RNA-seq to study HIV Infection in cells

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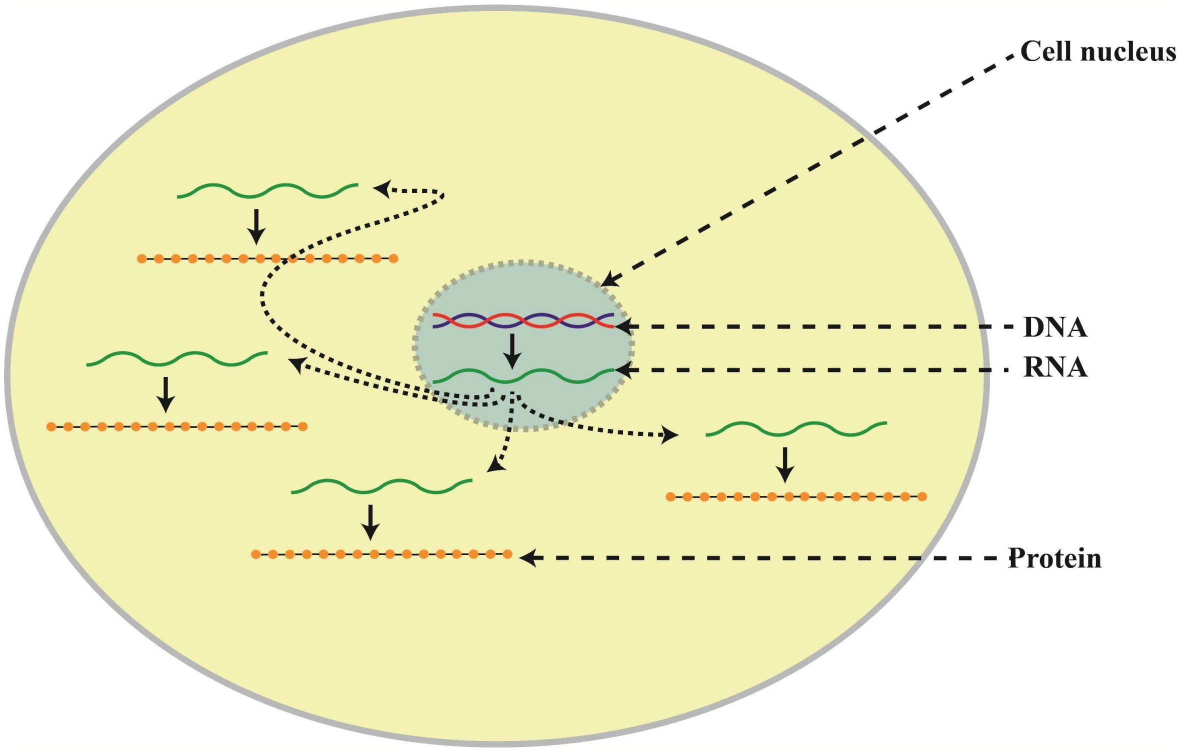
DNA and RNA in a cell

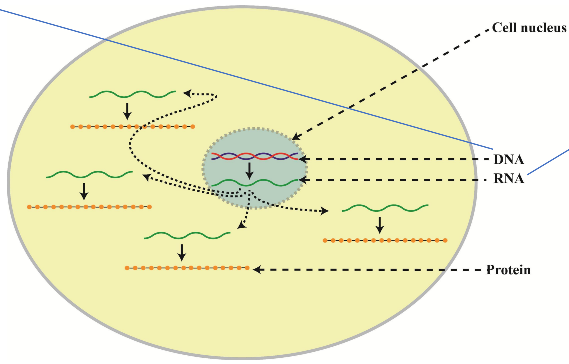


https://i0.wp.com/science-explained.com/wp-content/uploads/2013/08/Cell.jpg

Two common analysis goals

DNA Sequencing

- Fixed copy of a gene per cell
- Analysis goal:
  Variant calling and interpretation



RNA Sequencing

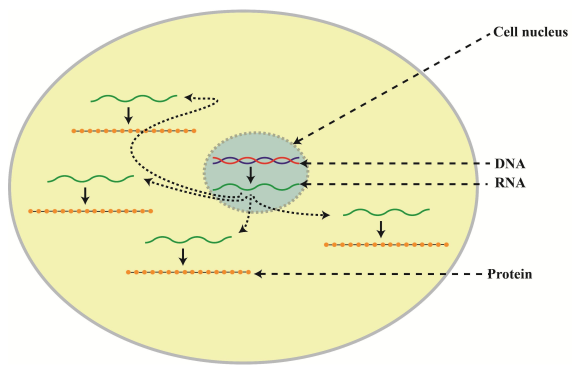
- Copy of a gene (mRNA) per cell depends on gene expression
- Analysis goal: Differential expression and interpretation

https://i0.wp.com/science-explained.com/wp-content/uploads/2013/08/Cell.jpg

Today we will cover RNA sequencing

DNA Sequencing

- Fixed c… f a g… per cell
- Analysis go…
  Variant c… interpretation



RNA Sequencing

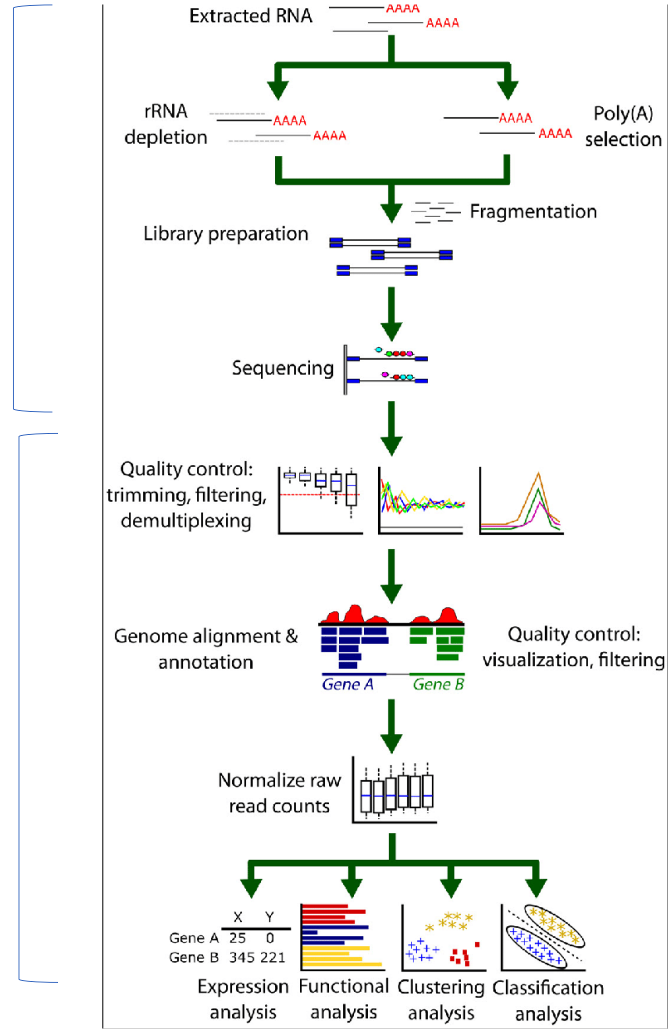
- Copy of a gene (mRNA) per cell depends on gene expression
- Analysis goal: Differential expression and interpretation

