# Automated RNA Purification from Salmonid Organs Using a Novel Paramagnetic Particle Technology

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## 1. Introduction

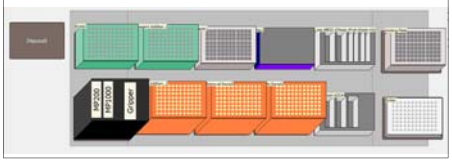
RNA viruses, including viral hemorrhagic septicemia virus, Salmonid alphaviruses (SAV) and infectious salmon anemia virus (ISAV), pose a significant threat to Atlantic salmon populations. Molecular techniques offer the means of monitoring for these viruses in fish farms as well as wild salmon populations. To facilitate rapid, reliable testing, we explore the use of magnetic particle-based purification technologies for automated extraction of RNA from salmon and trout organs.

Two automated methods were examined, one is the Maxwell® 16 Instrument with the LEV simplyRNA Tissue Kit and the second is a custom method and reagents that were created to adapt the purification chemistry to a 96-well format for laboratory liquid handlers such as the Biomek® 4000 Laboratory Automation Workstation. Using optimized reagents and protocols, we were able to extract more than 7.5µg of RNA from salmon brain, gill, and heart tissues, and more than 20µg of RNA from salmon kidney, liver and spleen tissues in both low throughput and high throughput automated processing. The RNA was of high integrity and purity and performed well in subsequent RT-qPCR assays . Two transcripts (β-actin and EIF1α) were monitored in all tissues and showed linear detectability across 5 orders of magnitude dilutions of the RNA samples.

These data support the use of the simplyRNA reagents on the Maxwell® 16 Instrument and customized for 96 well processing allowing automated purification of salmon organ RNA to streamline monitoring of salmon populations.

## 2. Biomek® 4000 Laboratory Automation Workstation Requirements

**Biomek® 4000 Laboratory Automation Workstation Deck Layout**



**Promega Materials Required:**

- ReliaPrep™ simplyRNA HT, Custom (AX1970)
- Proteinase K Solution (A5051)
- 2.2ml, Square-Well Deep Well Plate (V6781)
- Collection Plates (A9161)

**Biomek® Materials Required:**

- Biomek® Span P1000 Pipette Tips (B01124)
- Biomek® Span P250 Pipette Tips (379503)
- MP 1000 Tool
- MP 200 Tool
- Gripper
- Biomek® Modular Reservoir Quarter Module Divided By Length (372788)
- Biomek® Modular Reservoir Quarter Module (372790)
- Biomek® Orbital Shaker (379448)

**Other Materials Required:**

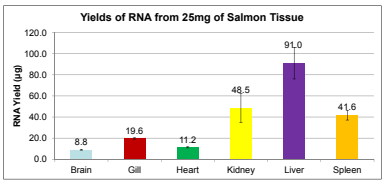
- Thermo Scientific™ Nunc™ 2.0mL Deep Well Plates (95040452)
- 80% Ethanol

## 3. Salmon Tissue Collection and Preparation

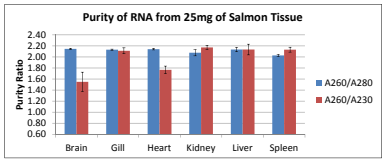
Chinook salmon tissue samples were collected from freshly euthanized fish from the Root River Steelhead Facility in Racine, Wisconsin. Brain, heart, kidney, liver, spleen, and gill tissues were removed from the fish, cut into small pieces (~0.5cm) and immediately placed in RNA*later*. Samples were stored for 24 hours in RNA*later* at 4°C. The RNA*later* was then removed from the vials and the samples were placed at -70°C for long term storage.

Tissue samples were homogenized using a Tissue-Tearor™ (BioSpec Products) in Homogenization Solution + 1-Thioglycerol at a final concentration of 125mg/ml. 25mg (200µl) of each tissue type was used for RNA purification.

## 4. High Yield and Purity Across a Range of Tissue Types



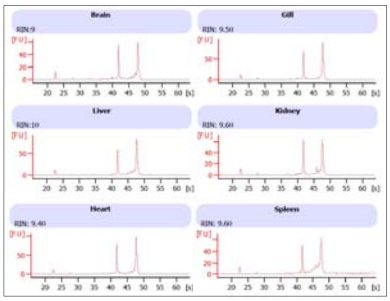

Following processing on the Biomek® 4000 Laboratory Automation Workstation with the magnetic particle-based purification technology, RNA from each tissue type (N=3 or 4) was analyzed using the NanoDrop 1000 spectrophotometer. >8ug of RNA was recovered from brain, gill and heart salmon tissue, while > 40ug of RNA was recovered from kidney, liver, and spleen tissue. The RNA was of high purity as denoted by the $A_{260}/A_{280}$ and $A_{260}/A_{230}$ purity ratios. Exceptions were $A_{260}/A_{230}$ ratios for brain (lipid-rich) and heart (fibrous) tissue, although this slight decrease in purity had no effect in RT-qPCR analysis




## 5. Automated Purification Delivers High Integrity RNA for Downstream Assays

| Average RIN Values | | | | | |
|---|---|---|---|---|---|
| Brain | Gill | Heart | Kidney | Liver | Spleen |
| 9.0 ± 0.3 | 9.3 ± 0.3 | 9.5 ± 0.1 | 9.9 ± 0.2 | 9.6 ± 0.3 | 9.2 ± 0.4 |

RNA integrity was determined using the Agilent 2100 Bioanalyzer. The purified RNA was consistently of high integrity with average RIN values ≥ 9.0 across all salmon tissue types tested.



