Introduction

The number of useable bases per SMRT Cell can exceed 1 Gb with the latest P6-C4 chemistry and 6-hour movies. For applications such as microbial sequencing, targeted sequencing, Iso-Seq full-length isoform sequencing and Roche NimbleGen’s target enrichment method, current PacBio RS II SMRT Cell yields could be in excess relative to project requirements.

To this end, barcoding is a viable option for multiplexing samples. For microbial sequencing, multiplexing can be accomplished by tagging sheared genomic DNA during library construction with SMRTbell barcoded adapters. Results from 2-, 4-, 6- and 8-plex will be presented.

For HLA typing, full-length HLA genes as large as 5 kb may be barcoded during amplification or during SMRTbell library construction. The preferred barcoding strategy depends on the user’s existing workflow and flexibility for changing and/or updating existing workflows. Five Class I/II genes (3.3 – 5.8 kb) x 96 patients can be multiplexed and typed in up to 4 SMRT Cells.

For Iso-Seq analysis, barcodes are incorporated during first-strand synthesis using oligo-dT tailed with 16-bp barcodes. Six maize RNA samples were barcoded, pooled and constructed into a single SMRTbell library.

Finally, for target enrichment using NimbleGen’s SeqCap EZ Target Enrichment method, linear barcodes are incorporated before the capture step. Results from a 12-plex target enrichment experiment will be presented.

Multiplexing for Microbial Assembly

| Sheared Microbe | 1, 3 |
| Single Tube End Repair and Ligation |
| PCR and Microbial Libraries |
| DNA Damage Repair |
| E. coli and V7 |
| Primer Annealing, Polymerase Binding and Selecting |

Figure 1:
A. Workflow for multiplexing microbial genomes
B. Barcodes are added to the SMRTbell adapters.
C. Recommended barcoded adapters available from PacBio.

MICROBIAL WHOLE GENOME ASSEMBLY OF MULTIPLEXED H. PYLORI STRAINS

Figure 2: N50 contig vs Multiplex level of sequenced H. pylori strains using 10 kb library preparations, P6-C4 chemistry, and sequenced on the PacBio RS II. Increasing P1 loading helped achieve higher multiplex level to 6, possibly 8.

Targeted Sequencing Multiplexing

<table>
<thead>
<tr>
<th>Sample Set</th>
<th>HLA Loci</th>
<th>Amplicon Size</th>
<th>Targeted Region</th>
<th>SMRT Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Class I (A/B/C)</td>
<td>~3.3 kb</td>
<td>Full Length</td>
<td>1</td>
</tr>
<tr>
<td>48</td>
<td>Class I (A/B/C)</td>
<td>~3.3 kb</td>
<td>Full Length</td>
<td>1</td>
</tr>
<tr>
<td>96</td>
<td>Class I (A/B/C)</td>
<td>~3.3 kb</td>
<td>Full Length</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>Class II (DR/DQ)</td>
<td>~3.3 to 5.8 kb</td>
<td>Partial</td>
<td>1</td>
</tr>
<tr>
<td>48</td>
<td>Class II (DR/DQ)</td>
<td>~3.3 to 5.8 kb</td>
<td>Partial</td>
<td>2</td>
</tr>
<tr>
<td>96</td>
<td>Class II (DR/DQ)</td>
<td>~3.3 to 5.8 kb</td>
<td>Partial</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 3: Barcoding options for targeted sequencing. A. Barcoded Universal Primers: Barcodes can be incorporated into the amplicon via a two-step tiled primer approach. Barcodes are commercially available from PacBio.
B. Barcoded Adapters: Barcodes are incorporated during ligation with barcoded adapters. Barcodes are commercially available from PacBio.
C. Locus-specific primers tagged with barcodes. Primers may be ordered from any oligo synthesis provider. The first 96 barcodes out of 384 available sequences are available.

Figure 4:
A. GenDx HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 are amplified and pooled for barcoding. All 96 wells are subsequently pooled into a single tube for SMRTbell library construction.
B. Recommendations for multiplexing NGS-go® amplicons.

Isoform Sequencing Multiplexing – cont.

GENOME ANNOTATION OF MULTIPLEXED MAIZE TISSUES

Figure 6: Bioanalyzer traces of barcoded cDNA from 6 Maize tissues prior to pooling.

Figure 7:
A. Full-length read distribution from pooled maize library.
B. Full-length yield summary per fraction per sample.

Target Enrichment Multiplexing

MULTIPLEXING WITH NIMBLEGEN NEUROLOGY PANEL

Figure 8: Target enrichment multiplex workflow. Sheared DNA samples are tagged with linear barcoded adapters. Workflow in development. For information: www.pacb.com/products-and-services/consumables/target-enrichment.

Figure 9: Multiplex of 12 samples. Number of mapped substrands of 5 kb target enrichment using Nimblegen’s neurology panel.

Figure 10: A 60 kb fragment showing intron-exon regions of the NPC1 gene.

Conclusions

- Variety of barcoding methods available for multiplexing samples for microbial whole genome sequencing, HLA typing, isoform sequencing, and targeted sequencing applications with PacBio Systems.
- Barcoding increases efficiency and sample throughput thus reducing the cost of library preparation and sequencing.

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