



SAGC

SOUTH
AUSTRALIAN
GENOMICS
CENTRE

SAGC BIOINFORMATICS WORKSHOP

RNAseq analysis using nf-core

Who should attend?

It is intended to be approachable to new users of RNAseq. The focus will be on understanding analysis options for RNAseq, although familiarity with command line tools (unix & R) will be necessary to run nextflow pipelines.

[Register Here](#)



A practical guide to RNAseq analysis using nextflow-core pipelines.

Recent development of computational biology tools and initiatives like nf-core have enhanced accessibility to analysis options for many genomics technologies including RNAseq. Discover how nextflow simplifies and streamlines RNAseq and other genomics analyses.

TOPICS



A hands-on walk-through of nf-core analysis pipelines for RNAseq

run nf-core bioinformatic pipelines using NGS data; QC metrics, read trimming, differential expression analysis, data visualisation, mRNA and small RNA.

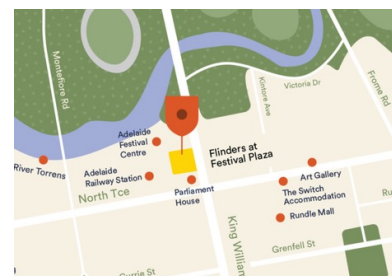


Description of the key metrics and analyses for RNAseq analysis

Gain familiarity with key bioinformatic tools and , and how to decipher and use the outputs.

