



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.

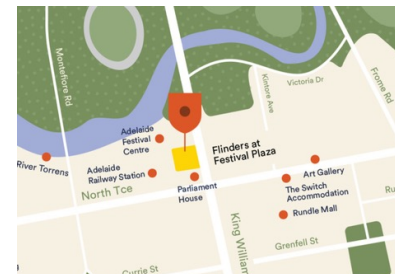


Description of the key metrics and analyses for RNAseq analysis

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

Why use RNAseq?

NGS

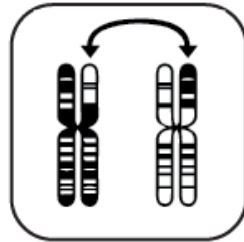


variant detection



SNV's
INDELS

structural variants



deletions
duplications
translocations
inversions

gene expression



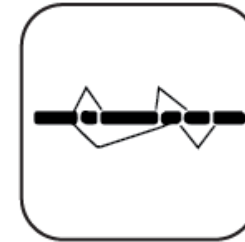
oncogene
lymphocyte surface
markers

antigen receptor



TCR
BCR

aberrant splicing



skipped exons
retained introns
fusion transcripts

metagenome



pathogens
virus's
bacteria

because it can do the work of dozens of other tests...

as long as you can analyse it.

Why nextflow and nf-core?

This is where bioinformatics comes in.



Made possible by the development and increasing popularity of *nextflow*, a workflow management system. Effectively making analysis options of many genomics technologies including RNAseq, more accessible, reproducible, and streamlined.

Recent initiatives to collect and publish curated analysis pipelines such as *nf-core*, are consolidating decades of software development.



And there is a growing list of , helping cement RNAseq as a staple methodology for molecular and genetic research .

Pipelines

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

RNA

Released 18 Under development 4 Archived 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.

rna 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 Chemirc (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

2.7.1 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 dseq2
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

facts-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-rbp-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

Incpipe ✂ ☆ 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

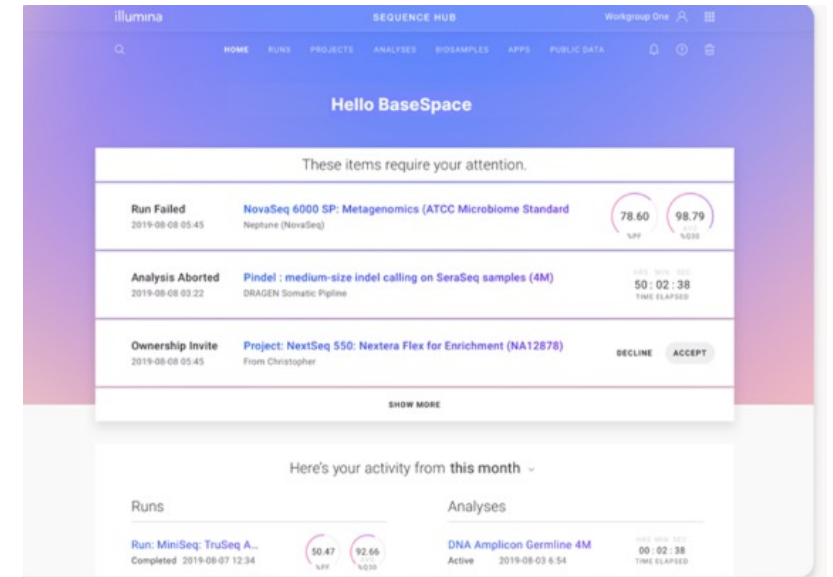
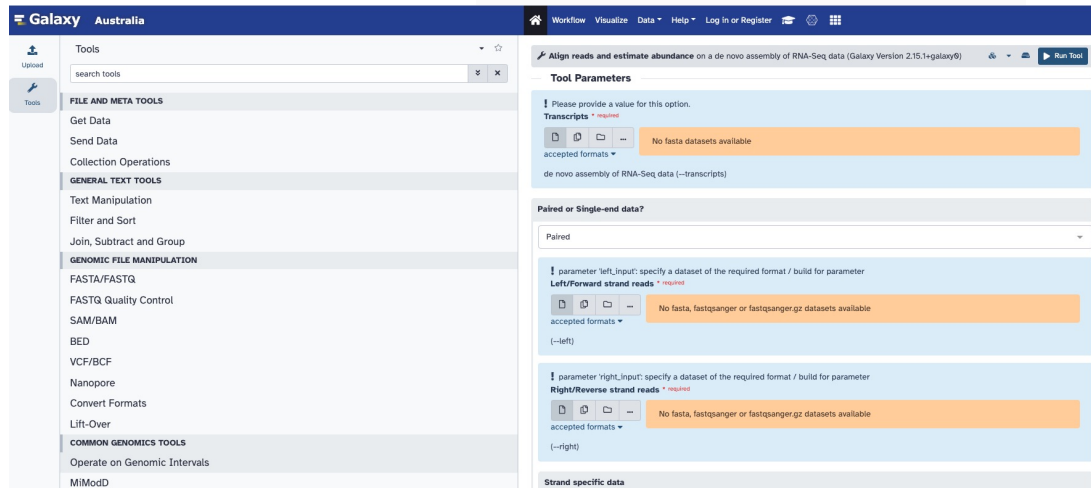
smartseq2 📄 ☆ 15

