Seek Extraction™: Introducing a Rapid, Versatile, and Transformative Technology for Nucleic Acid Extraction

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1 BACKGROUND

- Rapid and accurate molecular diagnostics (Dx) are critical for timely detection and treatment of infectious human, veterinary, and agricultural pathogens.
- Centralized laboratories dominate existing molecular diagnostic testing options and regularly cause delays and prohibit access for healthcare consumers.
- Healthcare consumers are increasingly favoring affordable and user-friendly technologies. Indeed, the Covid-19 pandemic highlighted the necessity for instrument-free, cost-effective molecular Dx technologies that are user friendly, deliverable, and can be rapidly configured for various disease targets. According to the US Centers for Disease Controls (CDC), 70% of today's medical decisions depend on diagnostic resuts.
- A key component of such molecular Dx technologies is nucleic acid extraction, the process of isolating and purifying DNA/RNA from a biological sample. Nucleic acid extraction is the first step and the greatest impediment.
- Despite notable technological advances, existing molecular Dx technologies are not adaptable to point-of-care (PoC) applications due to their dependence on laboratory methods for nucleic acid extraction including specialized laboratory equipment (e.g., centrifuges, incubators, spin-column kits, etc.) or hazardous reagents (e.g., phenol, Guanidinium thiocyanate, ethanol, etc.). Moreover, nucleic acid extraction typically involves complex biological samples (whole blood, tissue, and body fluid swabs), which present significant problems for adaptable PoC technologies as each require sample-specific extraction processes and reagents.
- Lab-based extraction technologies are not easily developed and deployed to PoC scenarios, leading to delays in diagnosis and treatment.

Here, we introduce Seek Extraction™, an innovative and efficient nucleic acid extraction platform that can extract qPCR-quality DNA/RNA across a variety of biological sample types in under 2 minutes without any specialized equipment or training. Seek Extraction yields highly concentrated, pure nucleic acid with output comparable to conventional laboratory extraction techniques. Its versatility makes it particularly suited for PoC or non-laboratory applications.

2 METHODS

Seek Extraction comprises the following steps to extract nucleic acid from biological samples (Fig. 1):

- Collect biological/environmental sample.
- Mix the biological sample with Lysis Buffer and transfer to Lysate Vial.
- Pour contents of Lysate Vial into Seek Extraction Unit.
- Pour the lysate solution back into the Lysate Vial and discard.
- Repeat steps 2 & 3 with the Elute Buffer and Vial.
- Pour the elute solution with the extracted and purified nucleic acid into the Elute Vial.
- The Elute Vial contains pure extracted nucleic acid and is now ready for downstream applications.

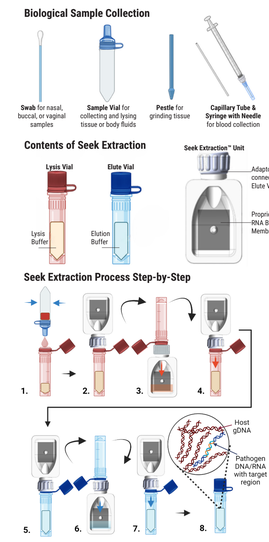

Figure 1. Seek Extraction process for all biological samples.

3 RESULTS

1. Internal validation demonstrates Seek Extraction successfully extracts nucleic acid from tissue faster than existing methods that require specialized equipment while preserving high concentration and quality.

DNA Extracted from Shrimp Tissue: Seek Extraction vs. Qiagen QiaAmp Kit

- Tissue processed from WSSV-infected shrimp with Seek Extraction took under 2 minutes while the Qiagen QiaAmp Kit took 3 hours (Fig. 2. B).
- Tissue processed with Seek Extraction showed similar qPCR results for WSSV infection levels for both muscle and pleopod samples when compared to Qiagen QiaAmp Kit (Fig. 2. C).
- Tissue processed with Seek Extraction showed similar DNA quality (Fig. 2. D) and DNA yield (Fig. 2. E) as the Qiagen QiaAmp Kit.

