**Introduction**

Deep, massively parallel sequencing of cDNA libraries (RNA-Seq) is rapidly gaining momentum for transcript profiling, discovery of novel transcripts, and identification of alternative splicing events. Methods for making next-generation sequencing specific cDNA libraries typically comprise preparing rRNA-depleted RNA followed by RNA fragmentation, cDNA synthesis, 5′-and 3′-adaptor ligation, and multiple clean-up steps. These methods are generally time-consuming, requiring about 2 days and significant hands-on time.

Here, we present an improved, more-automated version of the ScriptSeq™ v2 RNA-Seq method (ScriptSeq v2). The rRNA-depleted RNA from 100 ng to 5 µg was isolated from tissues using Ribo-Zero Gold Kit for removal of both intact and fragmented RNA samples. The purified rRNA-depleted RNA is then input into the ScriptSeq v2 workflow for directional RNA-Seq library generation. Following library synthesis, the purified di-tagged library is quantified and is readily ready for clustering and sequencing. The entire process can be readily completed in a standard work day.

**Methods Overview**

The ScriptSeq v2 process is completed in less than 8 hours, with no intermediate purification steps from RNA to di-tagged cDNA fragments.

**Results**

Efficient removal of rRNA from both intact and fragmented total RNA.

Dilute 3-µg aliquots of either intact or partially fragmented Universal Human Reference (UHR) total RNA was treated (single-pass) with the Ribo-Zero RNA Removal Kit (H/M/R). The rRNA-depleted RNA from 100 ng equivalent total RNA was then used as input for the ScriptSeq v2 kit. Each library was enriched by PCR for 15 cycles. Single-end sequencing (5′-nt reads) was performed on an Illumina® GAIIx instrument. The data were analyzed by CASAVA 1.7.

**Table 2. ScriptSeq™ v2 library metrics with Ribo-Zero™ rRNA-depleted FFPE RNA samples.**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Total RNA Yield</th>
<th>Uniquely Mapped Mitochondrial rRNA</th>
<th>Directionality (7000 genes)</th>
<th>Directionality (32928565 reads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFPE RNA (A)</td>
<td>138.0 ng (40.2%)</td>
<td>66.3%</td>
<td>95.13%</td>
<td>98.39%</td>
</tr>
<tr>
<td>FFPE RNA (B) + Ribo-Zero GOLD Kit</td>
<td>227.83 ng (65.8%)</td>
<td>66.3%</td>
<td>95.13%</td>
<td>98.39%</td>
</tr>
<tr>
<td>FFPE RNA (B) + Ribo-Zero GOLD Kit, Intact UHR (rep 2)</td>
<td>227.83 ng (65.8%)</td>
<td>66.3%</td>
<td>95.13%</td>
<td>98.39%</td>
</tr>
</tbody>
</table>

**Summary**

- **Ribo-Zero RNA Removal**
  - Highly efficient “single-pass” removal of rRNA from both intact and fragmented (e.g., FFPE) RNA samples (100 ng to 5 μg total RNA input).
  - Enables recovery of both polyA+ and nonribosomal polyA+ transcripts.
  - Excellent correlation (R = 0.925) between polyA+ selected and Ribo-Zero-treated RNA samples (data not shown).
  - Kits for human/mouse/tat (mammalants), bacteria, and plant are currently available (www.epicentre.com).

- **ScriptSeq v2 Library Preparation**
  - Simple and sensitive ligation-free, directional RNA-Seq library preparation method in under 4 hours from FFPE-depleted RNA.
  - High-quality RNA-Seq libraries from either intact or fragmented (e.g., FFPE) RNA samples.
  - Excellent strand preservation (>98%) and transect coverage.
  - High correlation (R = 0.925) with MAQC microarray data set.
  - Compatible with Illumina GAII and HiSeq® instruments with barcoding option available.