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

last changes over 3 years ago

Many options in running Bioinformatics Pipelines

With many pro's and con's.

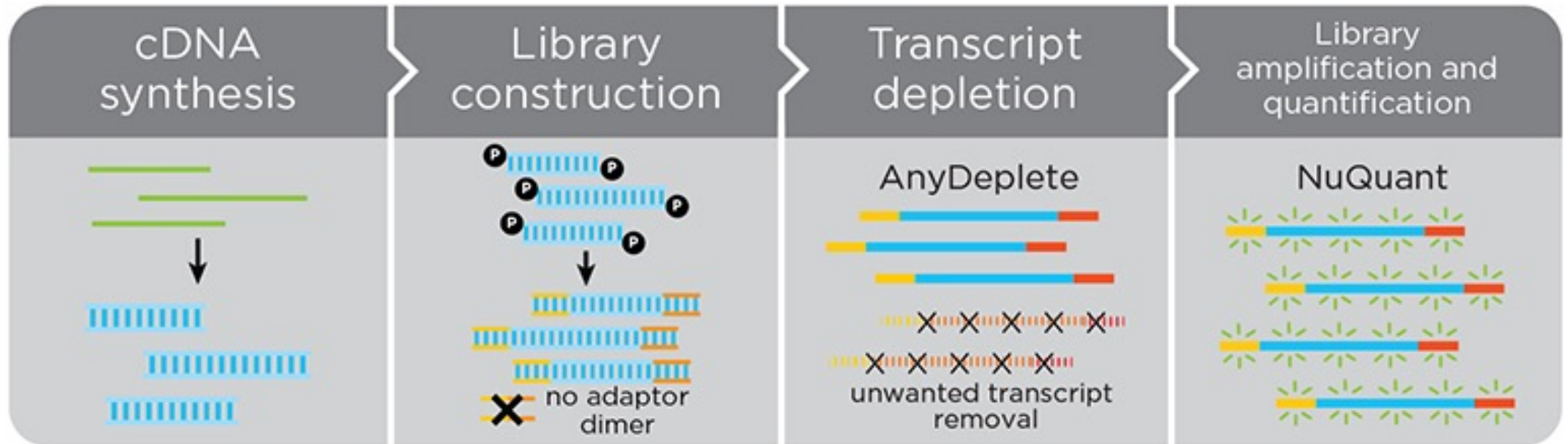