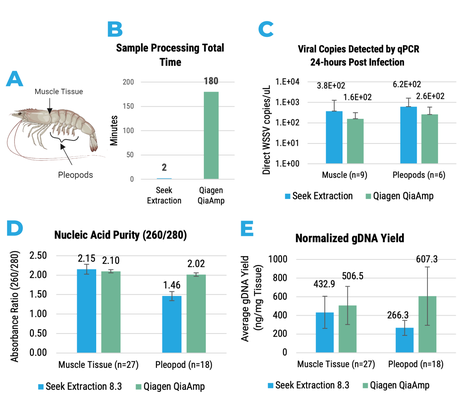

Figure 2. Internal analysis for WSSV-infected shrimp showing **(A)** locations of shrimp samples collected, **(B)** time of extraction, **(C)** infection levels via qPCR, **(D)** UV-Vis 260/280 absorbance ratio, and **(E)** average yield.

2. External validation demonstrates Seek Extraction successfully extracts nucleic acid from tissue faster than existing methods that require specialized equipment while preserving high concentration and quality.

External Validation of DNA Extracted from Shrimp Tissue: Seek Extraction vs. Promega Wizard Kit (performed independently at Animal Aquatic Health Lab (AAHL))

- Tissue processed with Seek Extraction took under 4 minutes compared to Promega Wizard Kit, which elapsed over 2-3 hours (Fig. 3. A).
- Tissue processed with Seek Extraction showed a statistically greater yield of extracted DNA compared to Promega Wizard Kit (Fig. 3. B).
- Tissue processed with Seek Extraction showed similar quality of extracted DNA compared to Promega Wizard Kit, as measured over 260/280 (Fig. 3. C).
- Sensitivity and specificity were measured with positive/negative results for WSSV detection on qPCR; Seek Extraction showed slightly lower sensitivity and significantly greater specificity compared to Promega Wizard Kit (Fig. 3. D).

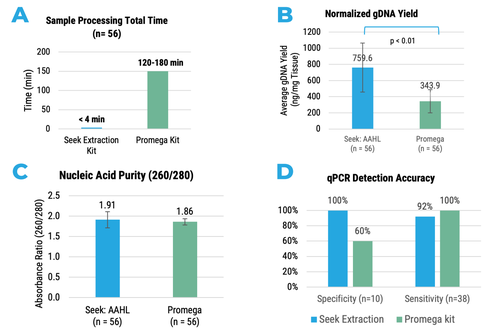

Figure 3. Animal Aquatic Health Lab (AAHL) analysis for WSSV-infected shrimp showing **(A)** total sample processing time **(B)** DNA yield normalized per mg of tissue, **(C)** UV-Vis 260/280 absorbance ratio, and **(D)** percentage accuracy for specificity and sensitivity derived from qPCR results.

3. Seek Extraction successfully extracts nucleic acid across a variety of animal tissue types with favorable concentration and seamless integration with qPCR amplification and detection.

DNA Extracted from Mouse Tissue: Seek Extraction vs. QiaAmp Kit

- Seek Extraction successfully extracted DNA from all mouse tissue types tested (Fig. 4. A).
- DNA extracted from mouse tissue with Seek Extraction successfully amplified mouse GAPDH gene on qPCR (Fig. 4. B).

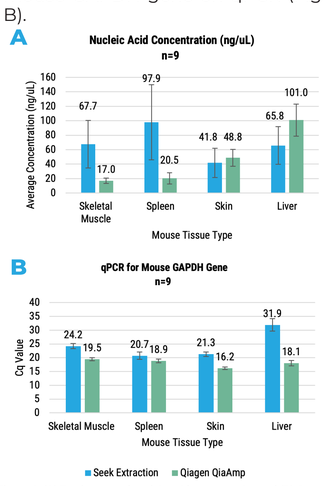

Figure 4. **(A)** Total extracted nucleic acid concentration and **(B)** Cq values from qPCR GAPDH detection for mouse skeletal muscle, spleen, skin, and liver tissue samples.

4. Seek Extraction successfully extracts nucleic acid across a variety of human sample types with high concentration and seamless integration with qPCR amplification and detection.