https://i0.wp.com/science-explained.com/wp-content/uploads/2013/08/Cell.jpg

RNA seq workflow

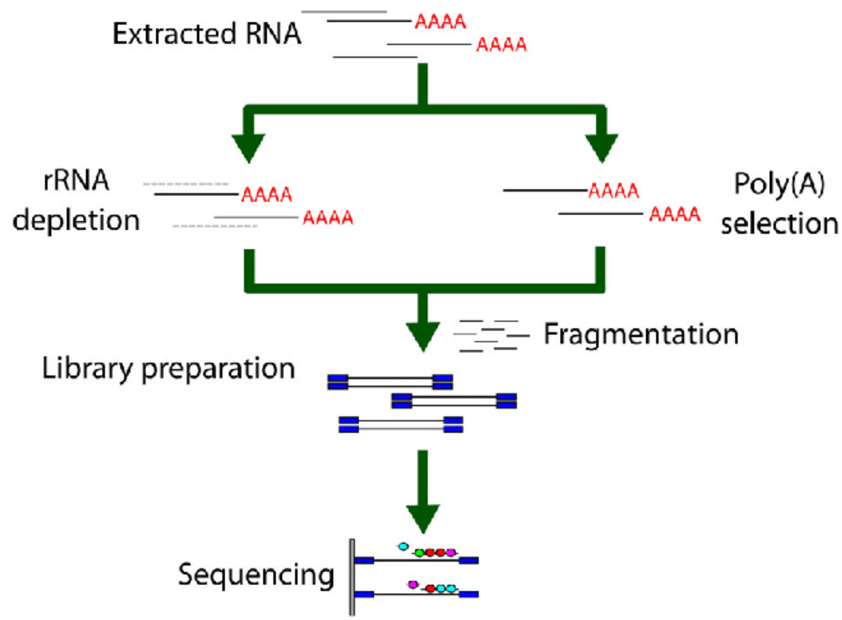
Library prep and sequencing

Bioinformatics



Good resource: Griffiths et al Plos Comp Bio 2015

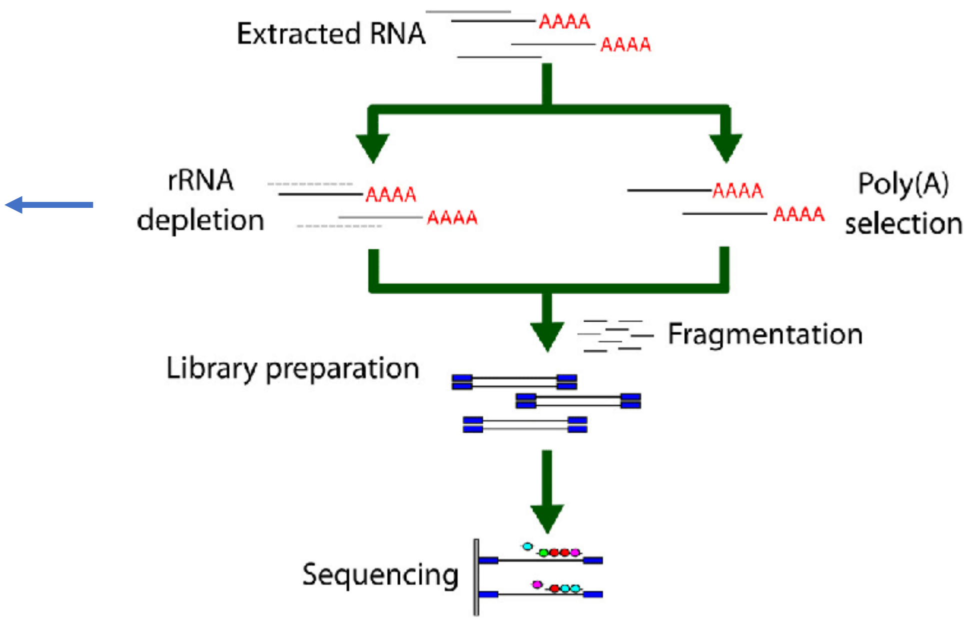
RNA seq library prep and sequencing



Good resource: [Griffiths et al Plos Comp Bio 2015]()

RNA seq library prep and sequencing

- Enrichment for mRNA
- In humans, ~95%–98% of all RNA molecules are rRNAs

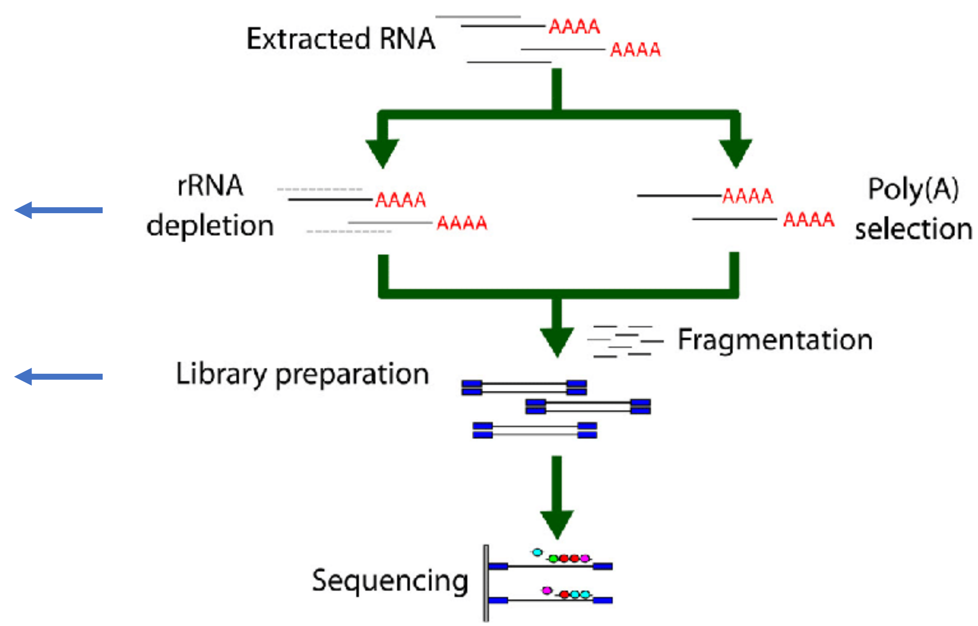


Good resource: Griffiths et al Plos Comp Bio 2015

RNA seq library prep and sequencing

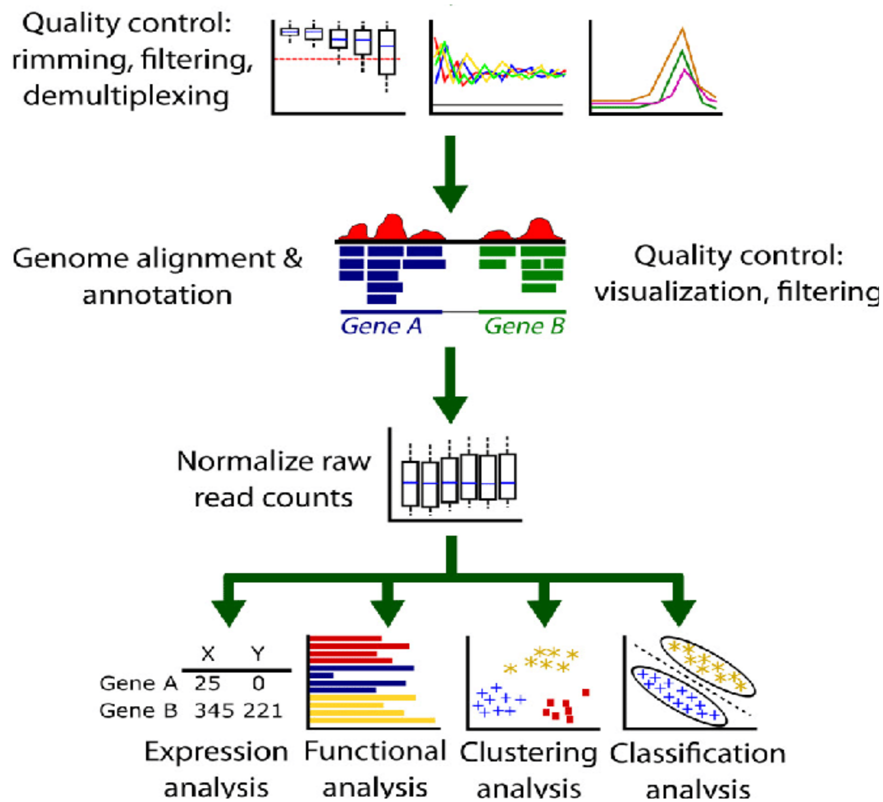
- Enrichment for mRNA
- In humans, ~95%–98% of all RNA molecules are rRNAs

