1.3.1.2 Bacterial Kidney Disease
Appendix 2
Quantitative PCR for Detection of
*Renibacterium salmoninarum*

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A. Reference
The assay detects a 69-base pair sequence of the gene encoding the p57 protein (MSA) of *Renibacterium salmoninarum*. The protocol is modified from:

B. Primer sets

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence 5’ to 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS 1238 F</td>
<td>GTGACCAACACCCAGATATCCA</td>
</tr>
<tr>
<td>RS 1307 R</td>
<td>TGCAGACCACCATTTACC</td>
</tr>
<tr>
<td>RS 1262 MGB probe</td>
<td>6FAM-CACCAGATGGAGCAAC-NFQ/MGB</td>
</tr>
</tbody>
</table>

1^The minor groove binder (MGB) probe is proprietary technology of Applied Biosystems Inc. (1 800-327-3002; www.appliedbiosystems.com).
C. Quantitative PCR amplification

Bulk reaction formulation for qPCR reaction

<table>
<thead>
<tr>
<th>PCR Reagents</th>
<th>Final Conc.</th>
<th>Stock Conc.¹</th>
<th>Vol. per Reaction</th>
<th>Vol. for ___ Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial Master Mix²</td>
<td>1X</td>
<td>2X</td>
<td>12 μL</td>
<td></td>
</tr>
<tr>
<td>Forward Primer</td>
<td>0.9 μM</td>
<td>45 μM</td>
<td>0.48 μL</td>
<td></td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>0.9 μM</td>
<td>45 μM</td>
<td>0.48 μL</td>
<td></td>
</tr>
<tr>
<td>TaqMan® MGB Probe</td>
<td>0.25 μM</td>
<td>6 μM</td>
<td>1.0 μL</td>
<td></td>
</tr>
<tr>
<td>Exo IPC Mix³</td>
<td>1X</td>
<td>10X</td>
<td>2.4 μL</td>
<td></td>
</tr>
<tr>
<td>Exo IPC DNA³</td>
<td>1X</td>
<td>50X</td>
<td>0.48 μL</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
<td>2.16 μL</td>
<td></td>
</tr>
<tr>
<td>DNA⁴</td>
<td>10-100 ng</td>
<td>-</td>
<td>5 μL</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total⁵</strong></td>
<td></td>
<td></td>
<td><strong>24 μL</strong></td>
<td></td>
</tr>
</tbody>
</table>

¹Change “stock concentration” parameters as necessary, depending on reagent source.
²Protocol assumes commercial master mix (e.g. TaqMan® Gene Expression Master Mix from Applied Biosystems Inc.) contains uracil-N-glycosylase (UNG or UDG) (Section 2, 6.2.A.9.a). Master mix should be tested for effects on PCR sensitivity.
³Commercial exogenous internal positive control (Exo IPC) is used to detect PCR inhibition that can lead to false negative results (Section 2, 6.2.A.8.c). Reagents include the Exo IPC mix (primers/probe) and the Exo IPC DNA (target DNA). Because the addition of an IPC creates a multiplex reaction that can reduce PCR sensitivity, the effect of an IPC on PCR sensitivity must be empirically evaluated. The IPC from Applied Biosystems Inc. (part # 4308323) does not reduce assay sensitivity and is suitable for use in this protocol.
⁴Always include a well with water only (no template control).
⁵This volume can be used for both 96-well and 384-well plates.

1. In the PCR reagent set-up area (Section 2, 6.2.A.6.a), add PCR reagents except the template DNA into the “Bulk Reaction” tube. See Table for bulk reaction formulation. Make extra master mix to compensate for loss associated with the repeat pipet.

2. Aliquot 19 μL of bulk reaction to each well using a repeat pipet into the 96 or 384-well plate. Add 5 μL of water to no template controls (NTC).

3. Move to DNA template area (Section 2, 6.2.A.6.b) and add 5 μL of extracted DNA to each well. Add DNA standards and seal plate.

4. Centrifuge plate for 3 minutes at about 1500 x g to eliminate air bubbles in wells.

5. Program real-time PCR instrument with the following parameters:
   Initial incubation of 50°C for 2 minutes (for activation of uracil N-glycosylase).

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Incubation at 95°C for 10 minutes (for deactivation of uracil N-glycosylase).
Amplification for 40 cycles of the following profile:
  a. Denaturing at 95°C for 15 seconds.
  b. Anneal/Extend at 60°C for 60 seconds.

6. For further discussion of controls that should be included, refer to PCR Quality Assurance/Quality Control (Section 2, 6.2.A.8).

D. Analysis

1. Refer to quantitative PCR documentation for the instrument platform used to analyze run results.

2. To analyze qPCR results for evidence of decreased amplification indicative of PCR inhibition, refer to the Exo IPC manufacturer’s instructions.

3. A procedure for preparation of absolute quantitative standards from *R. salmoninarum* genomic DNA extracted from a pure bacterial culture is described by Chase et al. (2006).

E. Disclaimer:

The use of trade, firm, or corporation names in this protocol is for the information and convenience of the reader. Such use does not constitute an official endorsement by the U.S. Geological Survey or the Department of the Interior of any product or service to the exclusion of others that may be suitable.