Benefits of Agilent Exomes
Agilent baits are of high quality as shown by the errors per kb

**Oligo Synthesis Fidelity**

\[
\%FL = (CY \times DY)^{nt}
\]

\%FL = %Full Length  
CY = Synthesis Cycle Yield  
DY = Depurination Cycle Yield

- Agilent baits have ~3x fewer errors per kb and are full length compared to conventional synthesis
- Every Agilent exome lot is going through Quality-Control (QC) to check for coverage, % on-target, dupe rates etc.

**No need to QC individual oligos as our bait production is robust and accurate**
Agilent exomes provide comprehensive coverage of relevant databases

With marginally more sequencing, Agilent V6 and CREv2 exomes provide better coverage of critical databases

Agilent exomes are more comprehensive
Agilent exomes target more disease-relevant content

For Research Use Only. Not for use in diagnostic procedures.
Agilent exomes efficiently capture GC-rich regions

- Vendor ID claims that only Vendor ID can get coverage of high GC exons, but they are only showing the data from older Agilent exome.
- Newer Agilent exomes provide robust coverage of high-GC regions.

76% GC

Agilent CREv2
Agilent exomes efficiently capture AT-rich regions

All exomes sequenced to the same average sequencing depth
Why do Agilent exomes have less GC bias?

Difference between Vendor ID and Agilent baits

Vendor ID bait design

Agilent bait design

Exon

How do we do our bait design?

Target

Find best region to place the bait for every given target based on: 1. Mappability; 2. GC content

This adds marginally more sequencing footprint but results in less GC bias
Agilent exomes have excellent on-target and duplicate rates

<table>
<thead>
<tr>
<th>Design</th>
<th>On Target Rate</th>
<th>Duplicate Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SureSelect V6</td>
<td>78%</td>
<td>8%</td>
</tr>
<tr>
<td>SureSelect CRE V2</td>
<td>76%</td>
<td>10%</td>
</tr>
<tr>
<td>Vendor R</td>
<td>62%</td>
<td>17%</td>
</tr>
<tr>
<td>Vendor ID</td>
<td>80%</td>
<td>13%</td>
</tr>
</tbody>
</table>

Mean of 8 samples with 100x sequencing; Agilent exomes represent SureSelect XT workflow.

- Vendor ID claims 30% difference in on-target but data shown is from older exomes.
- With new Agilent exomes, the difference is <5%.
- Agilent exomes have better duplicate rates.

High on-target rates and lower duplicate rates ensure maximum use of sequencing data.
Agilent exomes provide excellent depth of coverage

<table>
<thead>
<tr>
<th>Bases covered at</th>
<th>SureSelect V6</th>
<th>SureSelect CREv2</th>
<th>Vendor ID</th>
<th>Vendor R</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x</td>
<td>97%</td>
<td>96%</td>
<td>98%</td>
<td>97%</td>
</tr>
<tr>
<td>10x</td>
<td>95%</td>
<td>94%</td>
<td>97%</td>
<td>95%</td>
</tr>
<tr>
<td>20x</td>
<td>91%</td>
<td>89%</td>
<td>92%</td>
<td>85%</td>
</tr>
<tr>
<td>30x</td>
<td>83%</td>
<td>80%</td>
<td>82%</td>
<td>67%</td>
</tr>
</tbody>
</table>

Mean of 8 samples with 100x sequencing; Agilent exomes represent SureSelect XT workflow.

- Vendor ID claims better uniformity. However, older exome data is shown.
- New Agilent exomes provide comparable coverage at 20x and 30x depth of coverage.

Agilent Technologies
Agilent exomes provide better PPV as indicated by comparative analysis

Sensitivity or True Positive Rate = \( \frac{TP}{TP + FN} \) and PPV or Precision = \( \frac{TP}{TP + FP} \).

Desired: Lower FN, FP and Higher Sensitivity and PPV

- Vendor R library prep Vendor ID exome provides lower indel PPV (higher false positives) than Agilent exomes.
- SNP & indel sensitivity are similar across all exomes
Agilent exomes provide excellent overall solution to target enrichment needs

<table>
<thead>
<tr>
<th></th>
<th>Vendor ID</th>
<th>Agilent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bait Quality</td>
<td>ESI mass spec every probe because they have to.</td>
<td>Our high fidelity DNA printing process obviates the need to QC every oligo. And we robust QC of every lot of exomes ensures consistency.</td>
</tr>
<tr>
<td>Sequencing requirements</td>
<td>Needs less sequencing but the trade-off is incomplete disease-associated content and performance across low GC regions</td>
<td>With marginally more sequencing, Agilent exomes provide 1. better content, 2. bait design that helps in extreme high/low GC regions.</td>
</tr>
<tr>
<td>On-target and duplicate rates.</td>
<td>Slightly better on-target rates but higher duplicate rates</td>
<td>Excellent on-target and duplicate rates.</td>
</tr>
<tr>
<td>Uniformity</td>
<td>Comparable to Agilent exomes.</td>
<td>Excellent coverage and sensitivity/PPV.</td>
</tr>
</tbody>
</table>