and you can write your own tools



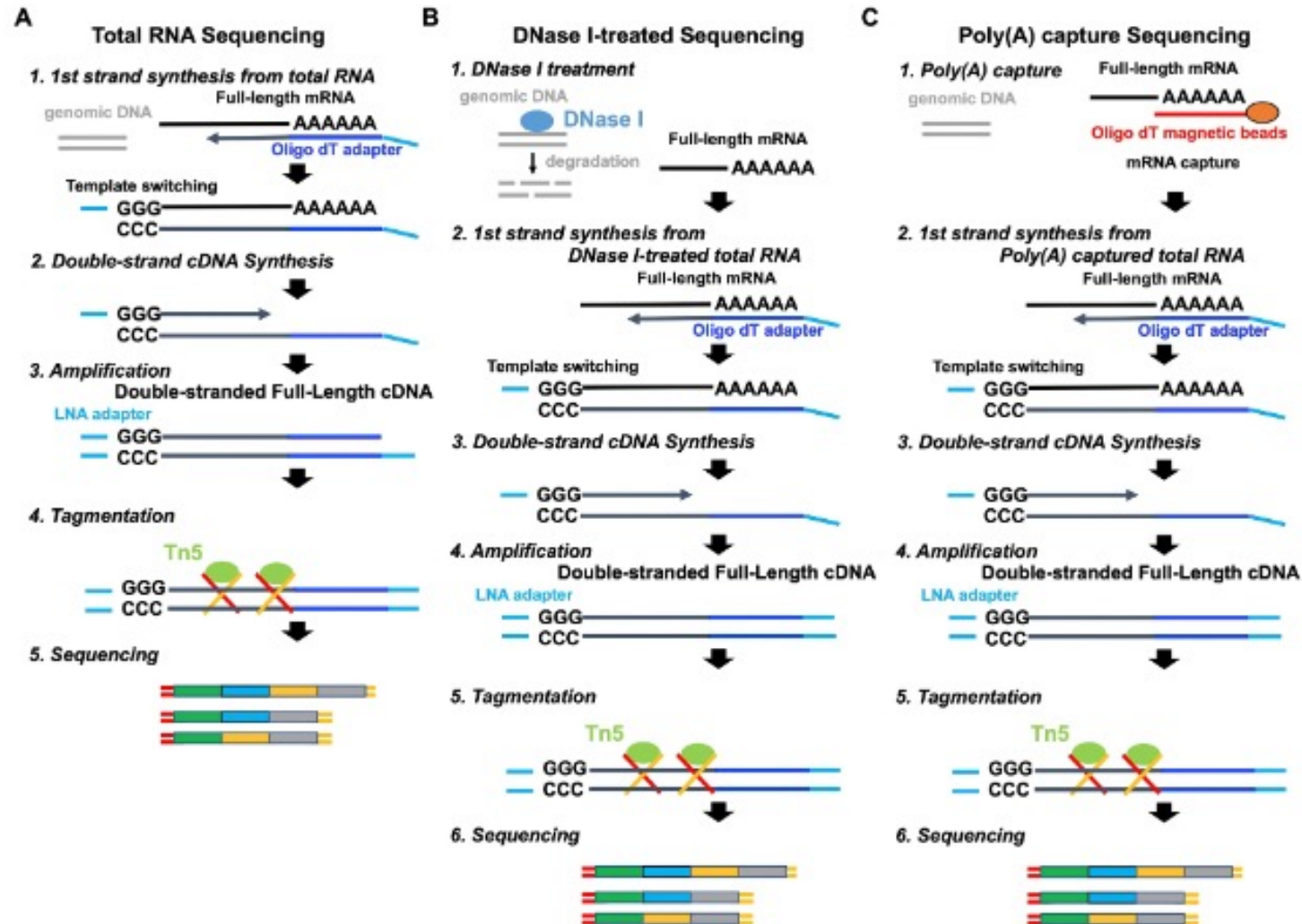
RNAseq library preparation

- Universal Plus Total RNA-Seq Library Preparation Kit



RNAseq Total RNA vs PolyA capture

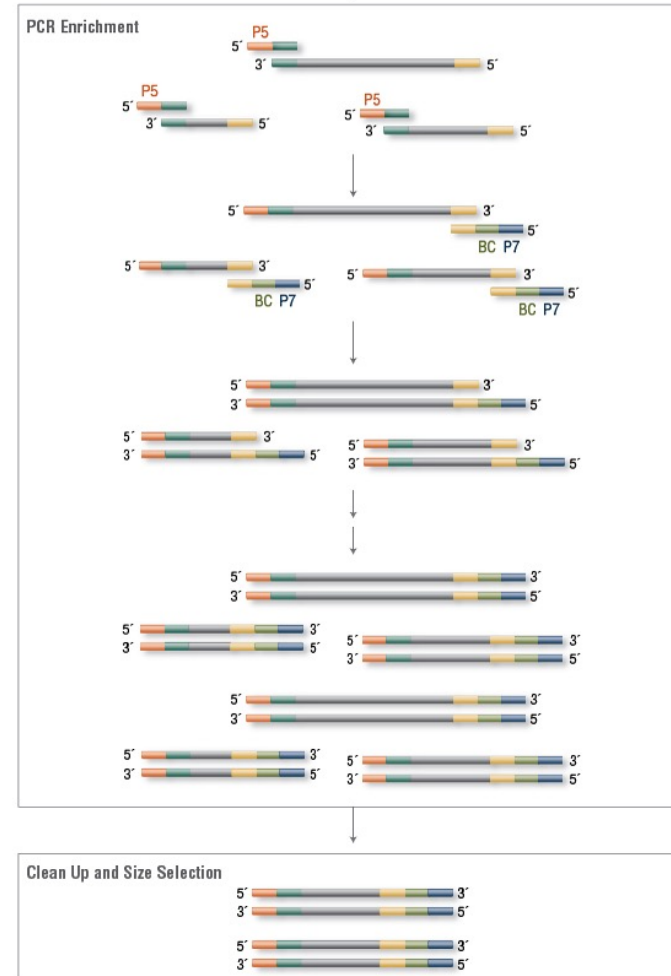
