Bulk RNA-Seq Analysis on the DNAnexus platform

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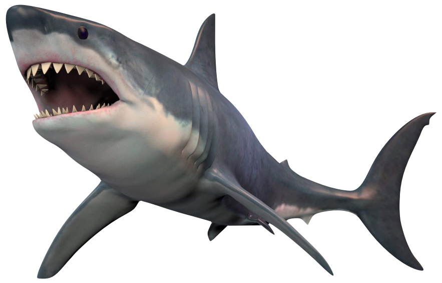
RNA-Seq Week



Specific Resource-Talk Goals

- Make you aware of the resource
- Explain what it does
    - Inputs
    - Outputs
- Who should use it
- Strengths and Weaknesses
- How to access it

Today’s Agenda

- Introduction to DNAnexus and Cloud Computing
- Introduction to Salmon - RNA-SEQ “mapping”
- RNA-Seq Workflow
    - Trimming - QC
    - Alignment/Mapping
    - Data Exploration
- Introduction to R-Shiny - Interactive tools
- How to gain access to these tools

DNAnexus

Cloud Computing made simple and safe

"**DNAnexus** provides a **cloud-based platform** optimized to address the challenges of security, scalability, and collaboration, for organizations that are pursuing genomic-based approaches to health, in the clinic and in the research lab."

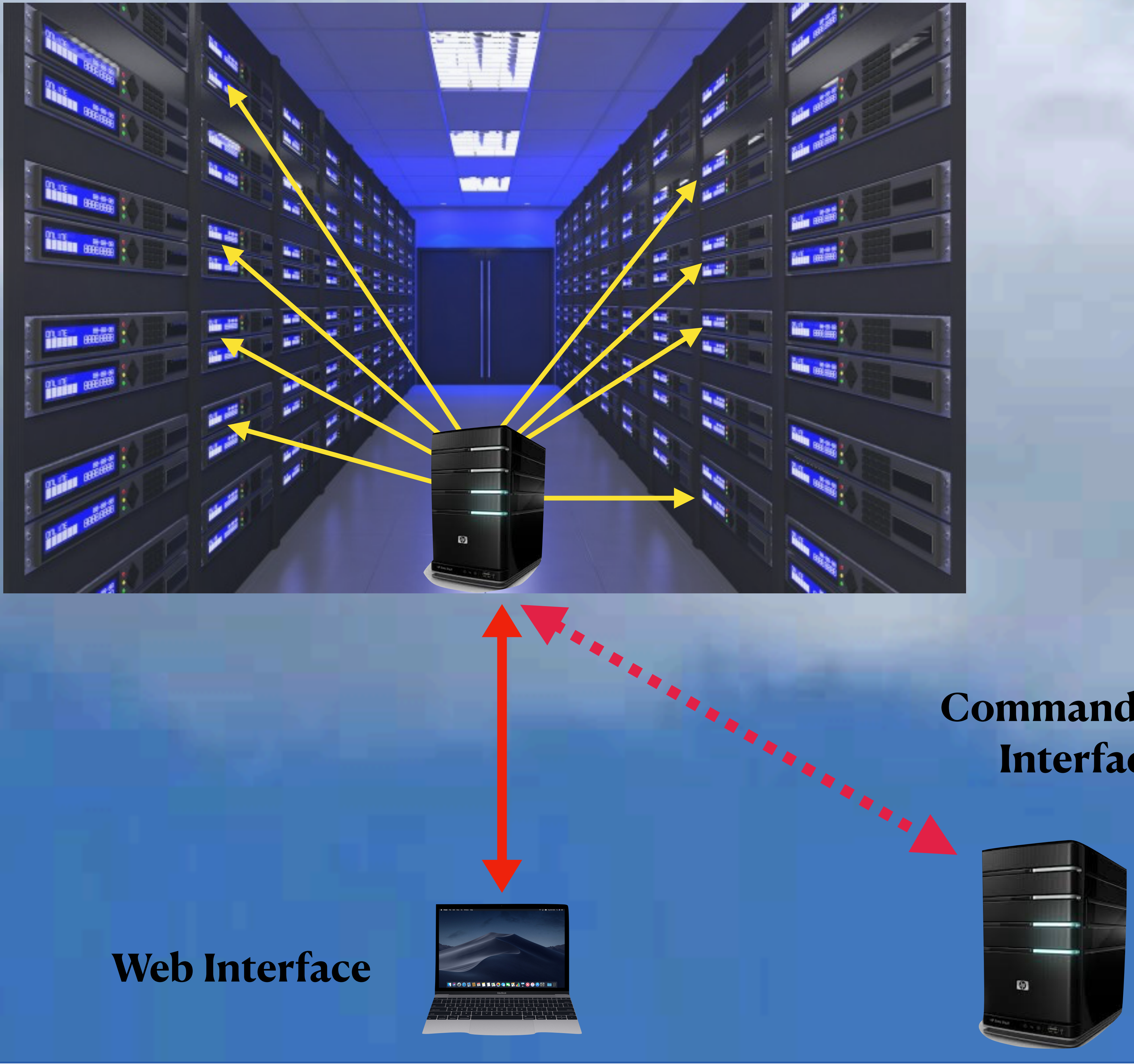
Practically this equates to a Web Interface to secure Compute and Storage facilities on the Amazon Cloud (AWS) within which CCR scientist can run a variety of application for the analysis of NGS data.
The typical interface is via the Web but the resources is also available through command line commands and scripts.

