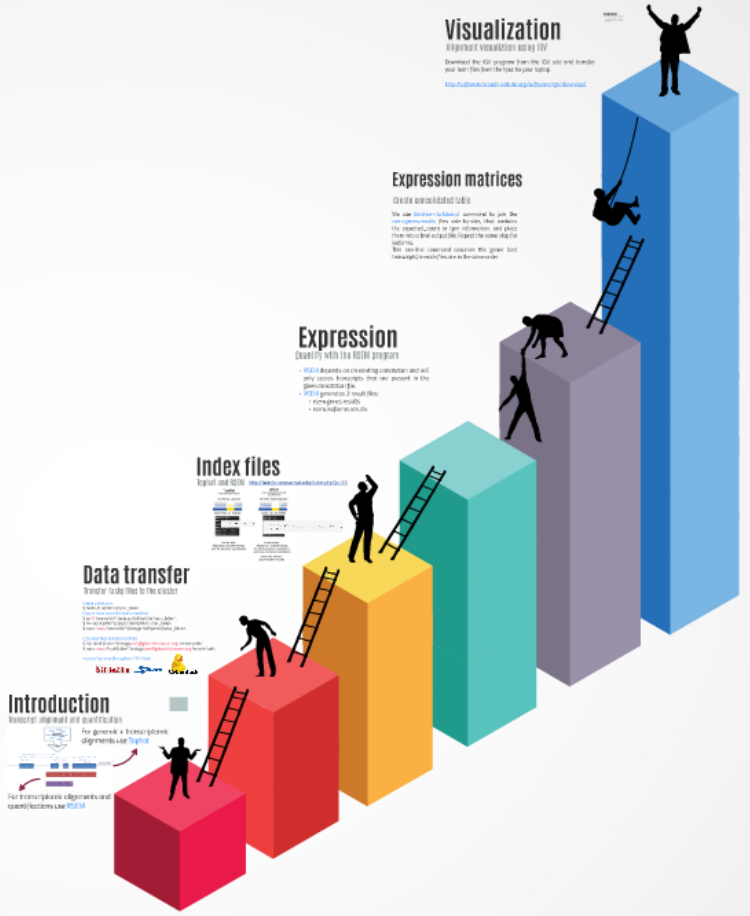
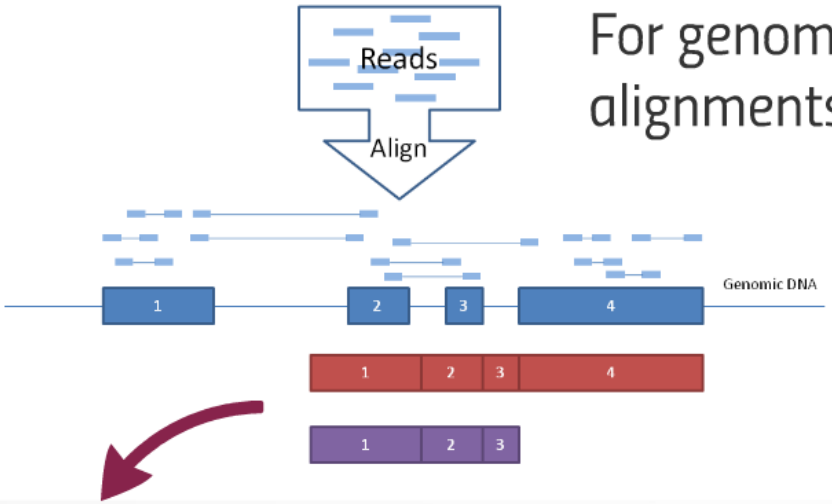
RNA-Seq