Location

[Flinders City Campus](#) (Festival Tower), Rm: 505



Lead Trainer

Dr Daniel Thomson SAGC

Time and Date

10:00am - 4:00pm
(Registration opens at 9:30 am)
Thursday, 10th October 2024

Cost

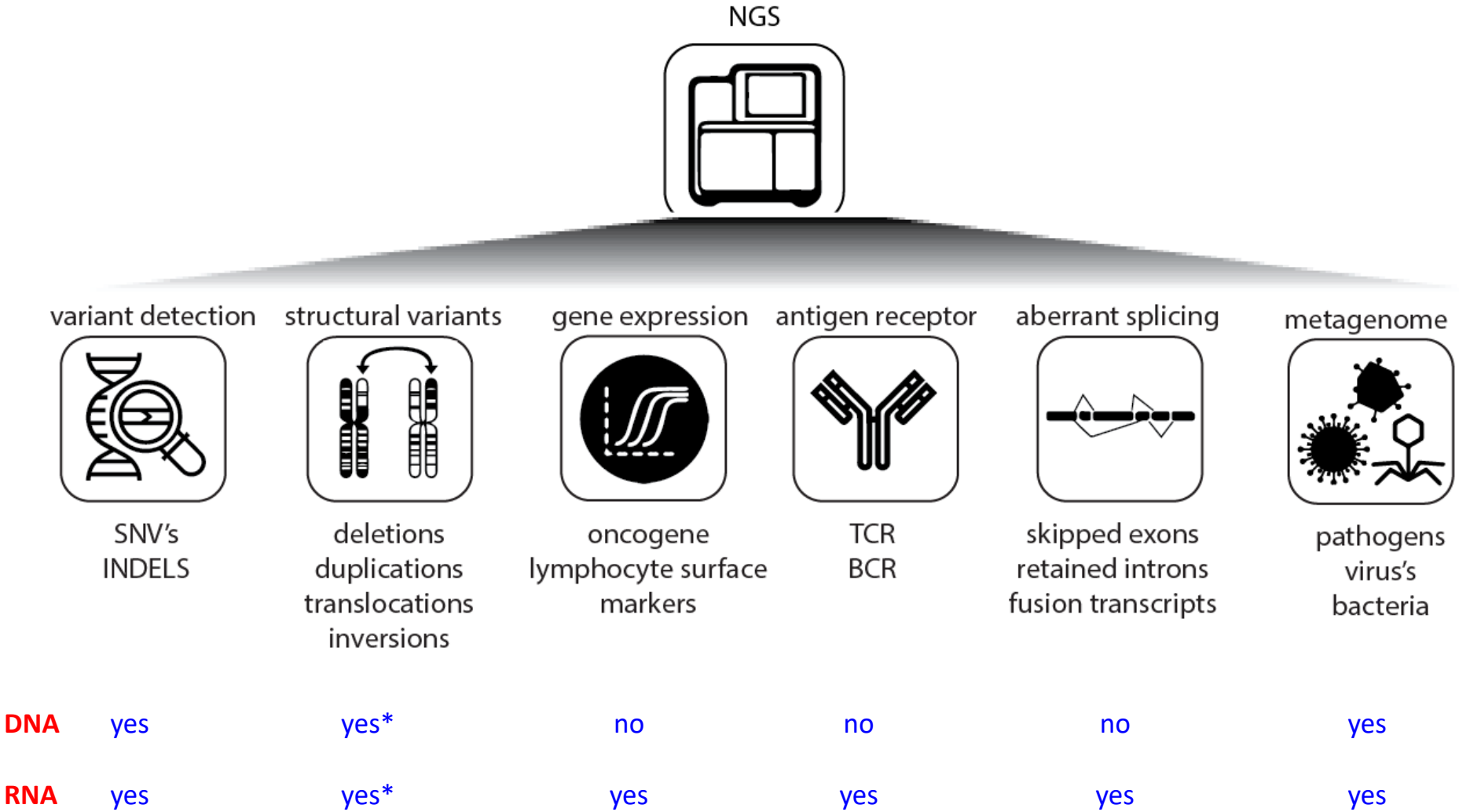
\$100 Non-Student \$50 Student

HEAPS
GOOD
GENOMICS.

RNAseq

Quick background

RNAseq is a versatile tool



Why nextflow and nf-core?

Pipelines

Browse the 113 pipelines that are currently available as part of nf-core.

Released 18Under development 4Archived 2

⌵ Last release ▾

⌵ ⌵

scnanoseq ✓ ☆ 15

New release!

Single-cell/nuclei pipeline for data derived from Oxford Nanopore and 10X Genomics

10xgenomics long-read-sequencing nanopore scrna-seq single-cell

🔍 1.0.0 released about 13 hours ago

rnaseq ✓ ☆ 880

RNA sequencing analysis pipeline using STAR, RSEM, HISAT2 or Salmon with gene/isoform counts and extensive quality control.

ma rna-seq

🔍 3.16.0 released 6 days ago

isoseq ✓ ☆ 28

Genome annotation with PacBio Iso-Seq. Takes raw subreads as input, generate Full Length Non Chimeric (FLNC) sequences and produce a bed annotation.

isoseq isoseq-3 rna tama ultra

🔍 2.0.0 released about 1 month ago

oncoanalyser ✓ ☆ 40

A comprehensive cancer DNA/RNA analysis and reporting pipeline

🔍 1.0.0 released about 1 month ago

scrnaseq ✓ ☆ 210

A single-cell RNAseq pipeline for 10X genomics data

10x-genomics 10xgenomics alevin bustools cellranger kallisto rna-seq single-cell star-solo

🔍 2.71 released about 2 months ago

denovotranscript ✓ ☆ 3

A pipeline for de novo transcriptome assembly of paired-end short reads from bulk RNA-seq

denovo-assembly rna-seq transcriptome

🔍 1.0.0 released about 2 months ago

ampliseq ✓ ☆ 182

Amplicon sequencing analysis workflow using DADA2 and QIIME2

16s 18s amplicon-sequencing edna illumina iontorrent its metabarcoding metagenomics microbiome pacbio qiime2 rrna taxonomic-classification taxonomic-profiling

🔍 2.11.0 released 2 months ago

rnasplice ✓ ☆ 42

rnasplice is a bioinformatics pipeline for RNA-seq alternative splicing analysis

alternative-splicing rna rna-seq splicing

🔍 1.0.4 released 5 months ago

differentialabundance ✓ ☆ 57

Differential abundance analysis for feature/observation matrices from platforms such as RNA-seq

atac-seq chip-seq deseq2 differential-abundance differential-expression gsea limma microarray rna-seq shiny