DNA Extracted from Complex Human Samples (Whole Blood, Skeletal Tissue, Formalin-Fixed Paraffin-Embedded (FFPE) Tissue): Seek Extraction vs. Commercial Kits

- DNA extracted from human sample types with Seek Extraction showed favorable yields when compared with commercial kits (Fig. 5. A).
- DNA extracted from human samples with Seek Extraction showed successful amplification of human GAPDH gene on qPCR (Fig. 5. B).

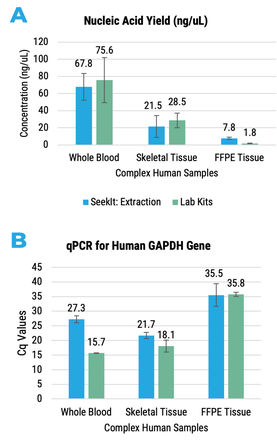

Figure 5. **(A)** Total extracted nucleic acid concentration and **(B)** Cq values for the qPCR detection of GAPDH for human whole blood, skeletal tissue, and FFPE tissue samples.

5. Seek Extraction directly integrates with existing sequencing technologies, which are critical for determining pathogenic variants.

NextGen Nanopore Sequencing: Seek Extraction vs. Promega Wizard

- DNA extracted with Seek Extraction shows favorable quality when compared with Promega Wizard Plasmid Extraction Kit (Fig. 6. B)
- Results from Nanopore sequencing show alignment between both pTOPO-VP28 sequences extracted with Seek Extraction and Promega Wizard Plasmid Extraction Kit (Fig. 6. C). Both Seek Extraction and Promega Wizard Plasmid Extraction Kit sequences show a 100% match with no errors compared to the expected sequence.

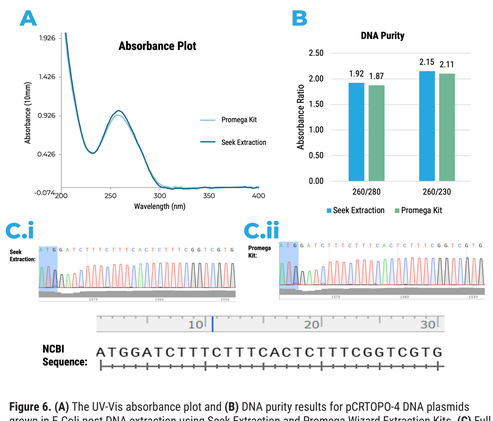

Figure 6. **(A)** The UV-Vis absorbance plot and **(B)** DNA purity results for pCRTOPO-4 DNA plasmids grown in E.Coli post DNA extraction using Seek Extraction and Promega Wizard Extraction Kits. **(C)** Full plasmid Nanopore sequencing from extracted materials without further cleanup after extraction from Seek Extraction **(C.i)** and Promega Wizard Extraction kits **(C.ii)**

4 FUTURE STUDIES

Ongoing studies are optimizing Seek Extraction for nucleic acid extraction from human pathogens. Ongoing work with Respiratory Syncytial Virus (RSV) and *Neisseria gonorrhoeae* (*N.g*) demonstrates Seek Extraction successfully extracts pathogenic DNA/RNA from artificially spiked human samples.

Seek Extraction Feasibility with Human Disease Pathogens: RSV and *N.g*.

- Extracted DNA and RNA from spiked samples showed positive amplification on qPCR (Fig. 7. A-B)

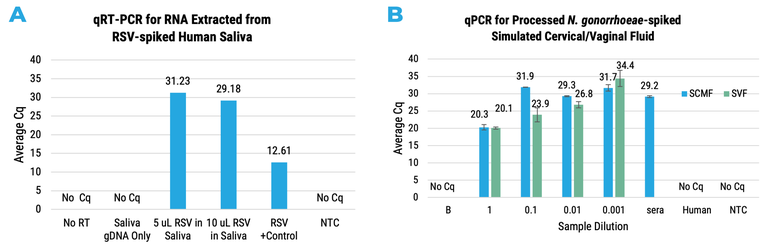

Figure 7. **(A)** qRT-PCR detection of Respiratory Syncytial Virus (RSV) RNA extracted from 1 mL saliva samples spiked with 0, 5 or 10 uL of cultured cells containing active RSV infection. **(B)** qPCR detection of *Neisseria gonorrhoeae* (*N.g*) extracted from Simulated Cervical Mucus Fluid (SCMF) or Simulated Vaginal Fluid (SVF) spiked with serial dilutions of N.g bacterial culture. Sera sample was positive control for *N.g.* while blank (B), human, and NTC are negative controls for Seek Extraction device, host gDNA, and NTC, respectively.

