

## Standard Operating Procedure (SOP)

### Title: DNA Extraction for PCR-based Detection of *Renibacterium salmoninarum*

**Number:** BACT-5

**Version:** 01 Created April 15, 2011

<b>Approval:</b>	<b>Date:</b>
Bacteriology Supervisor:	April 30, 2011

Effective Date:	April 30, 2011
Document Replaced:	
Reason for Revision:	
Area of Application:	

**Purpose:** Recommended DNA extraction procedure of fish tissues and ovarian fluids for PCR-based detection of *Renibacterium salmoninarum*

**Sections:**

- I. Background
- II. References
- III. Materials
- IV. Procedure

**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 or approval by the U.S. Department of Interior or the U.S. Geological Survey of any product or service to the exclusion of others that may be suitable.

## I. Background

This standard operating procedure (SOP) is a modification of the instructions provided by the manufacturer of the Qiagen<sup>§</sup> DNeasy Blood and Tissue Kit. This procedure incorporates lysozyme to enhance breakdown of the gram-positive cell wall. This procedure uses the single spin column format. Extraction in 96-well format is prone to cross-contamination. If users choose the 96-well format, they should carefully evaluate cross-contamination by interspersing positive and negative processing controls throughout the plate (processing controls described in Section II).

\*Safety: Buffer AL and Buffer AW1 contain guanidine hydrochloride and should not come in contact with bleach, acidic solutions or commercial DNA degrading solutions. Always clean spills with water first before applying bleach or DNA degrading solution. Poison information in English is available from Qiagen (Mainz, Germany) Tel: +49- 6131-19240.

## II. References

DNeasy Blood and Tissue Handbook; available one from Qiagen Inc<sup>§</sup>.

Chase, D. M., and R. J. Pascho. 1998. Development of a nested polymerase chain reaction for amplification of a sequence of the p57 gene of *Renibacterium salmoninarum* that provides a highly sensitive method for detection of the bacterium in salmonid kidney. *Diseases of Aquatic Organisms* 34:223-229.

Chase, D. M., D. G. Elliott, and R. J. Pascho. 2006. Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. *Journal of Veterinary Diagnostic Investigations* 18:375-380.

<sup>§</sup>Qiagen Inc.: 1-800-426-8157; [www.qiagen.com](http://www.qiagen.com)

### III. Materials

**DNeasy Blood and Tissue Kit:** Purchase commercially from Qiagen Inc<sup>§</sup> (part # 69506)

**95-100% ethanol:** Reagent quality non-denatured 95-100% ethanol. Maintain a dedicated stock of ethanol specifically for extraction.

**4X lysozyme Buffer:** Recipe for 40 mL of 4X lysozyme buffer. Hen egg white lysozyme is available from a variety of vendors. It is recommended that all buffers be made using disposable DNase-free plastic ware (disposable spatulas and flasks). Maintain a dedicated stock of reagents exclusively for this purpose to prevent introduction of contamination. Always use molecular grade (DNase-free) water to make Tris and EDTA stock solutions.

Reagent	Final Conc.	Amount added
Tris-CL pH 8.0 (1M)	80 mM	3.20 mL
EDTA (0.5 M)	8 mM	0.64 mL
Triton X-100	4.8%	1.92 mL
Lysozyme	80 mg/ml	3.2 g
Molecular grade water	-	34.24 mL

**Positive processing control:** Always include a positive processing control for each batch of extractions. This should be a known positive sample or sample spiked with *R. salmoninarum* culture.

**Negative processing control:** Always include a negative processing control for each batch of extractions. This can be water or a known negative sample that is carried through the entire extraction procedure.

<sup>§</sup>Qiagen Inc.: 1-800-426-8157; www.qiagen.com

## IV. Procedure

1. **Decontaminate work area.** Wipe down surfaces, pipets, tubes, racks and microcentrifuge rotor with a DNA degrading solution. Change gloves frequently between steps.
2. **Cell lysis.** Remember to include positive and negative processing controls.
  - a. ***Kidney Tissue:*** Cut 25 mg of tissue into small pieces and place in a 1.5 mL microfuge tube. Add 180  $\mu$ L of ATL Buffer and 20  $\mu$ L of Proteinase K solution. Incubate at 55°C for 1 hour or incubate at 37°C until tissue is completely lysed (typically overnight). Vortex mix occasionally.
  - b. ***Ovarian fluid:*** Pipet 50  $\mu$ L of ovarian fluid into a 1.5 mL microfuge tube. Add 180  $\mu$ L of ATL Buffer and 20  $\mu$ L of Proteinase K solution. Incubate at 55°C until tissue is completely lysed (usually 1 hour). Vortex mix occasionally.
  - c. ***R. salmoninarum culture:*** Pellet bacteria suspension by centrifugation at 7,000 x g for 15 minutes. Pour off supernatant and re-suspend pellet in 180  $\mu$ L of ATL Buffer and 20  $\mu$ L of Proteinase K solution. Incubate at 55°C for 1 hour. Vortex mix occasionally.
3. **Allow samples to cool to 37°C or below.**
4. **Briefly pulse-spin in a microcentrifuge. Add 50  $\mu$ L of 4 X lysozyme lysis buffer. Incubate at 37°C for 1 hour. Vortex mix occasionally.**
5. **Briefly pulse-spin in a microcentrifuge. Add 250  $\mu$ L of Buffer AL and incubate at 70°C for 10 minutes.**
6. **Centrifuge samples for 1 minute at 20,000 x g (14,000 rpm) to pellet any melanin in kidney samples.** Melanin has been reported to inhibit PCR.
7. **Transfer the supernatant to a new microfuge tube containing 250  $\mu$ L of 95% ethanol. Triturate samples to mix.** Pipet slowly to avoid aerosols.
8. **Place a QIAamp spin column in a 2 mL collection tube. Carefully apply the mixture to the spin column without moistening the rim, close the cap, and centrifuge at 6000 x g for 1 minute.** Note: All centrifugation is carried out at room temperature.
9. **Wipe down the inside of microcentrifuge with water followed by a DNA degrading solution before proceeding.**
10. **Place the spin column in a clean 2 mL collection tube and discard the tube containing the filtrate.**

11. **Carefully open the spin column and add 500  $\mu$ L of Buffer AW1. Centrifuge at 6000 x g for 1 minute. Place the spin column in a clean 2 mL collection tube and discard the collection tube containing the filtrate.**
12. **Carefully open the spin column and add 500  $\mu$ L of Buffer AW2. Centrifuge for 3 minutes at 20,000 x g (14,000 rpm). It is important to remove all residual ethanol.**
13. **Place the spin column in a clean collection tube and discard the collection tube containing the filtrate.** Carefully remove the column so that it does not come in contact with the flow-through, which results in residual ethanol. If this occurs, discard the flow-through and centrifuge the spin column for an additional 1 minute.
14. **Add 400  $\mu$ L of Buffer AE preheated to 70°C to the spin column and incubate for 1 minute at room temperature. Centrifuge at 6000 x g for 1 minute. SAVE ELUTION! If you want the DNA sample to be more concentrated, elute with only 200  $\mu$ L of Buffer AE.**
15. **Store DNA at 4°C for immediate use or at -20C for long-term storage.**