- Random priming and reverse transcription
- Double stranded cDNA synthesis
- Sequencing adapter ligation



Good resource: Griffiths et al Plos Comp Bio 2015

RNA seq bioinformatics



Good resource: [Griffiths et al Plos Comp Bio 2015]()

Goal of RNAseq

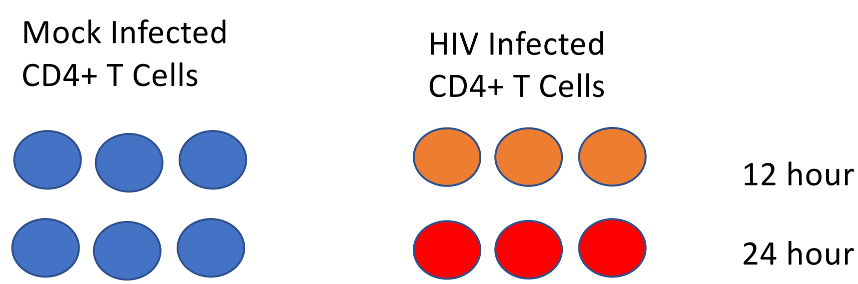
"How can we detect genes for which the counts of reads change between conditions **more systematically** than as expected by chance"

Oshlack et al. 2010. From RNA-seq reads to differential expression results. Genome Biology 2010, 11:220
http://genomebiology.com/2010/11/12/220

Our dataset

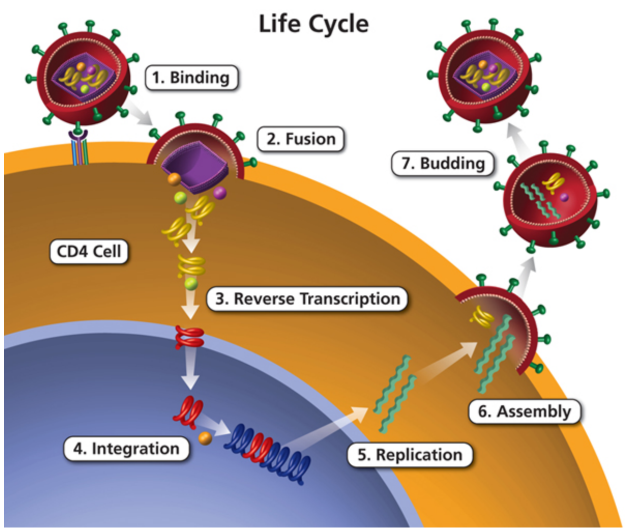
Next-Generation Sequencing Reveals HIV-1-Mediated Suppression of T Cell Activation and RNA Processing and Regulation of Noncoding RNA Expression in a CD4^{+} T Cell Line

Stewart T. Chang, Pavel Sova, Xinxia Peng, Jeffrey Weiss, G. Lynn Law, Robert E. Palermo, Michael G. Katze



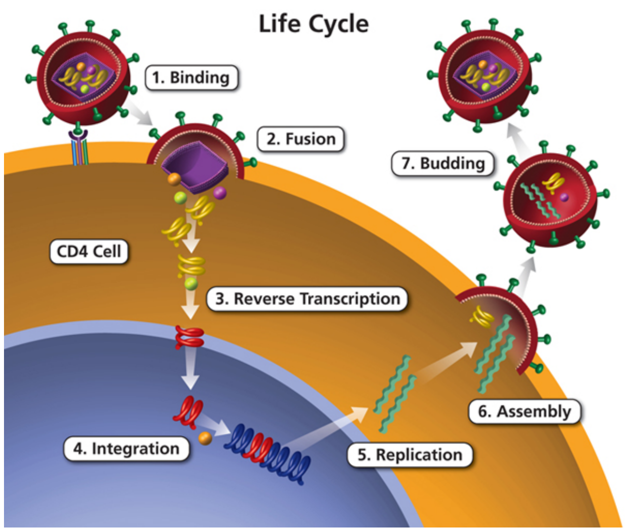
https://www.ncbi.nlm.nih.gov/pubmed/21933919