🔍 1.5.0 released 5 months ago

smrnaseq ✓ ☆ 73

A small-RNA sequencing analysis pipeline

small-rna smrna-seq

🔍 2.3.1 released 6 months ago

rnafusion ✓ ☆ 138

RNA-seq analysis pipeline for detection of gene-fusions

fusion fusion-genes gene-fusion rna rna-seq

🔍 3.0.2 released 6 months ago

nascent ✓ ☆ 18

Nascent Transcription Processing Pipeline

gro-seq nascent pro-seq rna transcription tss

🔍 2.2.0 released 7 months ago

marsseq ✓ ☆ 5

MARS-seq v2 pre-processing pipeline with velocity

facets-sorting mars-seq single-cell single-cell-rna-seq star-solo transcriptional-dynamics

🔍 1.0.3 released about 1 year ago

hlatyping ✓ ☆ 61

Precision HLA typing from next-generation sequencing data

dna hla hla-typing immunology optitype personalized-medicine rna

🔍 2.0.0 released almost 2 years ago

rnavar ✓ ☆ 35

gatk4 RNA variant calling pipeline

gatk4 rna rnaseq variant-calling workflow

🔍 1.0.0 released over 2 years ago

clipseq ✓ ☆ 19

CLIP sequencing analysis pipeline for QC, pre-mapping, genome mapping, UMI deduplication, and multiple peak-calling options.

clip clip-seq peak-calling rna-ribp-interactions

🔍 1.0.0 released over 3 years ago

dualrnaseq ✓ ☆ 18

Analysis of Dual RNA-seq data - an experimental method for interrogating host-pathogen interactions through simultaneous RNA-seq.

dualrna-seq host-pathogen quantification readmapping rna-seq

🔍 1.0.0 released over 3 years ago

cageseq ✓ ☆ 11

CAGE-sequencing analysis pipeline with trimming, alignment and counting of CAGE tags.

cage cage-seq cageseq-data gene-expression rna

🔍 1.0.2 released over 3 years ago

circrna 🏹 ☆ 44

circRNA quantification, differential expression analysis and miRNA target prediction of RNA-Seq data

circrna circrna-pipeline circrna-prediction circular-rna genomics miRNA mirna-targets ngs rna-seq

last changes 12 days ago

spatialvi 🏹 ☆ 49

Pipeline for processing spatially-resolved gene counts with spatial coordinates and image data. Designed for 10x Genomics Visium transcriptomics.

10x-genomics 10xgenomics image-processing microscopy rna-seq single-cell spatial spatial-transcriptomics st transcriptomics visium

last changes about 1 month ago

rnadnavar 🏹 ☆ 5

Pipeline for RNA and DNA integrated analysis for somatic mutation detection

last changes 3 months ago

scflow 📄 ☆ 23

Please consider using/contributing to <https://github.com/nf-core/scdownstream>

rnaseq single-cell single-nuclei single-nuclei-rna-sequencing

last changes 4 months ago

Incipie 🏹 ☆ 33

UNDER DEVELOPMENT--- Analysis of long non-coding RNAs from RNA-seq datasets

differential-expression lncrna long-non-coding non-coding rna rna-seq-analysis transcriptome