I believe the greatest asset of this system is that it gives researchers direct access to powerful open source tools in a controlled environment.

Key Features of DNAnexus

- DNAnexus provides ready access to a variety of prebuilt (by DNAnexus , local experts and other organizations) tools and workflows for the analysis of Genomic Data, within a “User friendly” Web interface.
- The platform is cloud based which means there is no hardware to buy or maintain, or data to backup
- Provides a ready means of sharing data, results and analytical methodology with intra and extramural colleagues
- Ready access to a vast computational resource, with multiple levels of parallelism
- Access to Web-based (Shiny etc) applications on dedicated “servers”
- As well as a Web interface all the applications on the system can be accessed via command line instructions or scripts.

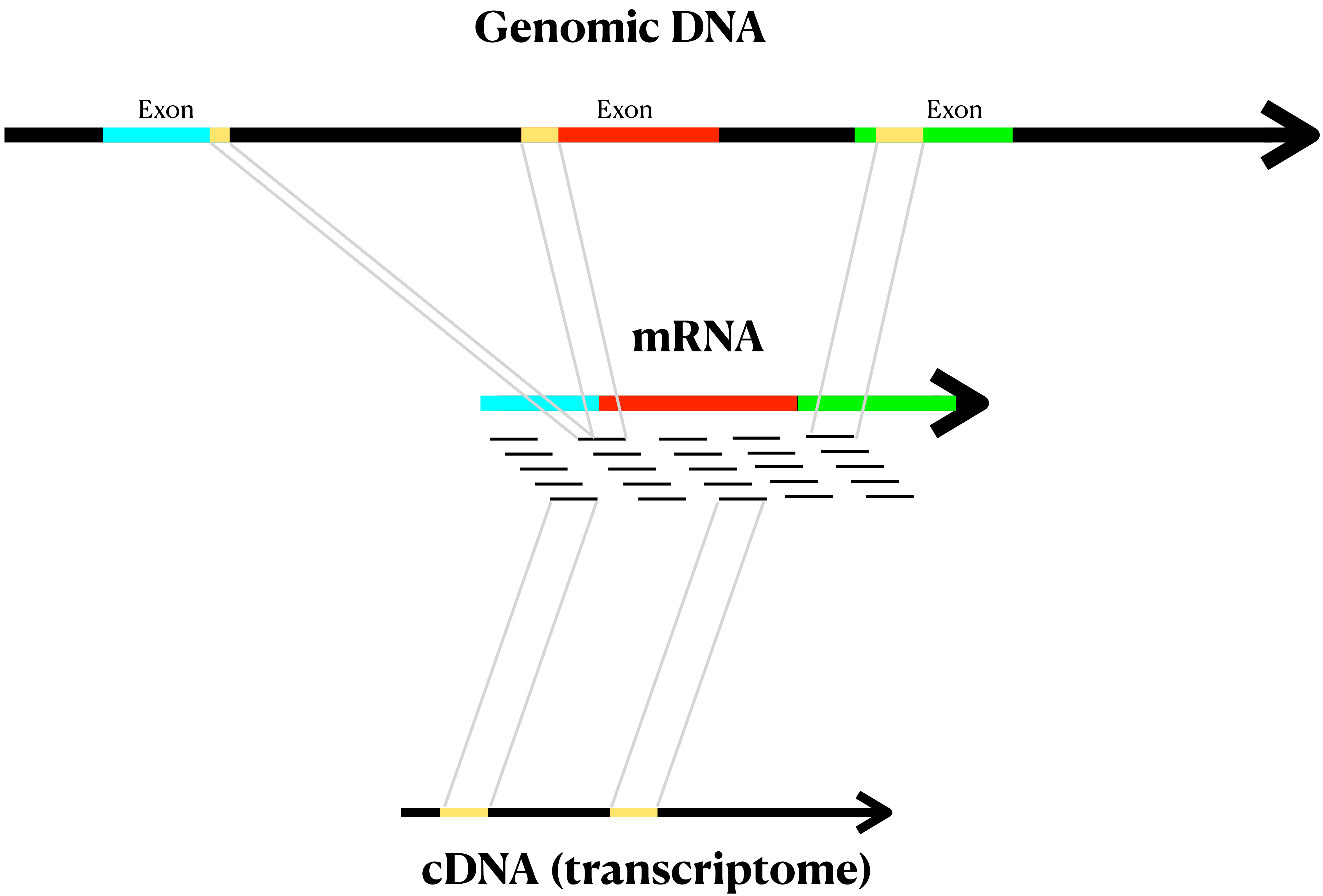
Cloud Computing



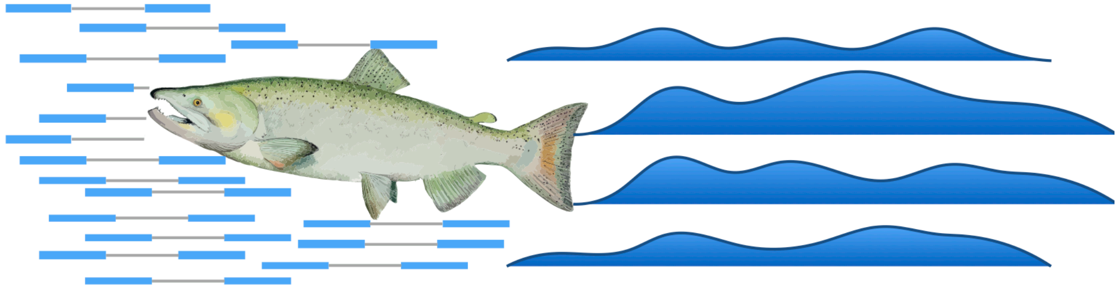
Command line Interface

Web Interface

RNA-Seq Mapping



Salmon



Fast, accurate and bias-aware transcript quantification from RNA-seq data

Three modes:

- Transcriptome only
- Transcriptome + similar regions of the genome (as background/decoy)
- Transcriptome + the rest of the genome (as background/decoy)