HIV lifecycle

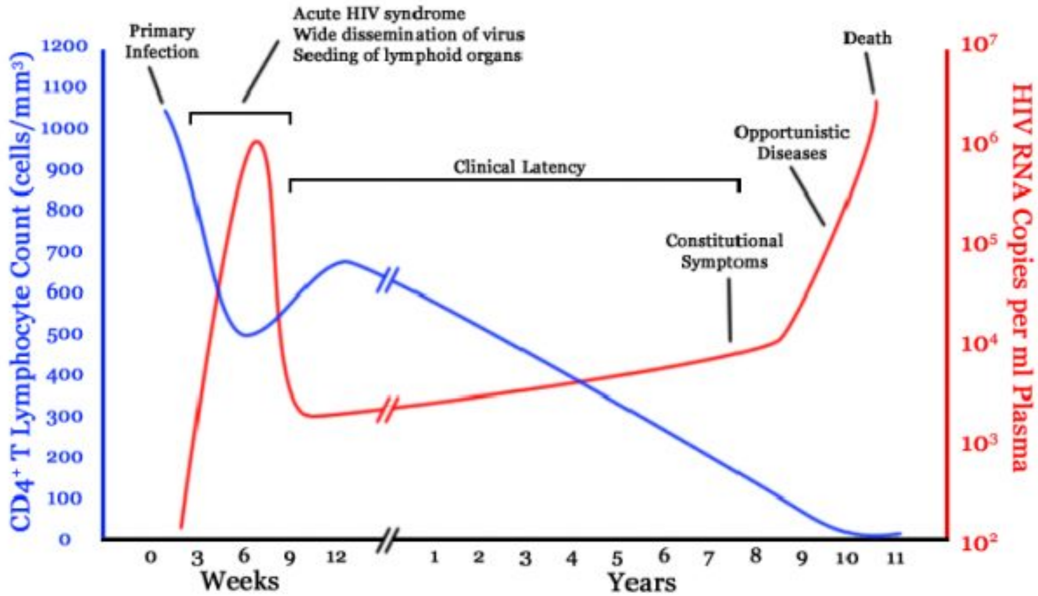


https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1596/life-cycle

HIV lifecycle



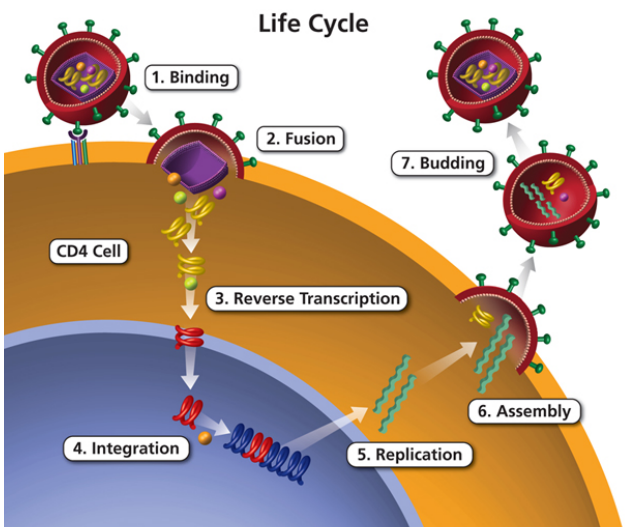
HIV infection in a human host

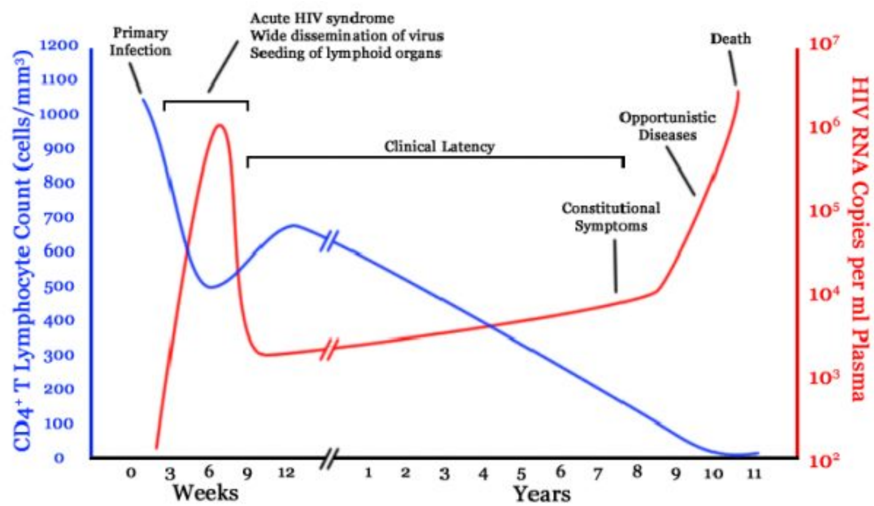


https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1596/life-cycle

The study question

What changes take place in the first 12-24 hours of HIV infection in terms of gene expression of host cell and viral replication levels?

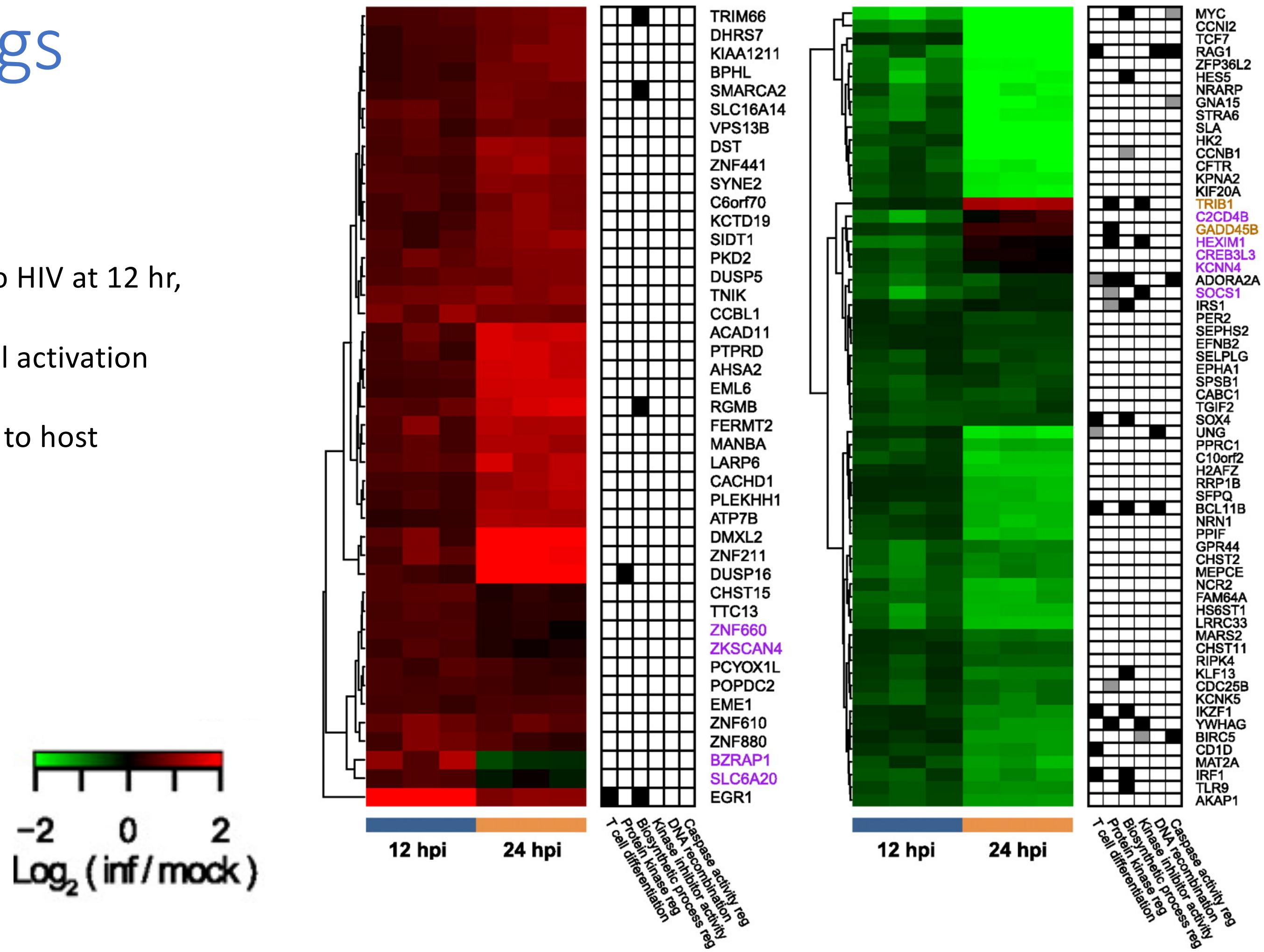




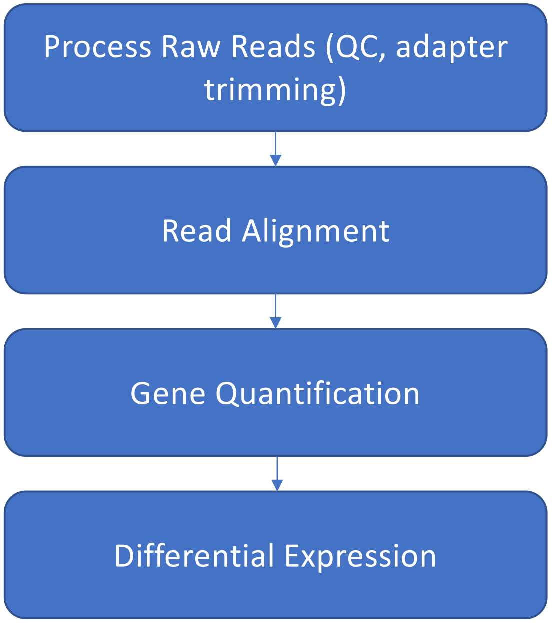
https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1596/life-cycle

Study findings

- 20% of reads mapped to HIV at 12 hr, 40% at 24 hr
- Downregulation of T cell activation genes at 12 hr
- 'Large-scale disruptions to host transcription' at 24 hr