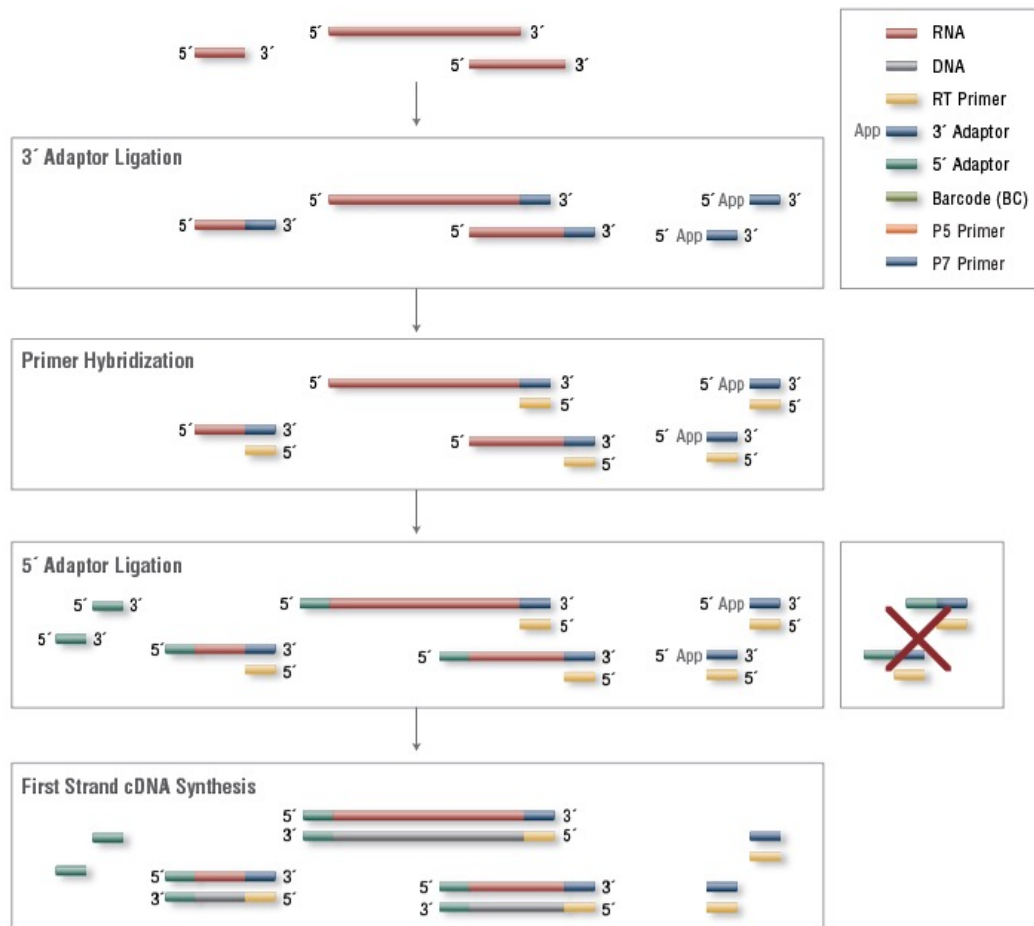
From: [Poly\(A\) capture full length cDNA sequencing improves the accuracy and detection ability of transcript quantification and alternative splicing events](#)