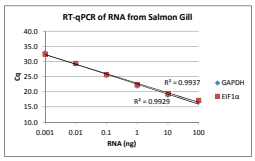

## 6. Linear Detectability Across 5 Orders of Magnitude Dilutions of RNA

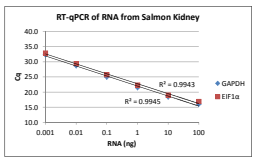
RNA was analyzed by RT-qPCR using the GoTaq® 1-Step RT-qPCR System on the BioRad CFX96 Real-Time PCR Detection System. Salmon specific primers targeting β-actin and EIF1α transcripts were used in a 20ul total reaction volume. The two transcripts were monitored in all tissues and showed linear detectability across 5 orders of magnitude dilutions of the RNA samples.

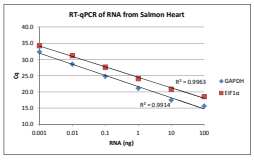
**β-actin***
For: CCAAAGCCAACAGGGAGAAG
Rev: AGGGACAACACTGCCTGGAT

**EIF1α***
For: TGCCCCTCCAGGATGTCTAC
Rev: CACGGCCCACAGGTACTG

*Olsvik, et al, "Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon", BMC Molecular Biology 2005 6:21

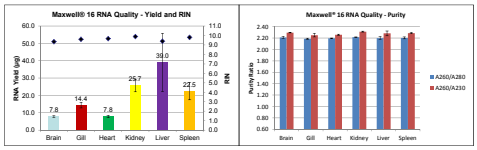


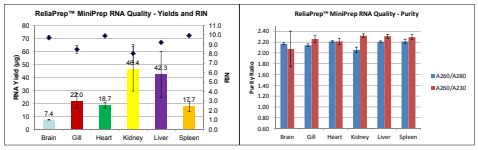




## 7. Lower Throughput Automated and Manual RNA Purification

The Maxwell® 16 is a low throughput instrument that extracts nucleic acid using the novel paramagnetic particles, allowing optimal capture, washing and elution of the target material. The instrument contains preprogrammed purification protocols, which, combined with pre-dispensed reagent cartridges, maximizes ease and convenience. RNA was purified from 25mg of salmon tissue using the Maxwell® 16 LEV simplyRNA Purification Kit (AS1280) in ~60 minutes (N=4).



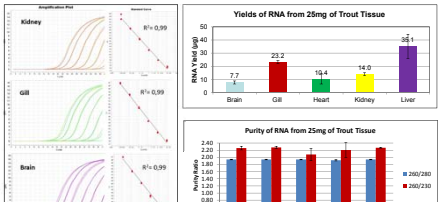

The ReliaPrep™ RNA Miniprep Tissue System (Z6111) utilizes a proprietary column/binding matrix that efficiently captures RNA from very small amounts of input material and can be eluted in a minimal volume (less than 15µl). Purification is achieved without the use of phenol:chloroform extractions or ethanol precipitations, resulting in pure RNA that does not require additional purification or concentration of the RNA for use in demanding applications. RNA was purified from 20mg of salmon tissue using the ReliaPrep™ RNA Miniprep Tissue System in ~30 minutes (N=3 or 4).




Using these lower throughput automated and manual options, RNA of high yield, integrity, and purity was purified from salmon tissue.

## 8. RNA Purification from Trout Using the Maxwell® 16

RNA was successfully purified from 25mg of various trout tissues stored in RNA*later* using the Maxwell® 16 LEV simplyRNA Purification Kit (N=4). RNA quality was assessed by the NanoVue spectrophotometer (GE Healthcare) as well as by RT-qPCR using the GoTaq® 1-Step RT-qPCR System on the 7500 Real-Time PCR System with universal primers targeting mitochondrial 12S rRNA.




**12S rRNA***
M13U12S-F: TGTAAAACGACGGCCAGTCAAACTGGGATTAGATACCC
M13U12S-R : CAGGAAACAGCTATGACCGAGGGTGACGGGCGGTGTGT

*Yang, et al, "Species identification through mitochondrial rRNA genetic analysis", Scientific Reports 2014 4:4089

## 9. Summary

- High yields of RNA can be isolated from fish tissues using a novel magnetic particle-based purification technology.
- The purified RNA is of high purity and integrity for use in downstream assays such as RT-qPCR.
- 96 samples can be processed on the Biomek 4000 Laboratory Automation Workstation using the ReliaPrep™ simplyRNA HT, Custom chemistry in under 3 hours.
- Lower throughput automated and manual methods are available that yield the same high quality RNA.
- For additional Promega support and information, please contact FSS@promega.com
- For additional Maxwell® 16 information, visit: http://www.promega.com/products/instruments/maxwell-systems/
- For additional ReliaPrep™ information, visit: http://www.promega.com/products/pm/rna-purification-from-cells-and-tissues/