Grants and Papers

Overview

This page includes terminology that you may find helpful when drafting grants and scientific papers for your lab. Below you will find a short explanation for each service we provide that may be included in your Materials and Methods sections. Additionally, there is a statement for acknowledging the HTSF in grants or papers. Please feel free to contact the HTSF customer service with any further questions.

Acknowledging the HTSF

"We gratefully acknowledge the technical support from the UNC High Throughput Sequencing Facility. This facility is supported by the University Cancer Research Fund, Comprehensive Cancer Center Core Support grant (P30-CA016086), and UNC Center for Mental Health and Susceptibility grant (P30-ES010126)."

Grant Support Request

If you are looking for a letter of support for a grant application please fill out the Requesting Grant Support Letter form on the website *Forms and Guides tab, Grant Information* and send it to htsf@med.unc.edu. Please allow 48 business hours (Mon-Fri) for a response.

HTSF Capabilities

See the ABOUT US Tab on the website for a discussion on this topic. There is a HTSF Capabilities white paper on the *Forms and Guide Tab, Grant Information*.

HTSF Rigor and Reproducibility

See the ABOUT US Tab on the website for a discussion on this topic. There is a HTSF Rigor and Reproducibility white paper on the *Forms and Guide Tab, Grant Information*.

Materials and Methods Section

Please note that the following passages may contain "XXX." These are values that are unique to a submission, as they are dependent on material type and library prep method. Please see Submissions tab on the website for more information about acceptable QAQC values.

Always feel free to contact HTSF customer service staff if you need greater details about your study. We will reference the processing databases to confirm what protocols your samples were run through. Please let us know what detail you need to confirm. It is best if you can supply the account name, TracSeq batch number, samples IDs. If you do not have the batch number, an approx. date range can work.

QAQC and Library Prep

- *Note_ highlighted italic statements indicate what should be filled in, where to find the information yourself and should be left out of final statement.*

QAQC of RNA Analytes

All RNA analytes were assayed for RNA integrity, concentration, and fragment size. Samples for total RNA-Seq were quantified on a TapeStation system (Agilent, Inc. Santa Clara, CA). RNA Integrity score (RIN) averaged XXX *(this can be pulled from your QAQC data sent to you OR found on the submission batch attachments in TracSeq)*. Samples with RINs > 7.0 were considered high quality. Input concentrations greater than XXX ng/ul *(this conc will be based on your library type)* were ideal and ranged between XXX-XXX ng/ul *(this is specific to your sample set, pull form your QAQc result on Tracseq).* Fragment size was used to determine if additional fragmentation was needed.

Total RNA-Seq Library Construction

Total RNA-Seq library construction was performed from the RNA samples using the TruSeq Stranded RNA Sample Preparation Kit and bar-coded with individual tags following the manufacturer's instructions (Illumina, Inc. San Diego, CA). Libraries were prepared on an Agilent Bravo Automated Liquid Handling System. Quality control was performed at every step and the libraries were quantified using a TapeStation system.

Sequencing

Illumina Sequencing Technology

Illumina sequencing technology provides a very high accuracy (99.9%) in a relatively quick manner compared to other methods. Though these machines are able to multiplex, or provide data for multiple samples simultaneously, the output reads are very short. For this specific research, short reads were sufficient so the *(insert specific technology)* was used.

MiSeq

- More tolerant of unusual library construction (i.e. low diversity libraries)
- Only platform that offers paired-end 300 cycle

HiSeq2500

- High output sequencing system
- Well suited for de novo and resequencing of small and large genomes
- **High throughput mode** will be able to generate up to 1Tb per 6 day run (with v4 chemistry)
- **Rapid mode** is capable of generating 180Gb in 40 hours

Hiseq4000

- Patterned flow cell technology
- Gives more reads per lane and generates twice the output in less time
- Well suited for exome, large whole genome, and whole transcriptome sequencing
- Generates up to 1.5Tb per 3.5 day run

NovaSeq6000

- Latest production-scale sequencer with patterned flow cell technology
- Generates unprecedented output in less than two days.

Total RNA Sequencing

Indexed libraries were prepared and run on *(insert specific platform set up here)* to generate a minimum of XXX million *reads (taken form your data, can fund on the lane metrics files in TracSeq for the run)* per sample library with a target of greater than XX% mapped reads *(from your data analysis, typically we see approx. 90%)*. Typically, these were pools of XX samples *(form you study details, can be found on the Tracseq submission d batch as approx. # at submission. Best to confirm on MY LANES in Tracseq which list all libraries demux from a pool).* The raw Illumina sequence data were demultiplexed and converted to FASTSQ files, and adapter and low-quality sequences were quantified. Samples were then assessed for quality by mapping reads to *(insert reference genome of you need)*, estimating total number of reads that mapped, amount

of RNA mapping to coding regions, amount of rRNA in sample, number of genes expressed, and relative expression of housekeeping genes. *(These later statements may not apply to your data analysis)* Samples passing this QAQC were then clustered with other expression data from similar and distinct tumor types to confirm expected expression patterns. Atypical samples were then SNP typed from the RNA data to confirm source analyte. FASTQ files of all reads were then uploaded to the GDC repository.

Relevant Grant, Paper and Presentation Forms and Guides

Requesting Grant Support Letter

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