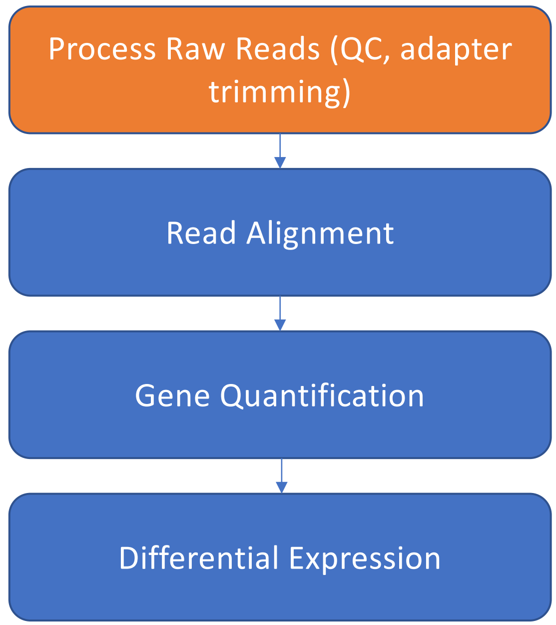


Workflow



Process Raw Reads (QC, adapter trimming)
Read Alignment
Gene Quantification
Differential Expression

Workflow


Process Raw Reads (QC, adapter trimming)
Read Alignment
Gene Quantification
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Raw reads in Fastq format

```
@SRR098401.109756285
GACTCACGTAACTTTAAACTCTAACAGAAATATACTA…
+
CAEFGDG?BCGGGEEDGGHGHGDFHEIEGGDDDD…
```

1. Sequence identifier
2. Sequence
3. + (optionally lists the sequence identifier again)
4. Quality string

Base Quality Scores

The symbols we see in the read quality string are an encoding of the quality score:

```
Quality encoding: !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHI
                  |         |         |         |         |
   Quality score: 0........10........20........30........40
```

A quality score is a prediction of the probability of an error in base calling:

Quality Score	Probability of Incorrect Base Call	Inferred Base Call Accuracy
10 (Q10)	1 in 10	90%
20 (Q20)	1 in 100	99%
30 (Q30)	1 in 1000	99.9%

https://www.illumina.com/science/education/sequencing-quality-scores.html

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Back to our read:

```
@SRR098401.109756285
GACTCACGTAACTTTAAACTCTAACAGAAATATACTA…
+
CAEFGDG?BCGGGEEDGGHGHGDFHEIEGGDDDD…
```