last changes about 2 years ago

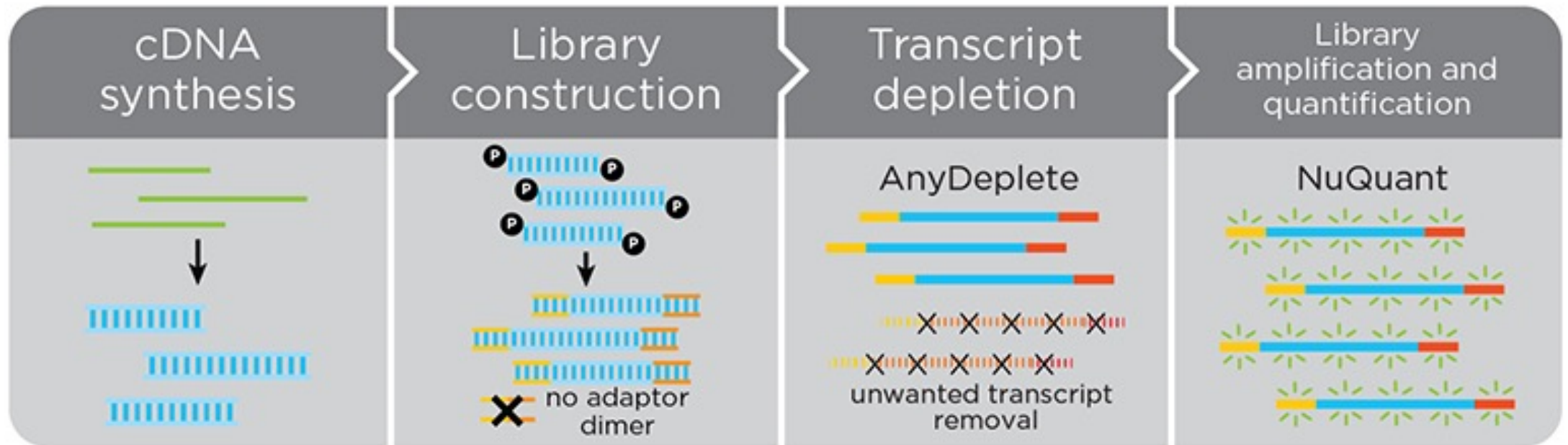
smartseq2 📄 ☆ 15

A pipeline for processing single cell RNA-seq data generated with the SmartSeq2 protocol.

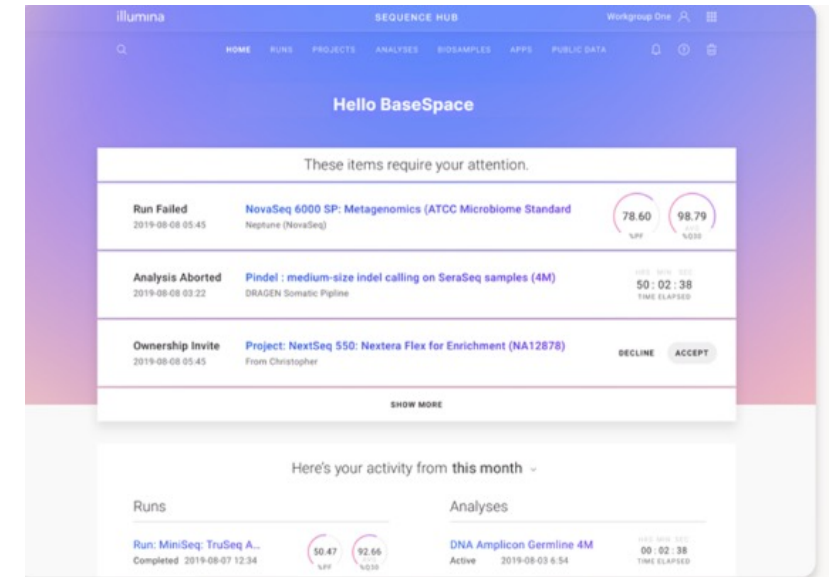
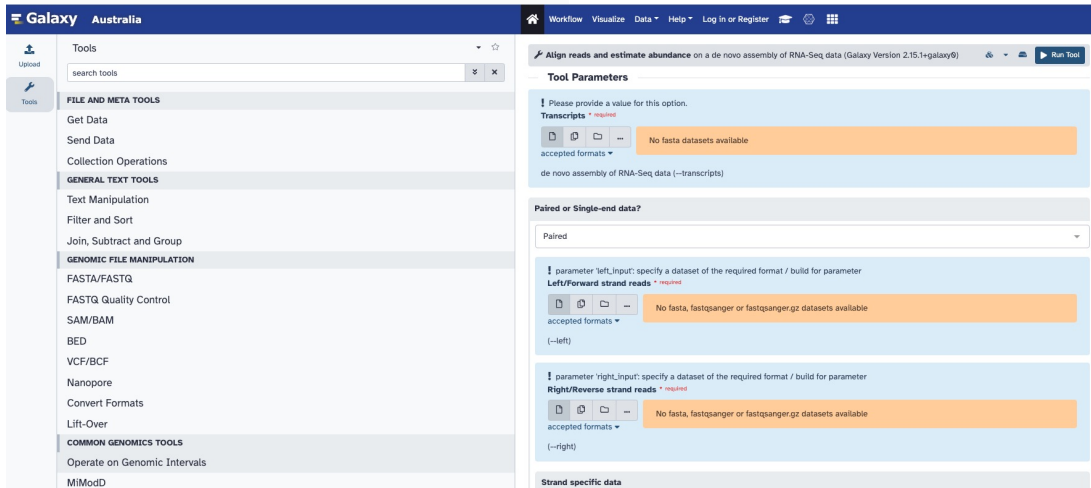
last changes over 3 years ago