Data processing



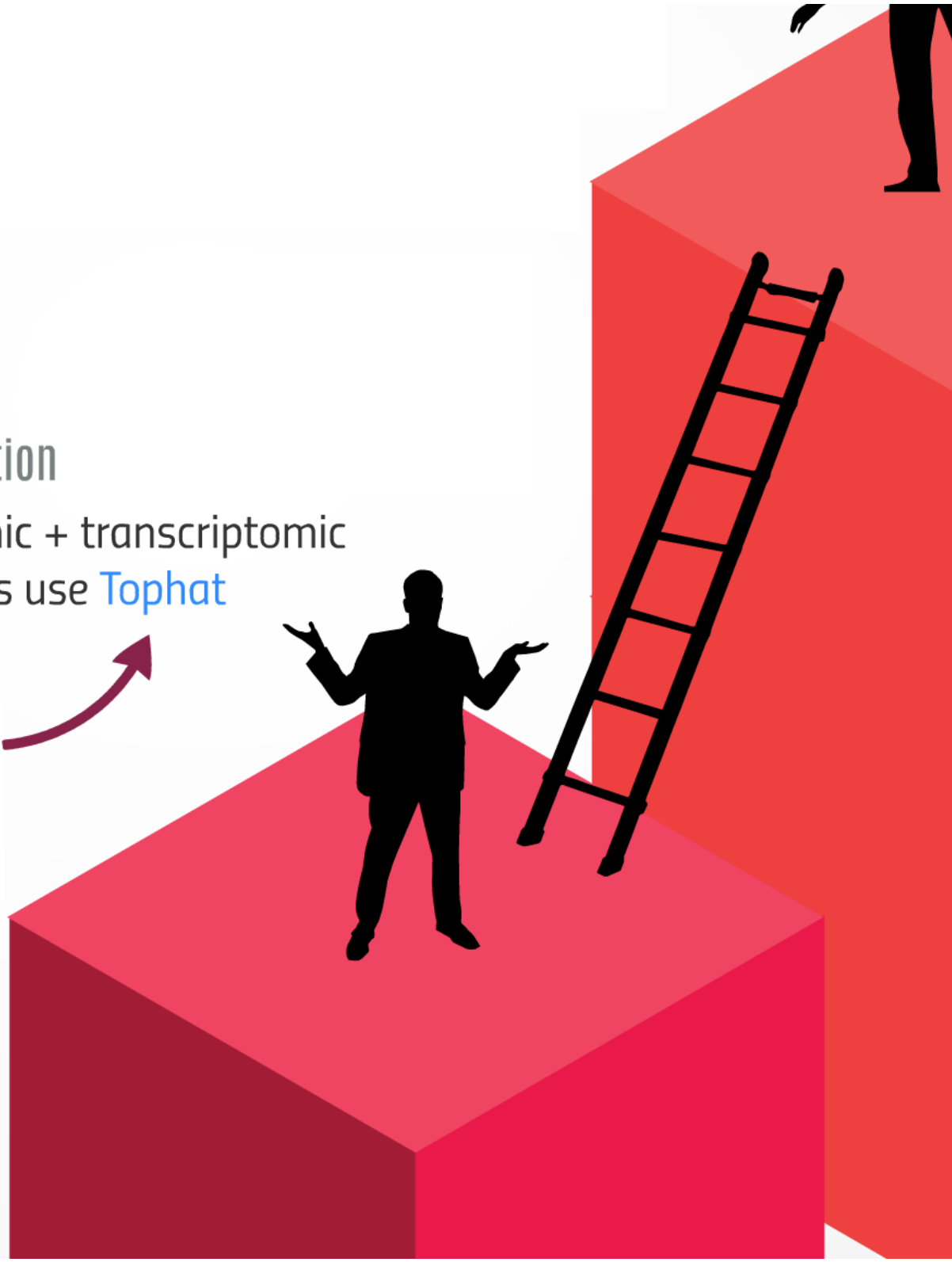
Introduction

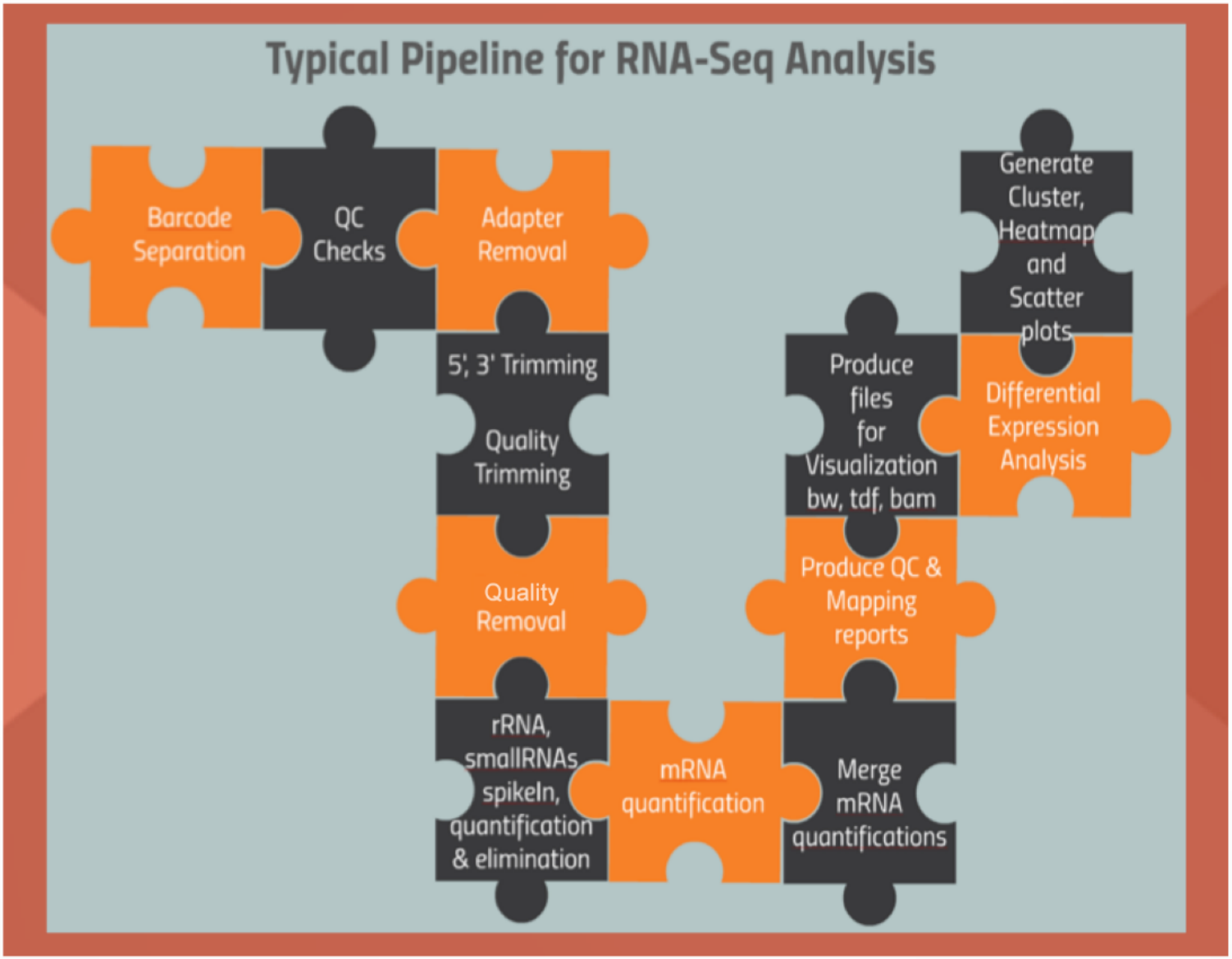
Transcript alignment and quantification



For genomic + transcriptomic alignments use Tophat

For transcriptomic alignments and quantifications use RSEM




Typical Pipeline for RNA-Seq Analysis
Barcode Separation
QC Checks
Adapter Removal
5', 3' Trimming
Quality Trimming
Quality Removal
rRNA, smallRNAs spikeIn, quantification & elimination
mRNA quantification
Merge mRNA quantifications
Produce files for Visualization bw, tdf, bam
Produce QC & Mapping reports
Generate Cluster, Heatmap and Scatter plots
Differential Expression Analysis

