The Power of Next Generation Sequencing

BRC-Seq

Sequencing Core at the Biomedical Research Centre
2222 Health Science Mall, UBC, V6T 1Z3
http://brc.ubc.ca/next-generation-sequencing-at-the-brc/
Email: BRCSeq@brc.ubc.ca
Use our instruments for a service fee or take advantage of our standard & custom workflows

- Illumina NextSeq
- Illumina Neoprep
- Illumina MiSeq
- Agilent 2100 Bioanalyzer
- Viia 7 Real-Time PCR System
Services at BRC-Seq

Standard mRNA sequencing services

mRNA sequencing from QC to analysis

Includes Bioanalyzer QC, Neoprep mRNA library preparation, Sequencing & Analysis

Options for deeper sequencing

Tertiary data analysis and training also available

Library Preparation Services

DNA and RNA TruSeq protocols on the Neoprep (0.1-100ng)

Lexogen QuantSeq 3’ RNA (0.1-5000ng and degraded)

New England Biolabs NEB NEXT DNA Ultra II (0.1-1000ng)

Bioanalyzer Sizing & QC
Standard mRNA-Seq Service Work-Flow Summary

Quality Control

RNA samples are run on the Agilent Bioanalyzer to ensure RNA is of sufficient quality & quantity for library preparation.

Library Preparation

RNA samples are prepared with the TruSeq Stranded mRNA Library Prep Kit & loaded onto the Neoprep for fragmentation, barcoding, and sequencer adaptor ligation.

Output libraries are quantified to ensure success.

Sequencing

Depending on experimental requirements, libraries are pooled and ran on either the MiSeq or NextSeq, with customizable sequencing fragment read length and depth.

Analysis
mRNA-Sequencing Service

Bioanalyzer Quality Control and RNA Integrity

Quality control testing is done by looking at 18s and 28s Ribosomal RNA fragment sizes. Non fragmented peaks are indicators for the integrity of mRNA.
Illumina Truseq Stranded mRNA chemistry is used on the nanofluidic neoprep instrument in order to do Poly-A selection of mRNA, Fragment, synthesize stranded cDNA, ligate adapters, barcode, and PCR amplify. Samples are then quantified and diluted for loading on the sequencer.
Library Preparation with the Neoprep

https://www.youtube.com/watch?v=n_BmE8nQEpQ
After samples are loaded onto the sequencer they bind to the flowcell and amplify at local regions called Clusters. Fluoro-conjugated nucleotides are then added 1 at a time, with pictures taken at the addition of each base, which records the sequence. This occurs simultaneously with of millions of fragments, with these fragment sequences known as Reads. Reads are then aligned to a reference genome and the number of times they fall on a particular transcript is counted. Sample counts are normalized for the total number of reads sequenced, and transcript lengths producing a normalized gene expression (known as RPKM or FPKM) are then used for comparisons or in further analysis.
Illumina’s Sequencing by Synthesis Technology

https://www.youtube.com/watch?v=HMyCqWhwB8E

http://www.illumina.com/technology/next-generation-sequencing.html
mRNA-Seq

Counts and differential expression analysis for whole coding transcriptome

Visualization of Transcript Structure

RNA-seq is a powerful experimental tool that allows for the determination of differential expression, demonstration of correlative properties, characterization by gene ontology, or examination of transcriptional structure and variants.
Heat maps demonstrate contrasts in gene expression within groups and across treatments.
Correlative analysis demonstrates transcriptional similarity or differences between samples.
Pathway Analysis/Ontology to characterize your model

SOX9 modulates the expression of key transcription factors required for heart valve development

Victoria C. Garside, Rebecca Cullum, Olivia Alder, Daphne Y. Lu, Ryan Vander Werff, Mikhail Bilenky, Yongjun Zhao, Steven J. M. Jones, Marco A. Marra, T. Michael Underhill, Pamela A. Hoodless

Development 2015 143: 4340-4350; doi: 10.1242/dev.125252
Advantages of Next Generation Sequencing

Sequencing is rapid, affordable, quickly developing, and powerful

Transition from a 10 year, $3 billion human genome to the $1000 human genome

Sequencing can be tailored to suit almost any experiment

Alternative technologies based on microarray, PCR, or other molecular probes are insensitive and limited by quality & quantity of sample and the genes queried.

Sequencing provides significantly more data, and the ability to compare directly to historical and published data.

Determination of population and microbiome makeup

Targeted querying

mRNA transcript quantification and gene expression

Novel gene discovery  SNP & splice site discovery

Characterize Chromatin structure  Medical Diagnostics

Many additional current and future applications

http://www.illumina.com/technology/next-generation-sequencing.html
Experiment Design: How replicates and sequencing depth can affect analysis

... but keep in mind, the statistics and best design will vary
About BRC-Sequencing Core

Located at the Biomedical Research Center, we are working on bringing greater sequencing accessibility to British Columbia and providing quality data from sample prep to analysis for researchers with quick turnaround time. We have standardized sample prep, sequencing, QC, and analysis assistance. We often work with challenging and unique experiments and cater to individual projects in order to offer the best quality and prices; please ask how we can help you.

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Other BRC Lab Services

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Our Team