C –> Q = 34 -> Probability < 1/1000 of an error

https://www.illumina.com/science/education/sequencing-quality-scores.html

Base Quality Scores

```
SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX......................
...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII......................
.................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL....................................................
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
|                         |    |        |                              |                     |
33                        59   64       73                            104                   126
0........................26...31.......40
                         -5....0........9.............................40
                               0........9.............................40
                                  3.....9..............................41
0.2......................26...31........41

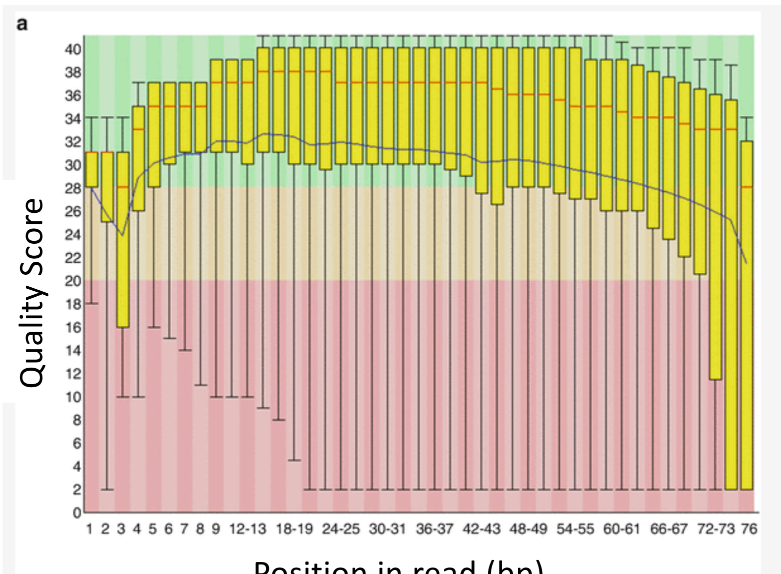
S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 41)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

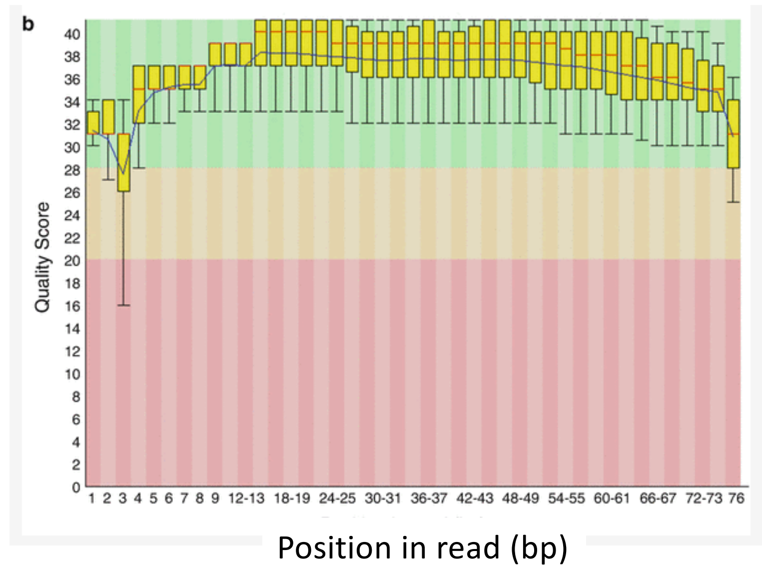
https://en.wikipedia.org/wiki/FASTQ_format

Raw read quality control

- Quality distribution over the length of the read
- GC content
- Per base sequence content
- Adapters in Sequence

FastQC: Sequence Quality Histogram

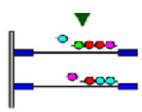




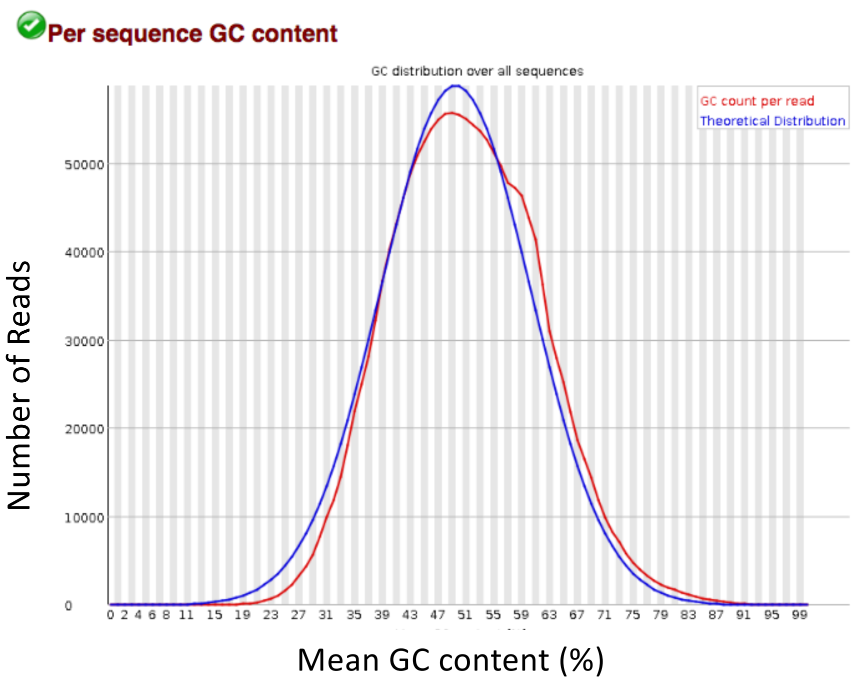
GOOD
High quality over the length of the read

BAD
Read quality drops at the beginning and end

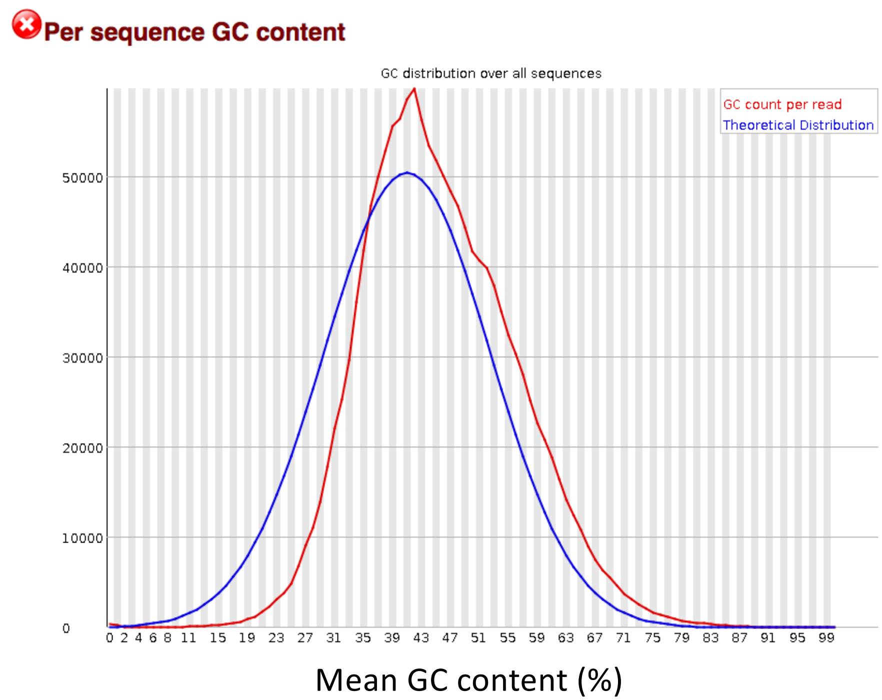
Sequencing



FastQC: Per sequence GC content

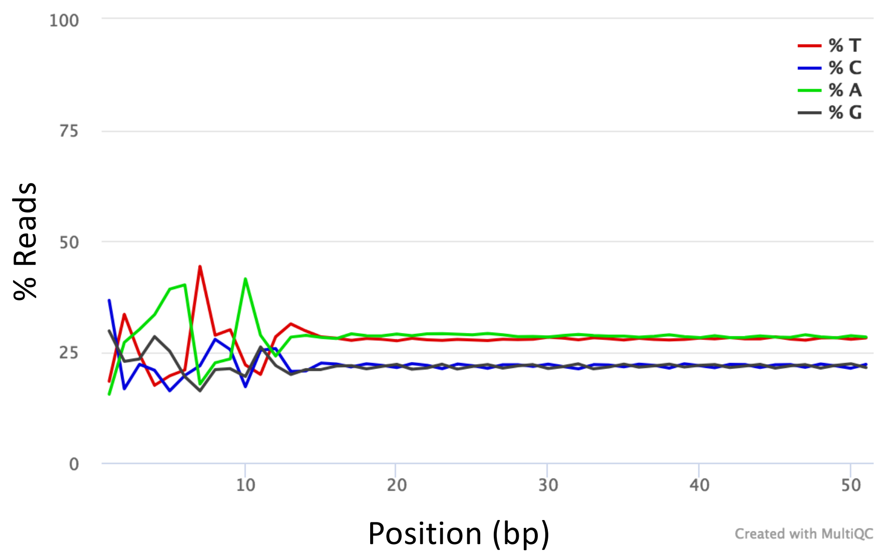


GOOD: follows normal distribution (sum of deviations is < 15% of reads)



BAD: can indicate contamination with adapter dimers, or another species

FastQC: Per Base Sequence Content

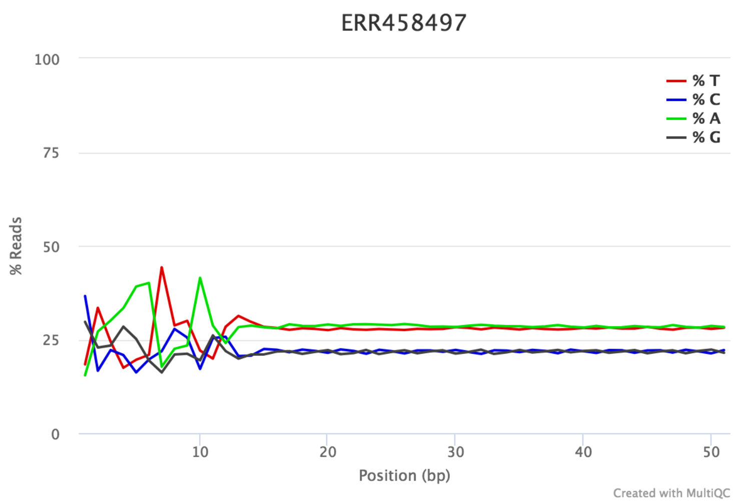


EXPECTED for RNAseq

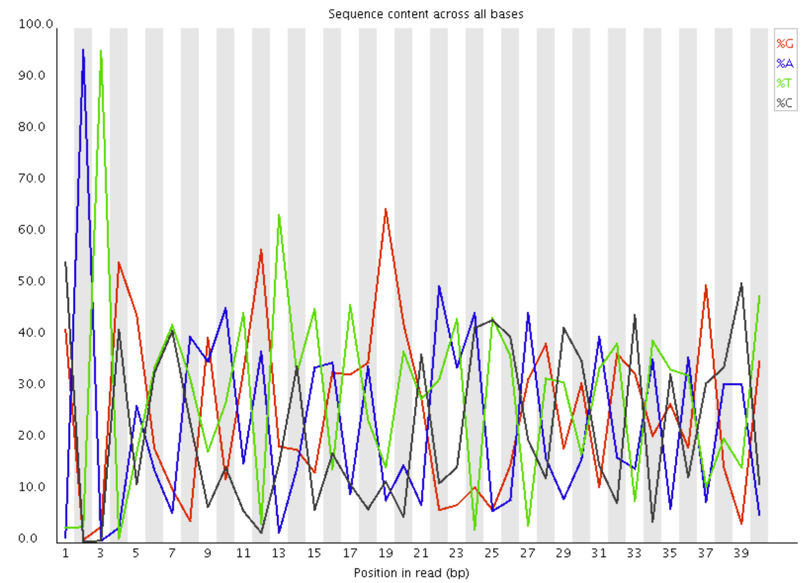
- Proportion of each position for which each DNA base has been called
- RNAseq data tends to show a positional sequence bias in the first ~12 bases
- The "random" priming step during library construction is not truly random and certain hexamers are more prevalent than others
- Studies have shown that this does NOT cause mis-called bases or drastic bias in sequenced fragments Read quality drops at the beginning and end

sequencing.qcfail.com

FastQC: Per Base Sequence Content



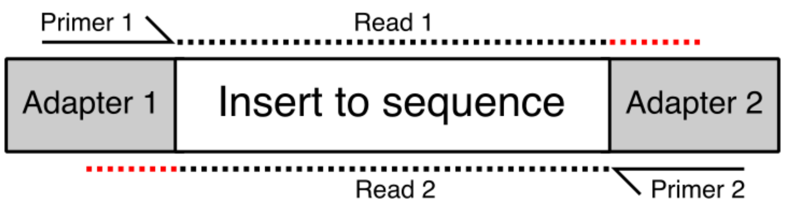
EXPECTED



BAD:
Shows a strong positional bias throughout the reads, which in this case is due to the library having a certain sequence that is overrepresented

FastQC: Adapter content

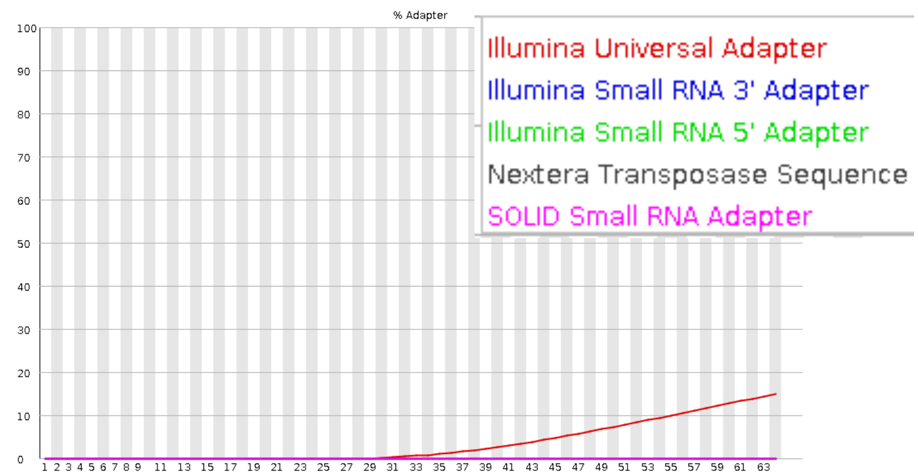