RNAseq library preparation

- Universal Plus Total RNA-Seq Library Preparation Kit



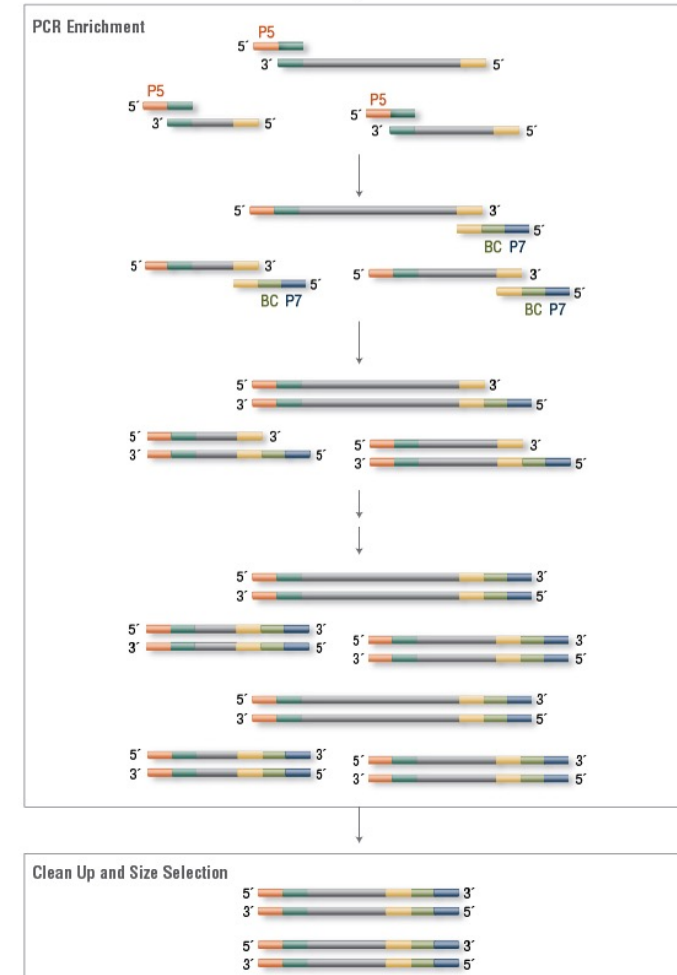
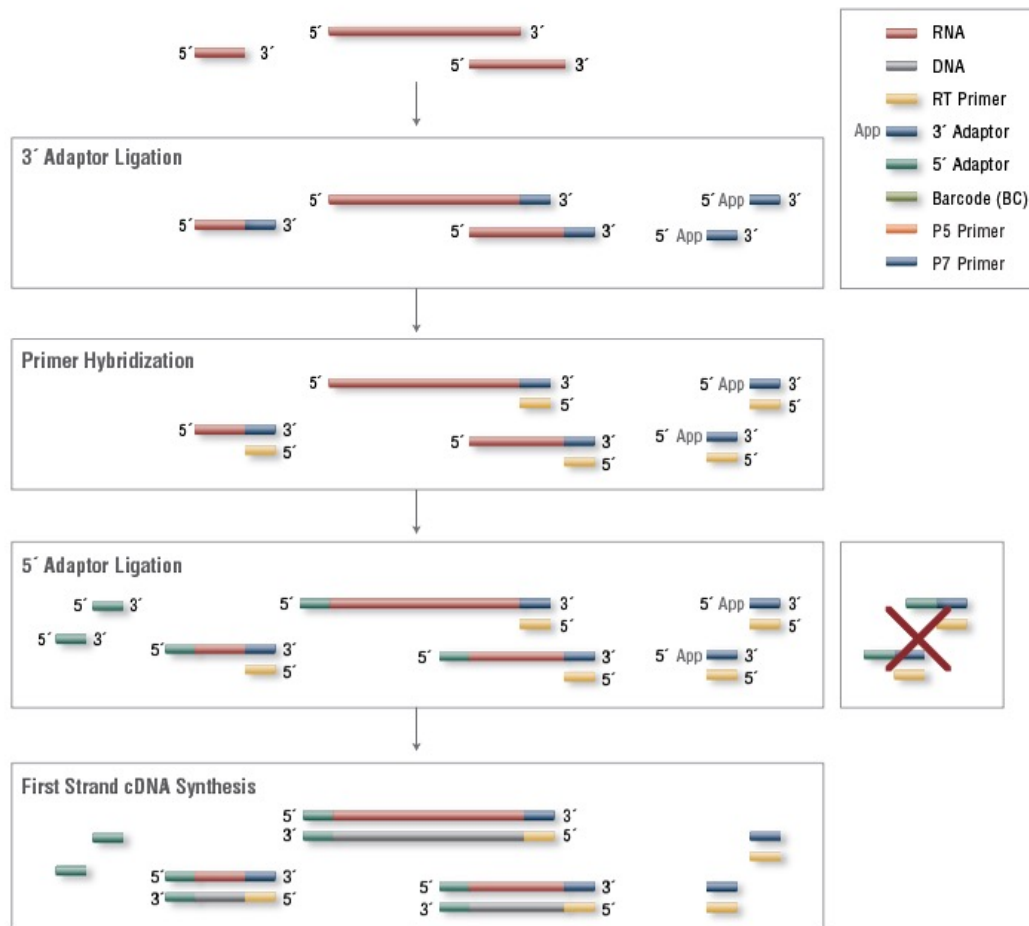
Other options in running Bioinformatics Pipelines