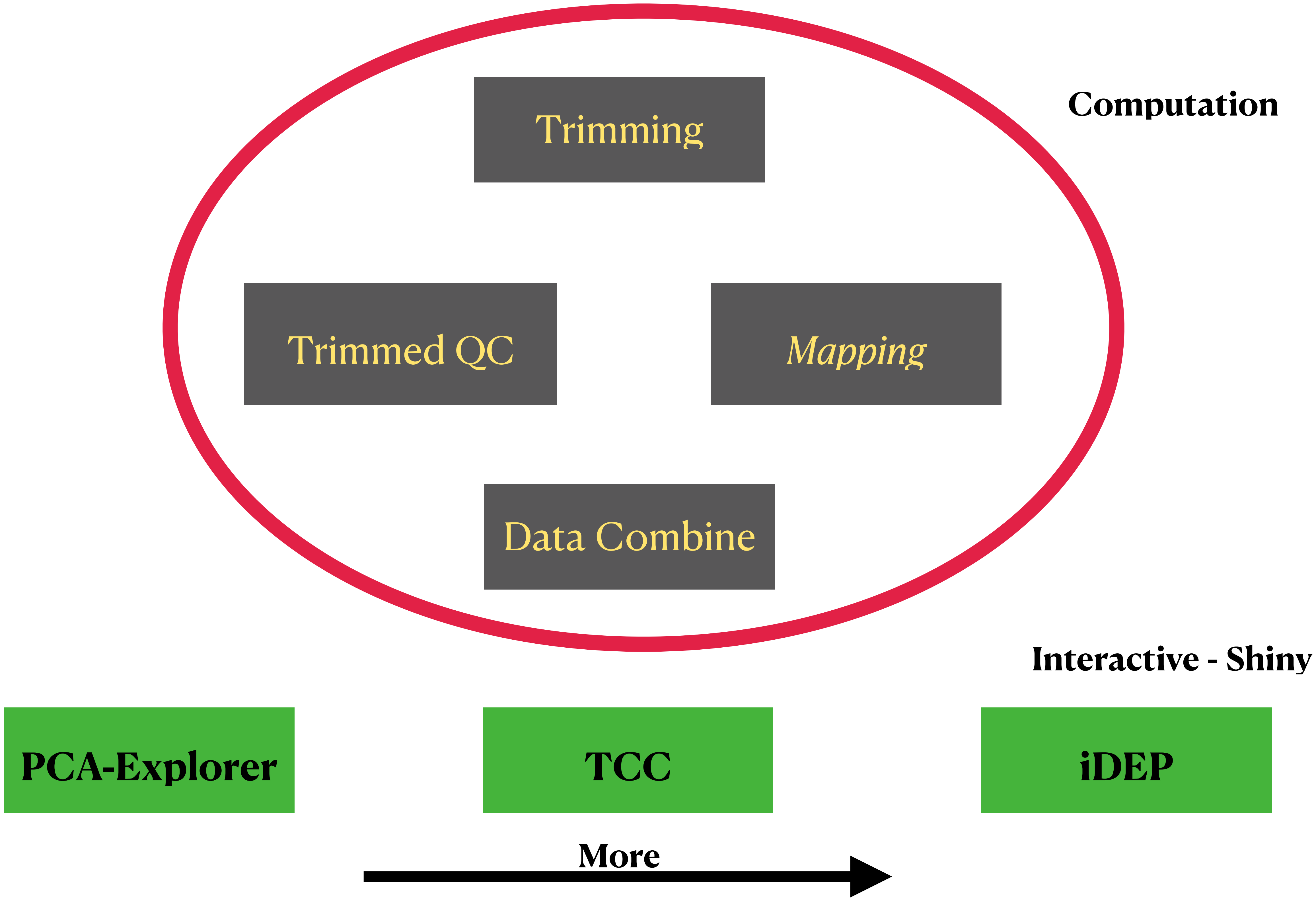
More Accurate ↓

Faster ↑

Genomes - Human, Mouse - Gencode annotation

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods.
https://www.nature.com/articles/nmeth.4197
https://combine-lab.github.io/salmon/
Srivastava, A., Malik, L., Sarkar, H. *et al.* Alignment and mapping methodology influence transcript abundance estimation. *Genome Biol* **21,** 239 (2020).
https://doi.org/10.1186/s13059-020-02151-8