Small RNAseq library preparation

- NEBNext Small RNA Library Prep Set for Illumina

Small RNA Library Preparation Workflow for Illumina



Range of Sequencing technologies

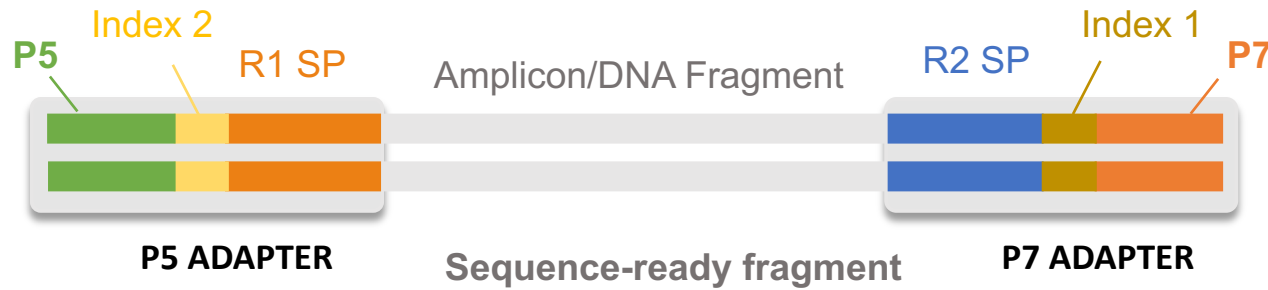


MGISEC-T7



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

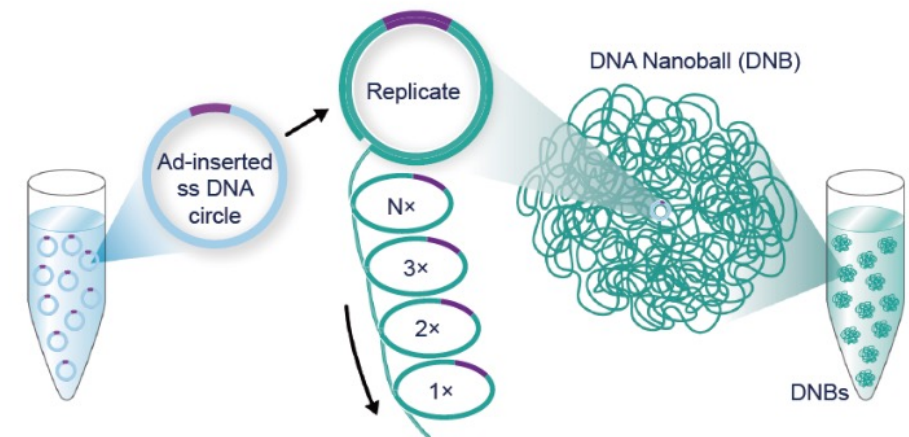
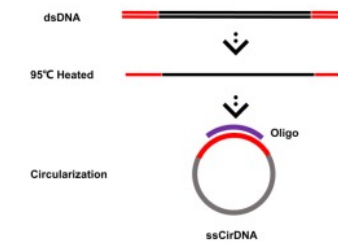
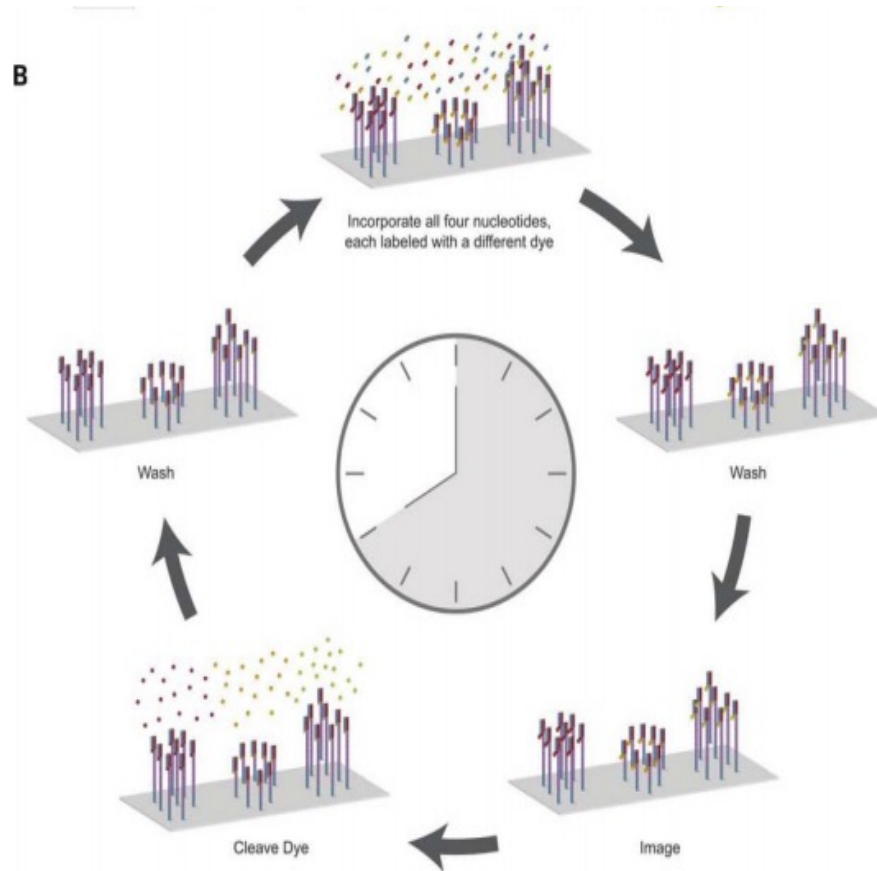
Read 1 & Read 2 Sequencing Primers are used to initiate sequencing