Small RNAseq library preparation

- NEBNext Small RNA Library Prep Set for Illumina

Small RNA Library Preparation Workflow for Illumina



Range of Sequencing technologies

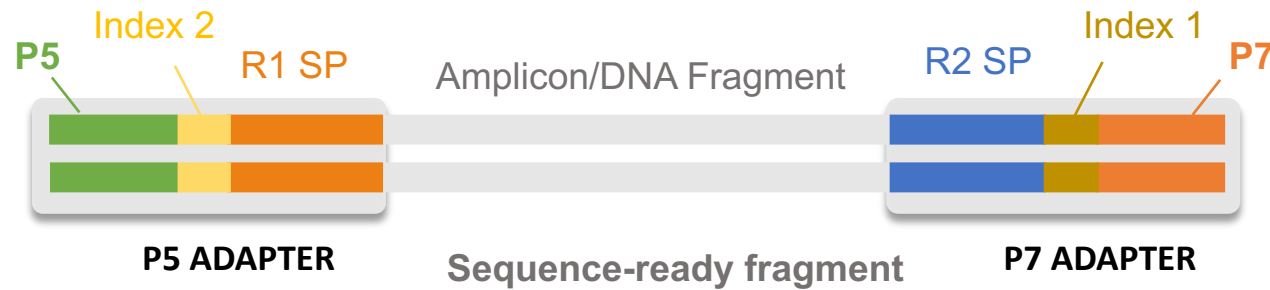


MGISEC-T7



Sequencing libraries

The aim of library prep is to obtain nucleic acid fragments with adapters attached on both ends



