Northwest Genomics Center
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Prepared Library Submission Information

Investigator Name: **Laura Spencer, Steven Roberts (PI)**

Investigator Institution: **Univ. of Washington**

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Quote Number: **2020-03_013**

Submission Date: **April 17, 2020**

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Sample Information: Please fill out as much of this form as possible. If this is your first submission with us, please give us a call at 206-685-9444 for a consultation!	
1. Pool/Project ID(s):	*Unknown*
2. Number of Samples:	146 (Batch #1 has 69, Batch #2 has 77)
3. Single samples or pre-pooled?	Single samples
4. Sample or Pool Concentrations in nM (Min= 5nM):	*Standard = 10nM;* Samples vary, see attached.
5. PhiX Spike % (for low base diversity samples)	
6. Sequencing Platform (HiSeq (4000 or X), MiSeq, NextSeq (HO or MO), NovaSeq (S2 or S4):	NovaSeq (*I do not know whether it is S2 or S4)*
7. Sequencing Read Length (ex. PE25, PE75, PE100):	Single Read 100 bp
8. Dual or Single index?	Single
9. Index Length (ex: +6, +8, +8+8, +10+10):	+6
10. Sequencing Depth (# of lanes needed):	2 lanes
11. Index Sequences (for demultiplexing):	See attached.
12. Primer Sequence (TruSEQ, Nextera, other):	Other – QuantSeq for Illumina platforms
13. Sample Type (genome, exome, RNA, other):	RNA
14. Sample Source (blood, bacterial, tissue, ect):	Oyster tissue

****Please only submit samples in 1.5mL – 2.0mL tubes with snap-caps or screw caps! Thank you!****

PLEASE NOTE THE FOLLOWING

***QC is included in the pricing and will be run in-house using the Qubit and Bioanalyzer for every submission. If you have any Bioanalyzer traces and/or MiSeq data available, please include with your sample submission.**

***Please include your sample barcode list with sample submission form prior to the sample run.**

***FASTQ is the standard data delivery type. If you require an alternative, please inquire before submitting as an additional charge may occur.**

**Email this page to nwgcseq@uw.edu when complete.
**Once emailed that your data is ready on Globus, you will have 120 days to access it before it is deleted indefinitely.
**If you do not have a Globus account already, please create one at https://docs.globus.org/how-to/get-started/ and send us the email addresses associated with their Globus login. We will email a link to the appropriate data share to those individuals. Please notify us if access for any individuals should be changed.

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Notes:

- Detailed sample information is attached.

- Important note: I submitted 2 PCR plates/batches, which need to be run on separate lanes since they have overlapping indices. Batch 1 has 69 libraries, and Batch 2 has 77 libraries.

- I included the average length (bp) of a subset of libraries, determined via a BioAnalyzer HS DNA Chip. I've summarized the average bp length by tissue source (Adult, Larval, Juvenile). If you plan to run more libraries on the bioanalyzer before pooling, I recommend doing some from the Adult and Larval categories, since those are underrepresented in my QC.

- I included three NTC samples that were prepped alongside libraries during amplification, but they do not have indices. Feel free to omit these NTC samples if needed.

- I prepped these libraries using the **QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina with i7 6 nt Indexing**; manufacturer information is available here: https://www.lexogen.com/quantseq-3mrna-sequencing/#quantseqdownloads

- A couple specific notes from the QuantSeq manual:
    - No other libraries from other vendors should be run in the same lane as these QuantSeq libraries
    - **"REMARK:** If an 8 nt i7 index (Index 1) needs to be entered into an Illumina sample sheet, add two nucleotides from the Illumina adapter sequence to the 3'end of the index. **EXAMPLE: 7001** would become CAGCGT**AT**, **7002** would become GATCAC**AT** and so on. These additional nucleotides are identical for all indices as they are derived from the Illumina adapter."