The cause: The “insert” sequence is shorter than the read, and the read contains part of the adapter sequence



FastQC will scan each read for the presence of known adapter sequences

The plot shows that the adapter content rises over the course of the read

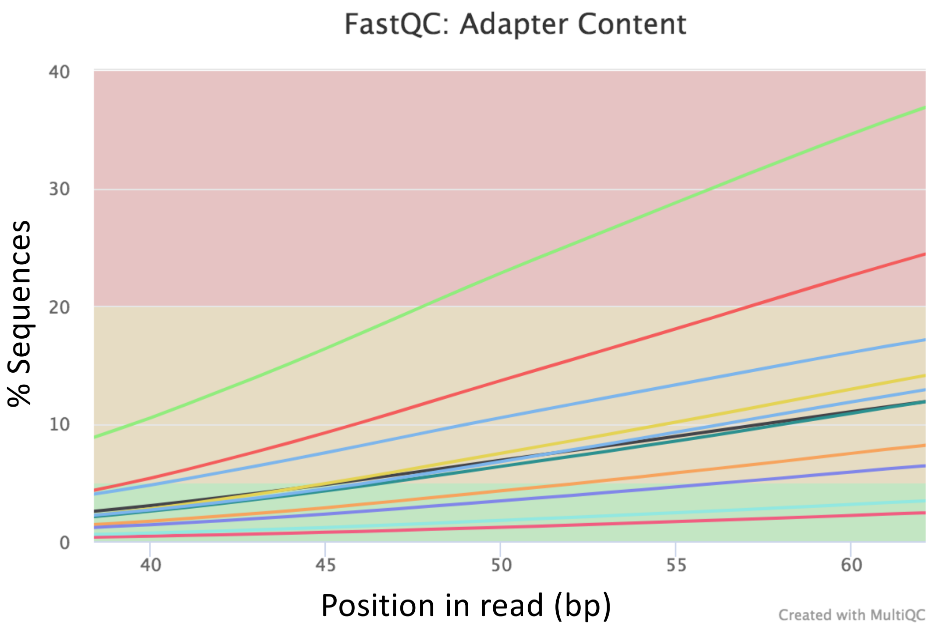
Solution – Adapter trimming!



sequencing.qcfail.com

FastQC -> MultiQC

Should view all samples at once to notice abnormalities for our dataset.

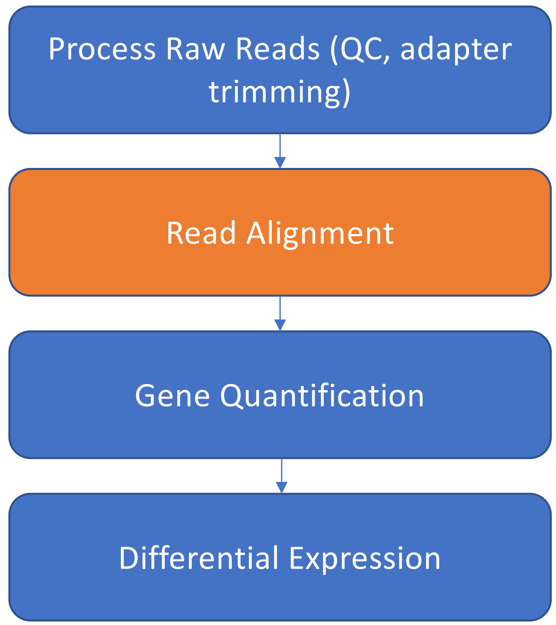


Adapter trimming

Trim Galore! is a tool that:

- Scans and removes known Illumina or custom adapters
- Performs read trimming for low quality regions at the end of reads
- Removes reads that become too short in the trimming process

Workflow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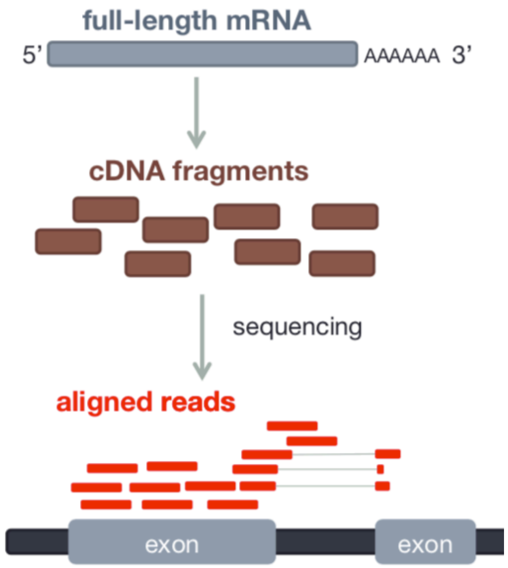
Read Alignment

- RNAseq data originates from spliced mRNA (no introns)
- When aligning to the genome, our aligner must find a spliced alignment for reads



Reference sequence

Reference-based vs Reference-free RNAseq

RNAseq can be roughly divided into two "types":

- **Reference genome-based** - an assembled genome exists for a species for which an RNAseq experiment is performed. It allows reads to be aligned against the reference genome and significantly improves our ability to reconstruct transcripts. This category would obviously include humans and most model organisms

- **Reference genome-free** - no genome assembly for the species of interest is available. In this case one would need to assemble the reads into transcripts using *de novo* approaches. This type of RNAseq is as much of an art as well as science because assembly is heavily parameter-dependent and difficult to do well.

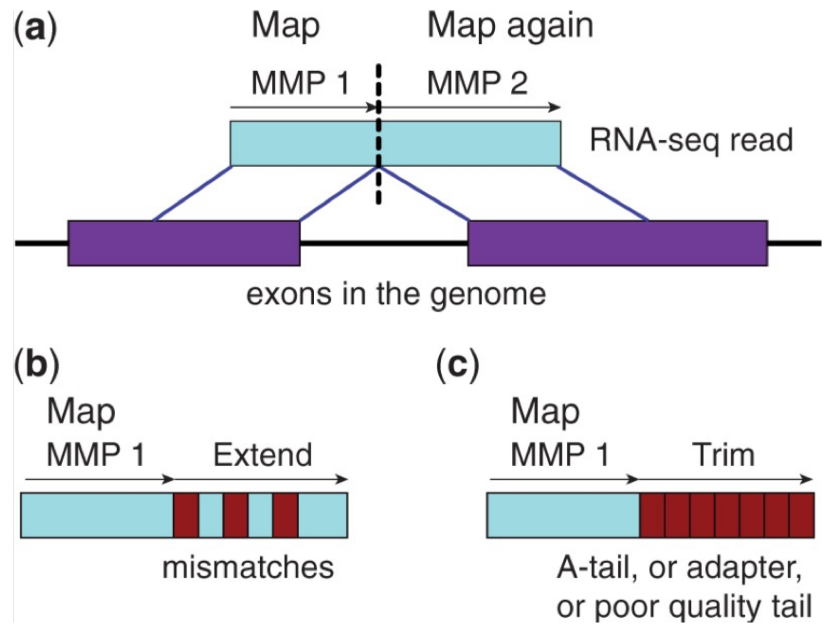
In this lesson we will focus on the **Reference genome-based** type of RNA seq.

https://galaxyproject.org/tutorials/rb_rnaseq/

STAR Aligner (Spliced Transcripts Alignment to a Reference)

Highly accurate, memory intensive aligner
Two phase mapping process

1. Find Maximum Mappable Prefixes (MMP) in a read. MMP can be extended by
   - mismatches
   - Indels
   - soft-clipping



[Dobin et al Bioinformatics 2013]

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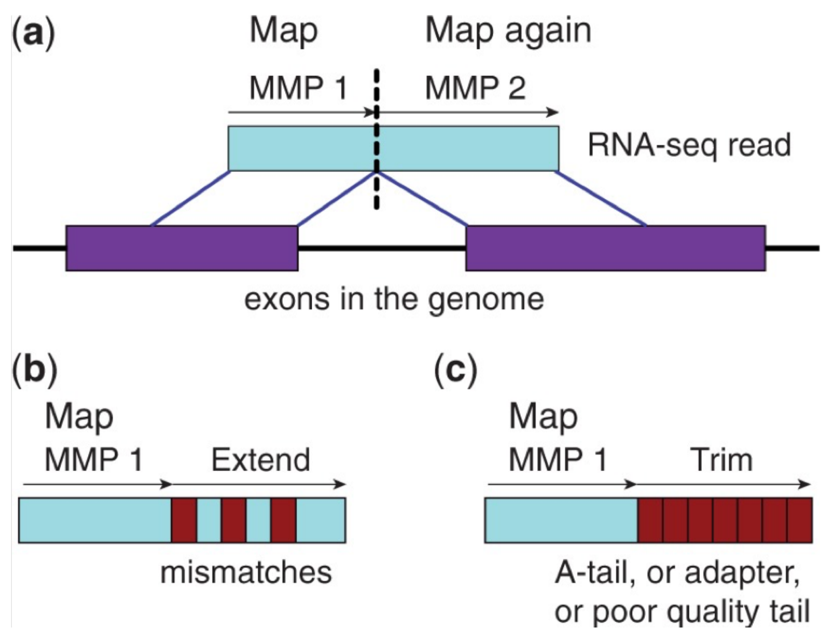
2. Clustering MMP, stitching and scoring to determine final read location



[Dobin et al Bioinformatics 2013](#)

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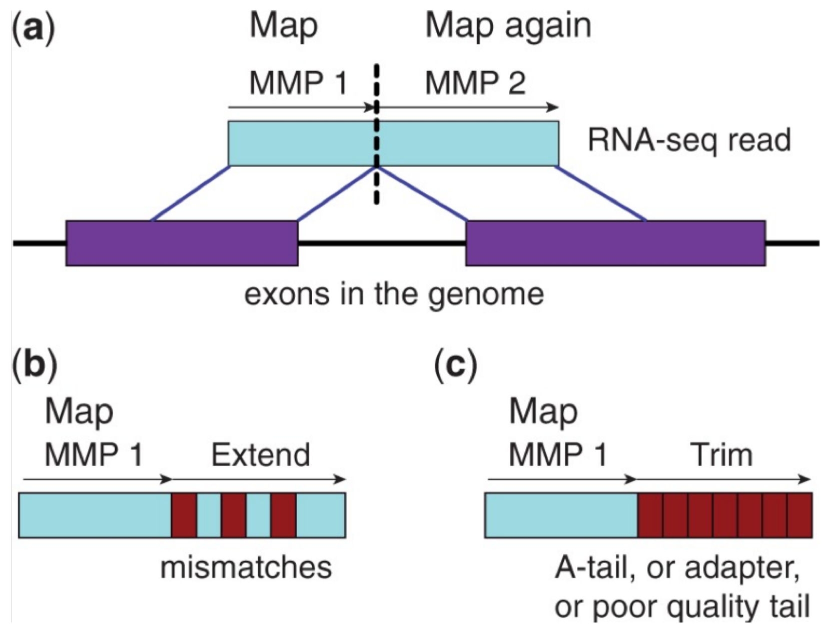
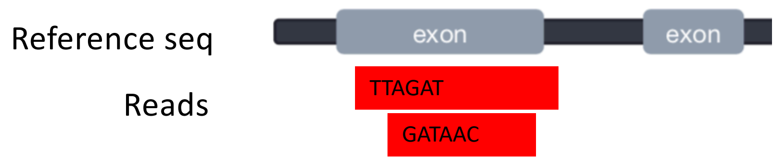
Output is a Sequence Alignment Map (SAM) file



Dobin et al Bioinformatics 2013

Sequence Alignment Map (SAM)



Header section

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
```