P5 and P7 regions are complementary to the oligos bound to the flow cell surface

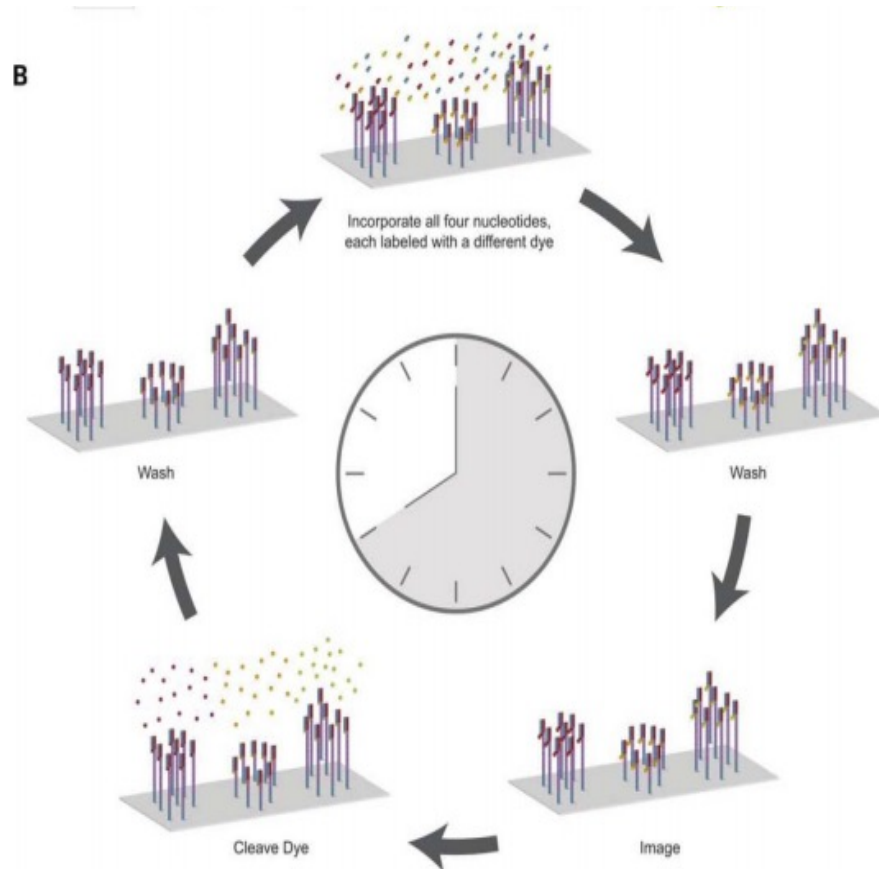
Index sequences are used to tag individual samples to allow for pooling

Read 1 & Read 2 Sequencing Primers are used to initiate sequencing

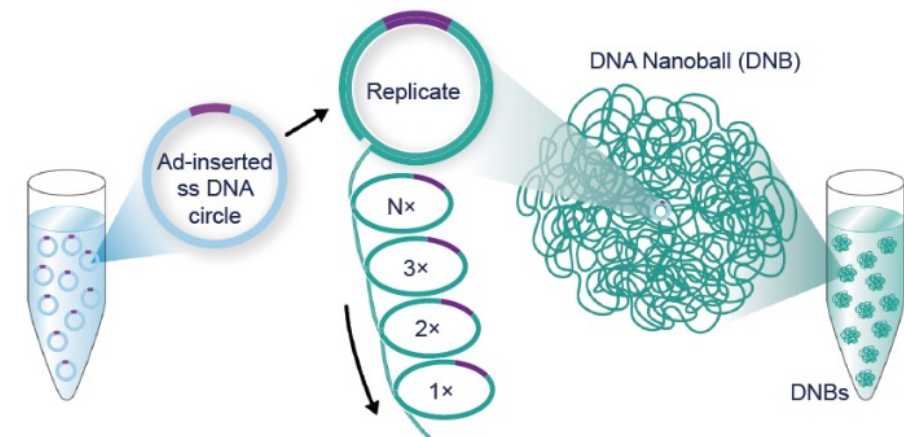
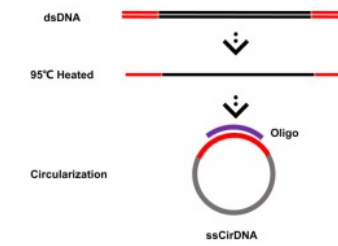
“short read” sequencing