RNA-Seq Anaysis on DNAnexus



Computation

Trimming

Trimmed QC

Mapping

Data Combine

Interactive - Shiny

PCA-Explorer

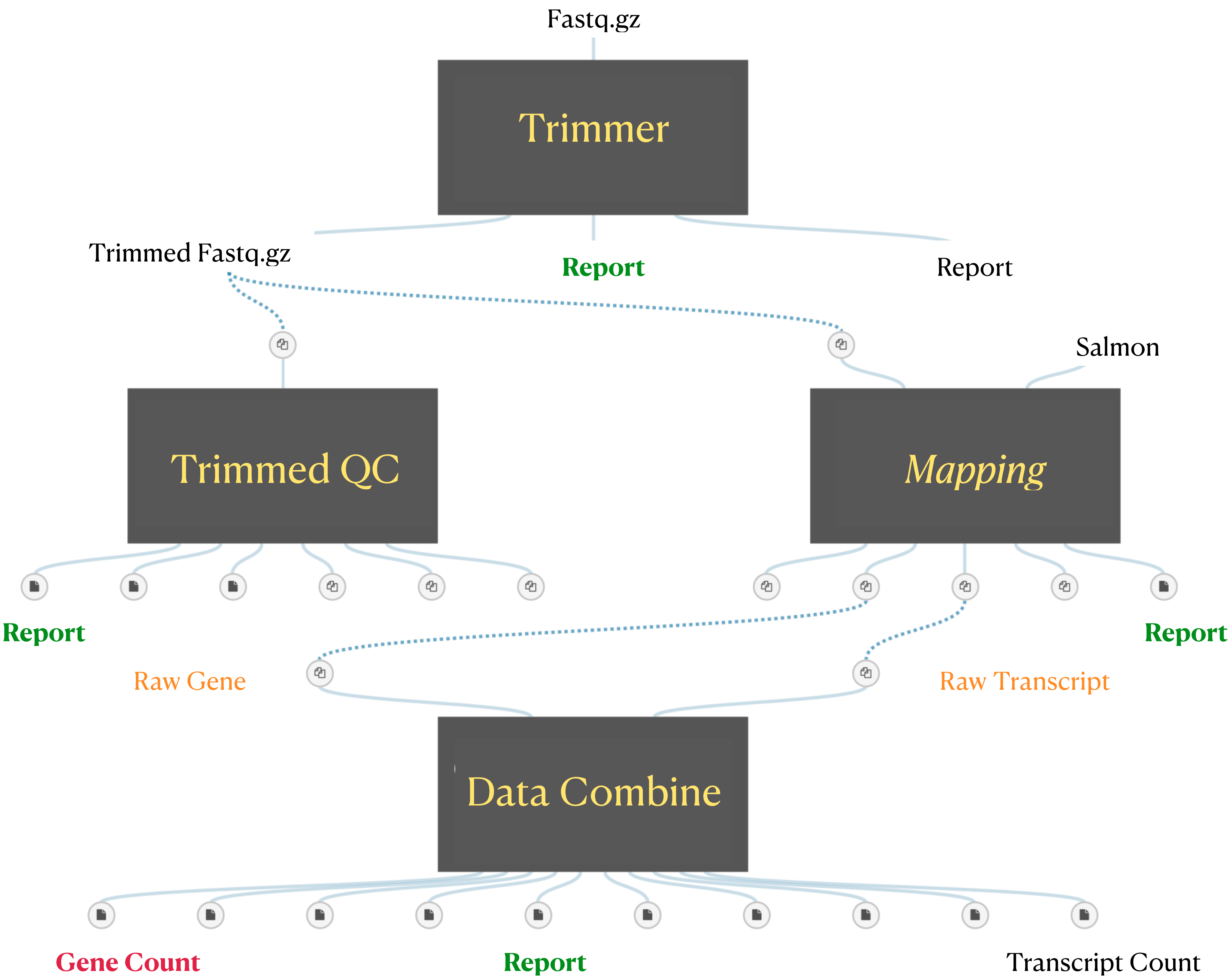
TCC

iDEP

More

(The DNAnexus implementation has largely been the work of Carl McIntosh)

RNA-Seq Workflow



Strengths and Weaknesses

RNA-Seq Workflow

- It is fast and lightweight - multi stages of parallelism
- Does a great job generating basic expression data - gene or transcript (*some argue that it is the most accurate method at the moment*).

- Cannot be used to look for novel splice variants (but will find annotated variants)
- Does not produce an alignment (bam file) and so is not readily manually verified

Inputs/Outputs

INPUT

- Fastq.gz files (adaptor trimmer or not)

OUTPUT

Main Workflow

- Raw counts for gene and transcripts
- TPM counts for gene and transcripts
- **Gene Matrix file** (with/without filter of zero count genes)
- Reports

Interactive Shiny Apps

- Normalized data
- Lists of Differentially Expressed Genes
- Heat Maps, MA plots, Volcano Plots etc. (Many plots are interactive)
- Reports

Inputs/Outputs

Salmon outputs *.quant.sf files with the following data

- **Name** — This is the name of the target transcript provided in the input transcript database (FASTA file).
- **Length** — This is the length of the target transcript in nucleotides.
- **EffectiveLength** — This is the computed *effective* length of the target transcript. It takes into account all factors being modeled that will effect the probability of sampling fragments from this transcript, including the fragment length distribution and sequence-specific and gc-fragment bias (if they are being modeled).
- **TPM** — This is salmon’s estimate of the relative abundance of this transcript in units of Transcripts Per Million (TPM). TPM is the recommended relative abundance measure to use for downstream analysis.
- **NumReads** — This is salmon’s estimate of the number of reads mapping to each transcript that was quantified. It is an “estimate” insofar as it is the expected number of reads that have originated from each transcript given the structure of the uniquely mapping and multi-mapping reads and the relative abundance estimates for each transcript.

