Due to the high optical efficiency of the Optical Adhesive Cover it is strongly recommended that you adjust the CCD camera exposure time on the 7700 Sequence Detection System prior to use. Failure to do so may cause CCD camera saturation resulting in compromised quantitation data.

Click the Show Analysis button. Under Diagnostics in the Instrument menu, select the Advanced Options dialog box.

Check the Set 7700 Exposure Time for Plates box and type in the value “10”. Click OK. Ignore the warning message if this is the only change made.

For the ABI PRISM 7000, 7700 and 7900HT Sequence Detection System:

- Launch the Sequence Detection Systems instrumentation software.
- Using the diagram below set up a real-time document with FAM as the reporter dye (make sure to set up the unknown populations as two separate 5K and 10K replicate populations). For further information on the plate set-up procedure refer to the appropriate Sequence Detection Systems instrumentation User Guide.

Set up the following Thermal Cycler Conditions:

<table>
<thead>
<tr>
<th>THERMAL CYCLER</th>
<th>TIMES AND TEMPERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INITIAL STEPS</td>
<td>EACH 40 CYCLES</td>
</tr>
<tr>
<td>MELT</td>
<td>ANNEAL EXTEND</td>
</tr>
<tr>
<td>HOLD 2 min 50°C</td>
<td>HOLD 10 min 50°C</td>
</tr>
<tr>
<td>ANNEAL 15 sec 60°C</td>
<td>CYCLE</td>
</tr>
</tbody>
</table>

- Always wear talc-free gloves when handling the RNase P plate.
- Remove the RNase P plate from the freezer and remove the foil packaging. (Note: remove the plate from the foil packaging immediately prior to use to minimize exposure to light).
- Briefly centrifuge the plate to force all reagents to the bottom of the wells and eliminate any air bubbles from the mixture.
• Place the compression pad (grey side down) on top of the sealed reaction plate. (7700 system ONLY)
• Transfer the sealed plate to the sequence detector sample block.
• Run the thermal cycling protocol.

Data Analysis
• After completion of the run analyze the data.
• Adjust the threshold value to the point within the exponential phase of the logarithmic scale amplification plot where the least variability is observed within replicate populations. The exponential phase occurs within the range of data points that increase linearly when graphed in this plot. (Note: to evaluate the threshold value where the least variability is observed it is recommended to look at each replicate population individually. Viewing all 96 data points at the same time may make an accurate adjustment of the threshold value difficult). For further information on data analysis refer to the appropriate Sequence Detection Systems instrumentation User Guide.
• The install specification of the 7700, 7900HT and 7000 systems demonstrates the ability to distinguish between 5,000 and 10,000 genomic equivalents with a 99.7% confidence level for a subsequent sample run in a single well. The following equation verifies the 7700, 7000HT and 7000 systems install specifications:

\[
[(\text{CopyUnk.1}) - 3 (\text{STDev.CopyUnk.1})] > [(\text{CopyUnk.2}) + 3 (\text{STDev.CopyUnk.2})]
\]

where:
* \(\text{CopyUnk.1} = \) The Average Copy Number of Unknown #1 (10K replicate population)
* \(\text{STDev.Unk.1} = \) The Standard Deviation of Unknown #1 (10K replicate population)
* \(\text{CopyUnk.2} = \) The Average Copy Number of Unknown #2 (5K replicate population)
* \(\text{STDev.Unk.2} = \) The Standard Deviation of Unknown #2 (5K replicate population)

Note: up to 6 wells from each replicate group can be ignored to meet specification.

*These values can easily be obtained from the experimental report window.

FOR MORE INFORMATION, VISIT OUR WEB SITE: www.appliedbiosystems.com

NOTICE TO PURCHASER: LIMITED LICENSE

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Safety Considerations
User releases Applied Biosystems from any claims or liability arising from user’s activities in the use, handling, or storage of reagents.
GUIDE LINE, INSERT FOLD INSTRUCTIONS

1. Specification is intended for Half Sheet inserts, two sheet max.
2. Material: 50# Offset (White)
3. Dimensions: Half Sheet (5 ½” x 8 ½”) folded size = 1.833” x 2.125” +/- .125”
4. Print on one sheet, front and back.
5. After final folding, upper left-hand corner of page 1 is to face out, with title and part number displayed.
6. Folded inserts to be packaged as follows:
   - Bundled in groups of 10ea.
   - Shrink-wrap 10ea groups for 100 inserts total.
   - Label shrink-wrapped bundles [Part# & Rev (font large enough to be legible), Qty, Manufacturer, and Barcode Part #128C].
   - Seal shrink-wrapped bundles in an appropriately sized corrugated shipper (not to exceed 25#, or have outside dimensions greater than 12” x 13 ½” x 24”).

NOTICE TO PRINTER
Print page(s) prior to this page ONLY.

This page is folding instructions which is NOT and MUST NOT BE PRINTED as part of this insert.