Data transfer

Transfer fastq files to the cluster

Create a directory:

```
$ mkdir /full/path/of/your_folder
```

Copy or move your files (same machine):

```
$ cp -R /source/dir/*.fastq.gz /full/path/of/your_folder/.
$ mv /source/dir/*.fastq.gz /full/path/of/your_folder/.
$ rsync -vazu /source/dir/*.fastq.gz /full/path/of/your_folder/.
```

Copy your files (remote machine):

```
$ scp /local/folder/*.fastq.gz user@ghpcc06.umassrc.org:/remote/path/.
$ rsync -vazu /local/folder/*.fastq.gz user@ghpcc06.umassrc.org:/remote/path/.
```

Use FTP clients to transfer the files

FileZilla WinSCP Cyberduck

Index files

Tophat and RSEM http://bioinfo.umassmed.edu/index.php?p=33

TopHat
A spliced read mapper

For Whole Genome

Index files for TopHat:

```
mm10.1.bt2
mm10.2.bt2
mm10.3.bt2
mm10.4.bt2
mm10.rev.1.bt2
mm10.rev.2.bt2
```

```
bowtie2-build -f mm10.fa mm10
```

Output file:
Alignments in BAM format
only for genomic coordinates

RSEM
Transcript alignment and quantification

For Only Transcriptome

Index files for RSEM:

```
mm10.rsem.1.bt2
mm10.rsem.2.bt2
mm10.rsem.3.bt2
```

```
rsem-prepare-reference \
--gtf ucsc.gtf --transcript-to-gene-map ucsc_into_genesymbol.rsem \
mm10.fa mm10.rsem
```

```
mm10.rsem.transcripts.fa
```

Output files:
Alignments in BAM format
for both genomic coordinates
and transcriptomic coordinates

Gene and isoform
quantification results

Expression

Quantify with the RSEM program

- RSEM depends on an existing annotation and will only scores transcripts that are present in the given annotation file.
- RSEM generates 2 result files:
  - rsem.genes.results
  - rsem.isoforms.results

Expression matrices

Create consolidated table

We use bin/rsem.to.table.pl command to join the rsem.genes.results files side-by-side, that contains the expected_count or tpm information, and place them into a final output file. Repeat the same step for isoforms.

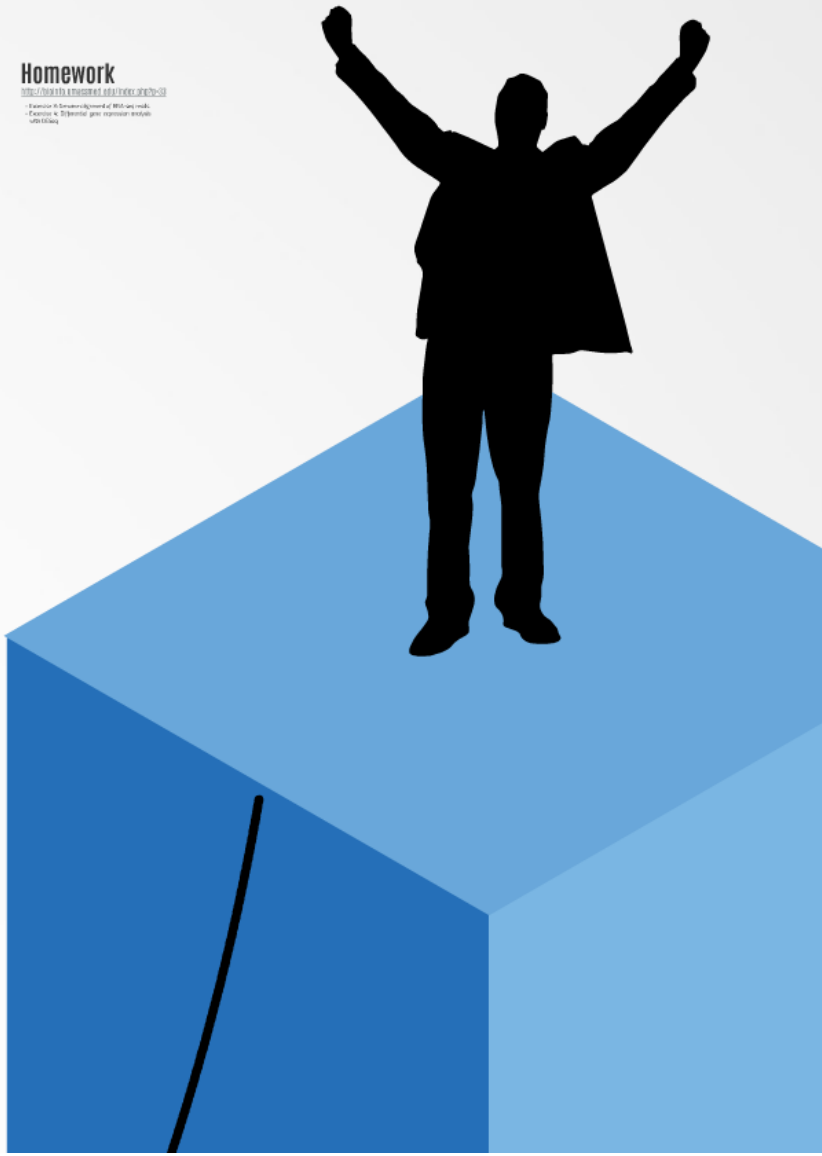
This one-line command assumes the genes (and transcripts) in each files are in the same order.