Name	Length	Effective Length	TPM	NumReads
AJ271736.1	773	604.234	0	0
IL9R	1944	1775.16	0.070881	1
TRPC6P	880	711.196	0.471496	2.665
VAMP7	2357.99	2189.18	50.6254	880.802

Inputs/Outputs

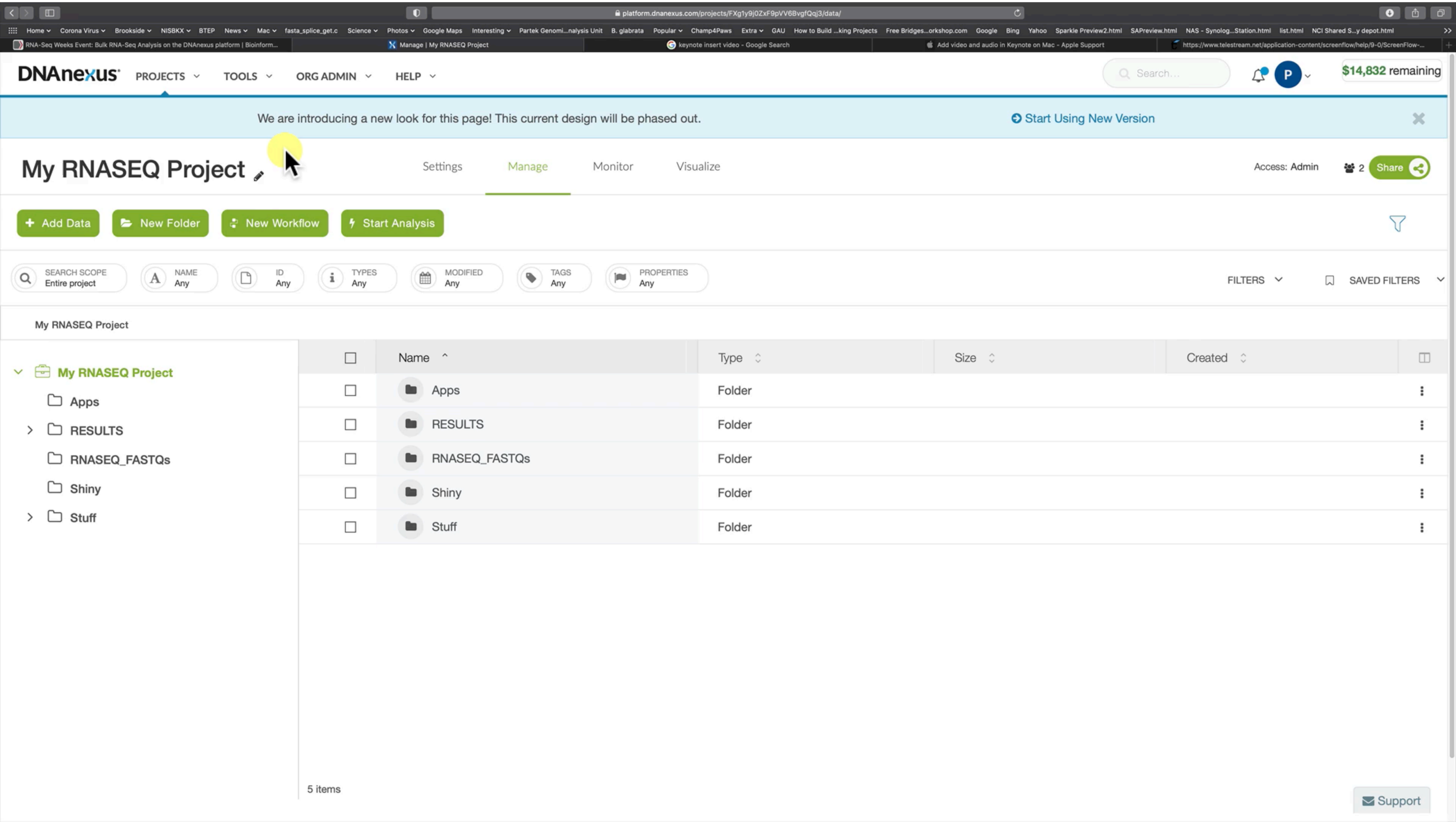
The gene matrix file us the ultimate output of this workflow

