IonTorrent at LUMC

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OVERVIEW
A brief summary of the Ion Torrent PGM technology

- Release of hydrogen ion as a by-product of nucleotide incorporation by DNA polymerase
- Charge from ion changes the pH of the solution
- The base is called based on the incorporated nucleotide and the voltage change
- Multiple incorporation will be reflected in a higher voltage change
- Empty well is identified by the signal delay compared to the neighbouring wells during washing NaOH (Sodium Hydroxide)
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Signal processing, base calling, and quality assessment
→ Nucleotide incorporation signal is a Poisson distribution of empirically derived nucleotide-specific parameters (see table below)

→ **Base position** within the read from the start of the sequence
→ **Local noise** in the immediate neighbourhood of a given base
→ **Read noise** as a peak-normalised expression of all 1-mers & 0-mers avg. and std.
→ **Multiple incorporation weights**, 1 for all (n-1)mers and n for the last incorporated base
→ **Phase error**, the number of incorporations of the same nucleotide in the previous flow
→ **Environment noise** in the larger (±10) neighbourhood of a given base

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T</th>
<th>A</th>
<th>C</th>
<th>G</th>
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</thead>
<tbody>
<tr>
<td>$K_{r\text{default}}$</td>
<td>15</td>
<td>16</td>
<td>20</td>
<td>25</td>
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<tr>
<td>$K_r$</td>
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<td>1.252</td>
<td>1.252</td>
<td>1.252</td>
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<tr>
<td>$K_m$</td>
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<td>20</td>
<td>17</td>
<td>18</td>
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<tr>
<td>$D$</td>
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<td>189.618</td>
<td>227.021</td>
<td>188.48</td>
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<tr>
<td>$t_0$ delay</td>
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<td>1.78</td>
<td>0</td>
<td>0.17</td>
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<tr>
<td>$\sigma_{mult}$</td>
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<td>1.124</td>
<td>1</td>
<td>0.8533</td>
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Genome Engineering by TALENs
Engineering of biological systems that recapitulate human genetic disorders relies on efficient manipulation of the genome. Transcription activator-like effector nucleases (TALENs) have shown promising potential in site-specific genome editing. We have targeted intron 52 of hDMD transgene in TALENs-transfected mouse Embryonic Stem Cells.
TALENs
Targeting Intron 52 of hDMD

TALENs:
→ Modular structure: enables design and simple construction
→ Specificity: recognise virtually any DNA sequence
→ Initiate double strand break that is repaired by non-homologous end-joining, introducing a large variety of mutations
→ Variable efficacy due to lack of selection procedure
TALENs
Targeting Intron 52 of hDMD

PGM Sequencing and Analysis:
→ 135bp locus of hDMD was PCR-amplified and sequenced for 100,000 TALENs transfected and non-transfected cells
→ Coverage of > 450,000x
→ Assembled the occurrence and coupling of mutations introduced by TALENs compared to controls
TALENs
Targeting Intron 52 of hDMD

Editing events:
→ Mainly small insertions and deletions
→ 4-fold higher rate of observed mutations as compared to the control experiment
Clinical Genetics: Variant Detection/Validation
6 loci of interest, looking for mutations

100 patients, efficient barcoding and pooling of samples

Single barcode setup: $6 \times P1 + (6 \times A) \times 100$ barcode = 606 primers
6 loci of interest, looking for mutations

100 patients, efficient barcoding and pooling of samples

New barcode setup: $6 \times \text{A-BC-M13} + 60 \times \text{M13-F-BC} + 6 \times \text{R-P1} = 76$ primers
6 loci of interest, looking for mutations

100 patients, efficient barcoding and pooling of samples

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<thead>
<tr>
<th>Mutation</th>
<th># Patients</th>
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<tr>
<td>Type 02</td>
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<tr>
<td>Type 03</td>
<td>1</td>
</tr>
<tr>
<td>Type 04</td>
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<tr>
<td>Type 14</td>
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<td>Normal</td>
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Bizarre Systematic-Errors in IonTorrent
Acknowledgement

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Johan den Dunnen