Visualization

Alignment visualiztion using IGV

Download the IGV program from the IGV site and transfer your bam files from the hpcc to your laptop.

http://software.broadinstitute.org/software/igv/download



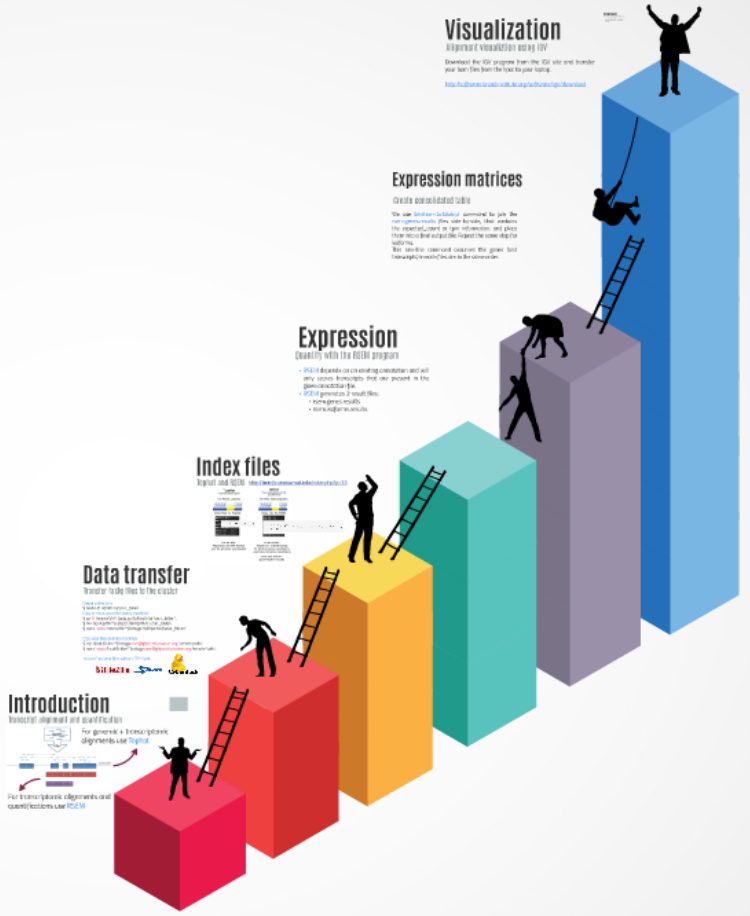
Homework

http://bioinfo.umassmed.edu/index.php?p=33

- Excercise 3: Genome alignment of RNA-seq reads.
- Excercise 4: Differential gene expression analysis with DESeq

RNA-Seq

Data processing



Thanks! Questions?

http://bioinfo.umassmed.edu