illumina®

Sequencing by Synthesis



MGI



DNBSEQ™

Fastq files – raw sequence data

label @A00119:119:HMJWYDSXX:3:2678:28094:37059 3:N:0:ANATGTAC
sequence GAGAAACTGAAGCATTCTAAGACAAAGGAAAACCTTTTAAAAAGCCTCTAACAGGCCGGA
separator + Views - IGV
Q score FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFF
(ASCII chars)

eg. Base=T, Q=':'=25

- Read information for each sample stored as FASTQ files
 - Unmapped raw sequence file
 - Text format (.fastq.gz)
 - Has a 4-line entry for each read
 - Contains base call information and quality scores for each base
 - Contains reference information for each read

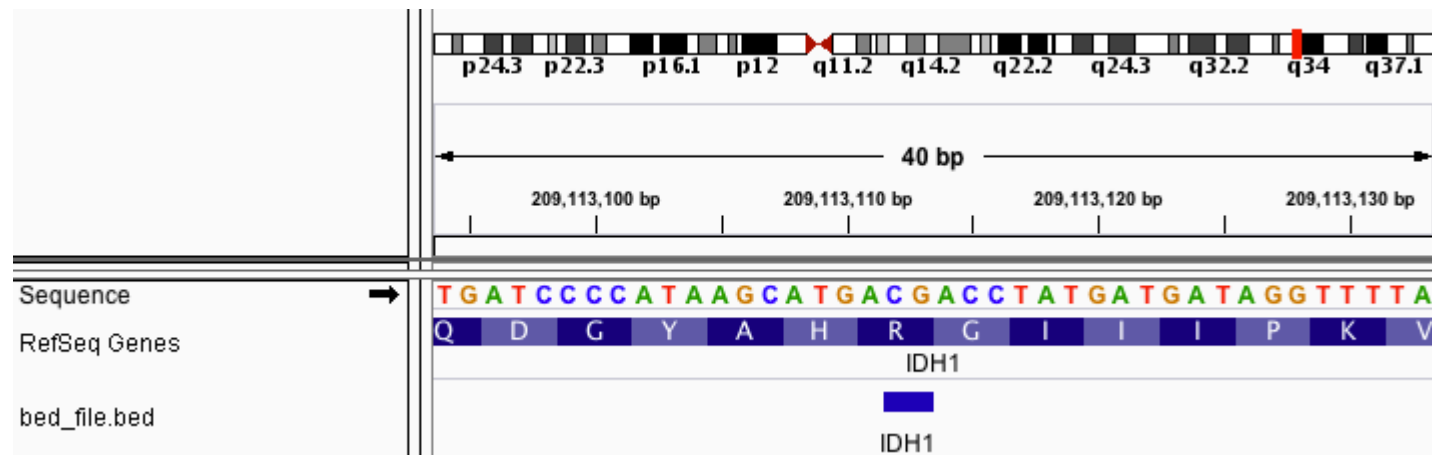
BED file

- Browser Extensible Data (BED) file
- 4 -12 columns

chromosome	start	stop	name
chr21	37518705	37518706	CBR3
chr2	209113111	209113113	IDH1
chr3	37053566	37053567	MLH1

tab - delimited

IGV – genomics viewer



BIOINFORMATICS PIPELINES

The SAGC provides a suite of analysis pipelines developed both externally and in-house, based on community best practises.

Workflows designed for SAGC sequenced libraries with set endpoints for quick turnaround.



Whole-Genome (WGS)



Whole-Exome (WES)



RNA seq



DNA Methylation, or Whole-Genome Bisulfite (WGBS)



Single Cell RNA (scRNA-seq)



Spatial Transcriptomics



Chromatin Immunoprecipitation Sequencing (ChIP-seq)



Small RNA-seq



Assay of Transposase Accessible Chromatin (ATAC-seq)



Metagenomics



Microbial Profiling (Microbiome)



Amplicons