“short read” sequencing

illumina®

Sequencing by Synthesis

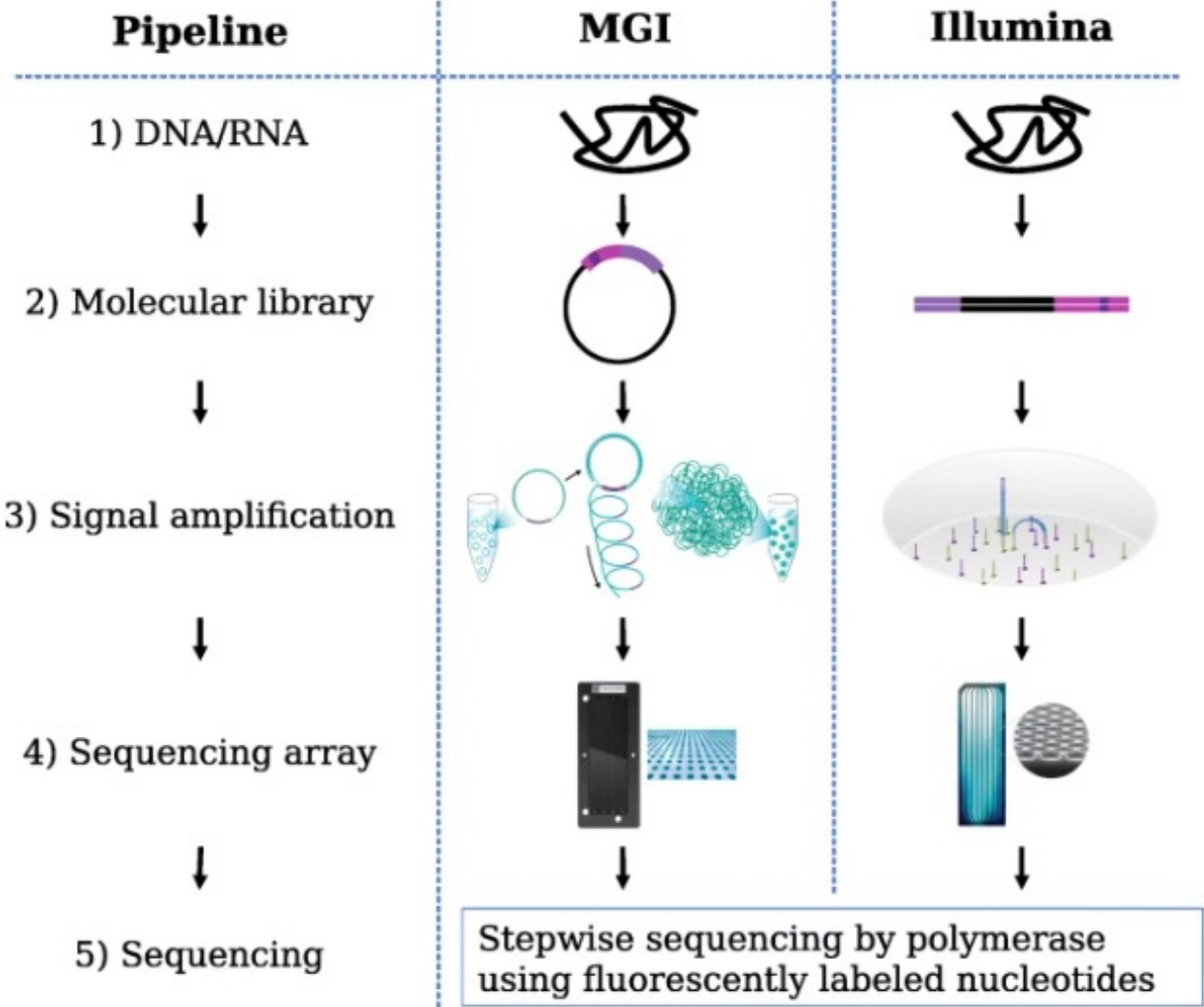
MGI



DNBSEQ™

Differences between MGI and Illumina

Fig. 1



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