The file contains read counts for all genes in each sample

Gene	A_rep1	A_rep2	A_rep3	B_rep1	B_rep2	B_rep3
RAB39B	713	715	571	0	0	2
VBP1	1529	1748	957	637	697	737
BRCC3	485	481	284	186	74	203
FUNDC2	728	1180	457	1929	2562	2044
F8A1	435	248	211	65	197	118

RNA-Seq Workflow
Demo
FASTQ - > Count Matrix

Pre-recorded



□	Name	Type	Size	Created
□	Apps	Folder		
□	RESULTS	Folder		
□	RNASEQ_FASTQs	Folder		
□	Shiny	Folder		
□	Stuff	Folder		

R Shiny

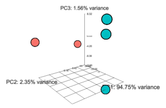
Shiny is an R package that makes it easy to build interactive web apps straight from R.

Shiny provides access to many R tools via simple to use Graphic User interfaces

DNAnexus provides the means of running R Shiny apps on a dedicated server (made available on demand) without the hassle of worrying about installation, dependencies or coding.

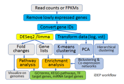
Interactive Data Exploration

There are currently three R-Shiny applications on the Platform that facilitate the exploration and biological interpretation of RNA-Seq data:



pcaExplorer: Interactive Visualization of RNA-seq Data Using a Principal Components Approach

🔬TCC-GUI: Graphical User Interface for TCC package



Read counts or FPKMs
Remove lowly expressed genes
Convert gene IDs
DESeq2 /limma
Transform data (log, vst)
Fold changes
Gene lists
K-means clustering
PCA
Hierarchical clustering
Pathway analysis
Enrichment analysis
Biclustering & Co-expression networks
Visualize on genomes
GO terms, KEGG pathways, TF target genes, miRNA target genes
iDEP workflow

iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data

pcaExplorer

pcaExplorer: Interactive Visualization of RNA-seq Data Using a Principal Components Approach

This package provides functionality for interactive visualization of RNA-seq datasets based on Principal Components Analysis. The methods provided allow for quick information extraction and effective data exploration. A Shiny application encapsulates the whole analysis.

Marini, F., Binder, H. pcaExplorer: an R/Bioconductor package for interacting with RNA-seq principal components. *BMC Bioinformatics* **20,** 331 (2019). https://doi.org/10.1186/s12859-019-2879-1

