samples:

■ A: aSTRs
■ B: aSTRs
■ A: Y,X STRs
■ B: Y,X STRs



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Flow cell capacity: Kit A vs Kit B

| Run | Comments | # of samples | Experiment: samples: DNA input | Primer Mix |
|-----|---------------------------------|--------------|--|-----------------------------------|
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| C | Same samples as in runs A and B | 32 | Sensitivity: 3M and 3F: 800pg, 400pg, 200pg, 100pg, 50pg | Kit B (Lot#3206C086), not expired |

Total # of markers:

Kit A: 152

Kit B: 228

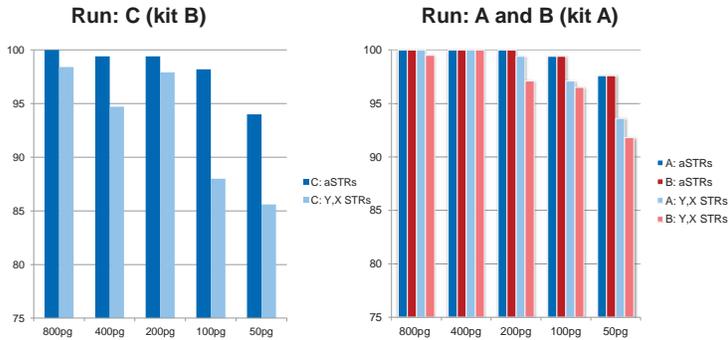


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Flow cell capacity: Kit A vs Kit B

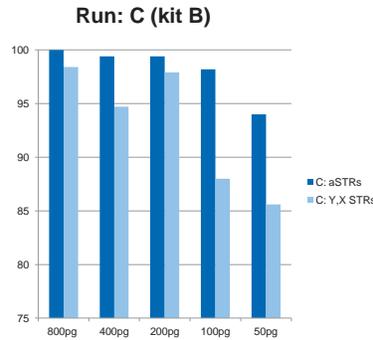


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Flow cell capacity: Kit A vs Kit B



- 200pg DNA lowest input that showed 100% correct outcome
- Drop outs: PentaD, D1S1656, DX10103, DYS385a-b, and DYF387S1



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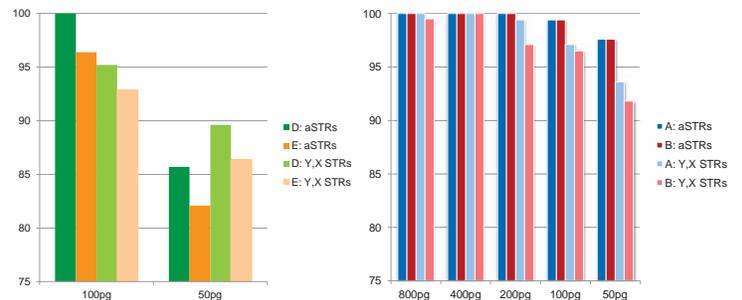
Flow cell capacity: Total DNA/run

- Recommended number of samples per run for casework: 32, including positive (M2800) and negative (H₂O) controls
- 31 samples at 1ng DNA = 31ng DNA (genomic DNA input per run)

| Run | Comments | # of samples | Total DNA [pg] | Experiment: samples: DNA input | Primer Mix |
|-----|---------------------------------|--------------|----------------|---|----------------------------------|
| A | | 32 | 10,300 | Sensitivity: 3M and 3F: 800pg, 400pg, 200pg, 100pg, 50pg | Kit A (Lot#3206C085) not expired |
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| D | | 32 | 27,300 | Sensitivity: 1M and 1F: 100pg, 50pg 26 samples: 1000pg | Kit A (Lot#3206C085) not expired |
| E | Repeat of D | 32 | 27,300 | Sensitivity: 1M and 1F: 100pg, 50pg 26 samples: 1000pg | Kit A (Lot#3206C085) not expired |

Flow cell capacity: Total DNA/run

Run: D and E (kit A: 27,300pg DNA) Run: A and B (kit A: 10,300pg DNA)



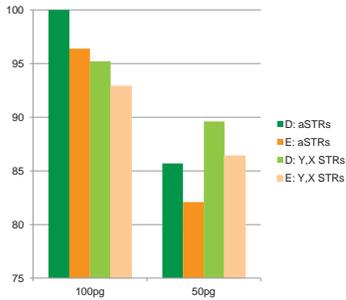
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Flow cell capacity: Total DNA/run

Run: D and E (kit A: 27,300pg DNA)



- Allele drop out and locus drop out occurred more often
- Amelogenin, vWA, PentaE, CSF1PO, TPOX, and DXS10103



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Outcomes

- Concordance was verified for all 16 samples for the common 24 STR loci using PowerPlex Fusion
- The ForenSeq™ DNA Signature prep Kit can be used reliably to obtain full outcomes from lower DNA inputs than the recommended 1000pg DNA.
- The outcomes of single samples do not merely depend on their DNA input but also on the number of targets (Kit A vs Kit B) and on the total DNA used for one experimental run.



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Thank you



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