5 APPLICATIONS

Seek Extraction is capable of integration with multiple downstream amplification and detection diagnostic technologies to optimize and complement molecular diagnostic processes. Here, we highlight three specific applications for Seek Extraction integration: Seek Amplification™, qPCR, and NextGen Sequencing. Future studies could illuminate integrations with additional existing amplification and detection techniques.

Seek Extraction seamlessly integrates with Seek Amplification, a novel isothermal amplification method developed in-house at Seek Labs.

Seek Amplification is a self-contained, isothermal (20°C – 40°C) amplification method capable of amplifying a single copy of target DNA to a detectable range (10^7) in under 30 minutes without any equipment.

- WSSV DNA extracted from infected shrimp tissue samples with Seek Extraction and integrated with Seek Amplification yields increased target amplification with increasing concentration of Extraction elute in total reaction (Fig. 8).

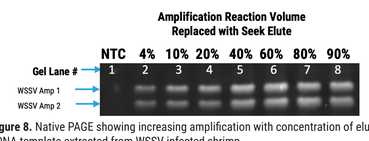

Figure 8. Native PAGE showing increasing amplification with concentration of eluted gDNA template extracted from WSSV-infected shrimp.

Seek Extraction seamlessly integrates with PCR & qPCR, the laboratory gold standard methodology for nucleic acid amplification and detection.

- Nucleic acid extracted from multiple human and animal samples (shrimp tissue, mouse tissue, whole human blood, human skeletal tissue, and human FFPE tissue) shows successful amplification on qPCR compared to standard extraction methods (Fig. 9).

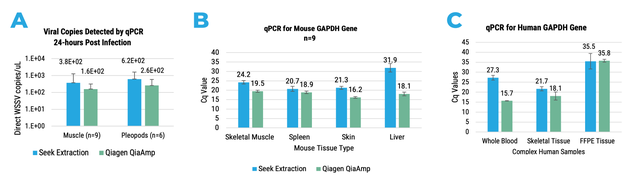

Figure 9. Seek Extraction integration to qPCR with **(A)** shrimp tissue, **(B)** mouse tissue, and **(C)** human samples.

Seek Extraction is compatible with Next Gen sequencing using Nanopore technology for identification of nucleotide sequences.

- DNA extracted from a cloned bacterial culture using Seek Extraction successfully integrates with Nanopore sequencing (Fig. 10).

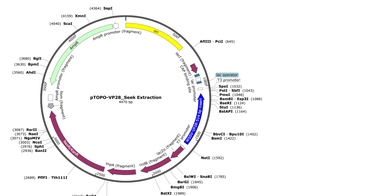

Figure 10. Full plasmid Nanopore sequencing from extracted materials without further cleanup after extraction using Seek Extraction.

6 CONCLUSION

Seek Extraction is primed for rapid development in laboratory and PoC settings.

- Nucleic Acid Extraction, the first step in molecular diagnostics, is a major impediment to rapid and accurate diagnostic technologies. Existing extraction technologies rely on laboratory equipment, trained technicians, hazardous reagents, etc. In order to develop molecular diagnostic technologies for the point of care and non-laboratory applications, optimized extraction technologies that solve bottlenecks are required.
- Seek Extraction is a rapid, highly adaptable, and affordable sample processing system that extracts pure nucleic acid across a variety of biological samples.
- Seek Extraction operates inside or outside of the laboratory and does not require additional equipment, incubation, or specialized training and can extract pure nucleic acid in under 4 minutes.
- Finally, Seek Extraction can integrate with direct detection, amplification, and Nanopore sequencing.

7 ACKNOWLEDGEMENT

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