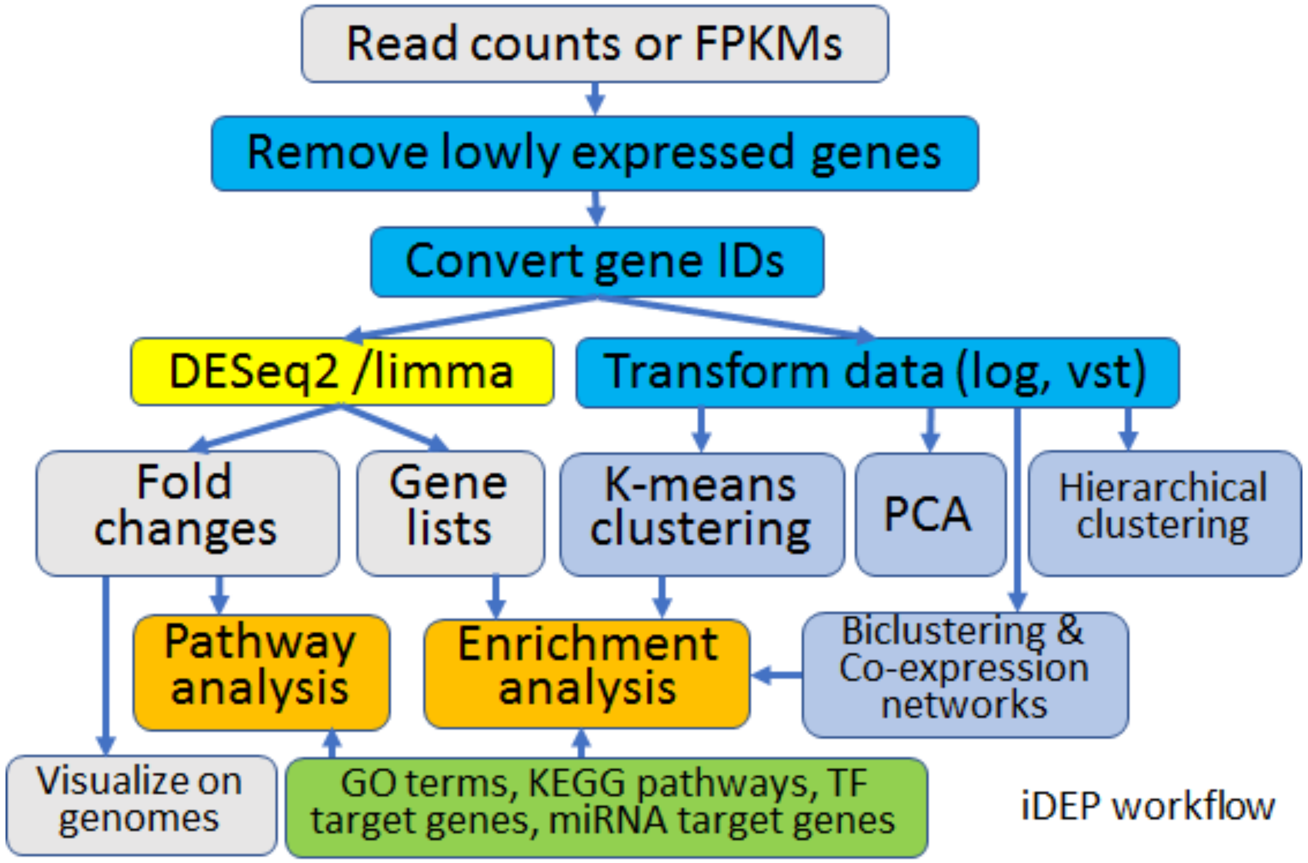
TCC[1] is a R/Bioconductor package provides a series of functions for performing differential expression (**DE**) analysis from RNA-seq count data using a robust normalization strategy (called **DEGES**).

🔬TCC-GUI: Graphical User Interface for TCC package

The basic idea of **DEGES** is that potential differentially expressed genes (**DEGs**) among compared samples should be removed before data normalization to obtain a well-ranked gene list where true **DEGs** are top-ranked and **non-DEGs** are bottom ranked. This can be done by performing the multi-step normalization procedures based on **DEGES** (**DEG elimination strategy**) implemented in TCC. TCC internally uses functions provided by **edgeR**[2], **DESeq**[3], **DESeq2**[4], and **baySeq**[5] . The multi-step normalization of TCC can be done by using functions in the four packages.

Su, W., Sun, J., Shimizu, K. et al. TCC-GUI: a Shiny-based application for differential expression analysis of RNA-Seq count data. BMC Re
Notes 12, 133 (2019). https://doi.org/10.1186/s13104-019-4179-

iDEP92



iDEP workflow

Ge, S.X., Son, E.W. & Yao, R. iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data. *BMC Bioinformatics* **19,** 534 (2018). https://doi.org/10.1186/s12859-018-2486-6

Strengths and Weaknesses

Shiny Apps

- Great for **exploring** the data and or parameter choice effects.
- Generate interactive graphics
- Generate multipage reports
- Can quickly provide biological inside
- Great for pilot experiments

- Not the best for *reproducible science*
- *Slow startup*
- *Finite lifetime and Shiny inactive state termination… not the best for interrupted work*

RNA-Seq Workflow
Demo
Interactive DEG Apps

Pre-recorded

Intended Audience

- People with RNA-SEQ data - self generated or public data
- Anyone who wants rapid, accurate gene-count data from RNASeq data
- People in a hurry to **explore** their data
- People who want to tinker with parameters to understand their effect
- People who are using RNA-Seq in a pilot experiment or as a validation or exploration of an idea or hypothesis.

How to Access DNAnexus

CCR has a group license for DNAnexus and is currently funding all reasonable projects

If interested in pursuing the use of this platform send email to:

Peter Fitzgerald
fitzgepe@nih.gov

DNAnexus also host many different NGS workflows, including many custom built analysis packages for select tasks