Alignment section

```
r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

CIGAR: summary of alignment, e.g. match, gap, insertion, deletion

Mapping Quality

Position

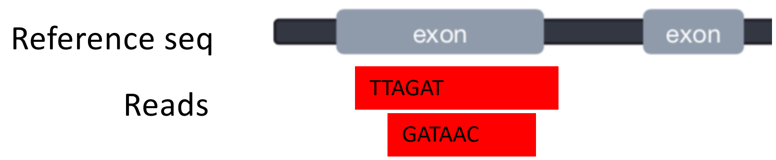
Ref Sequence name

Flag: indicates alignment information e.g. paired, aligned, etc
https://broadinstitute.github.io/picard/explain-flags.html

Read ID

www.samformat.info

Sequence Alignment Map (SAM)



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r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

Paired end info

Sequence

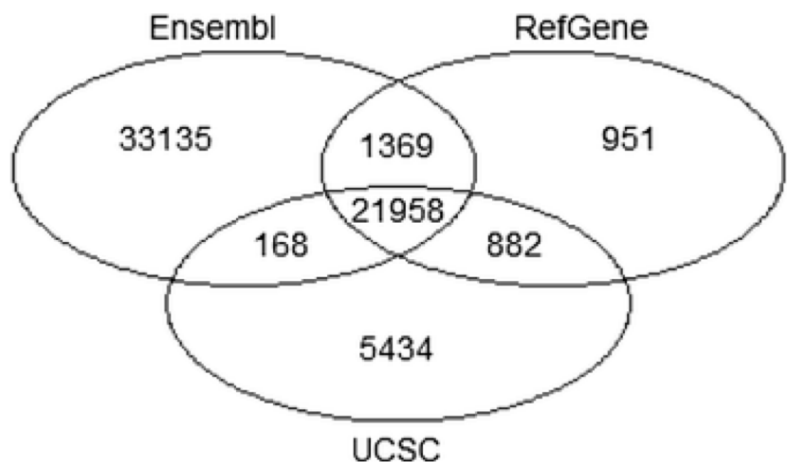
Quality Score

Optional Fields

www.samformat.info

Genome Annotation Standards

- STAR can use an annotation file gives the location and structure of genes in order to improve alignment in known splice junctions
- Annotation is dynamic and there are at least three major sources of annotation
- The intersection among RefGene, UCSC, and Ensembl annotations shows high overlap. RefGene has the fewest unique genes, while more than 50% of genes in Ensembl are unique
- Be consistent with your choice of annotation source!



[Zhao et al Bioinformatics 2015]

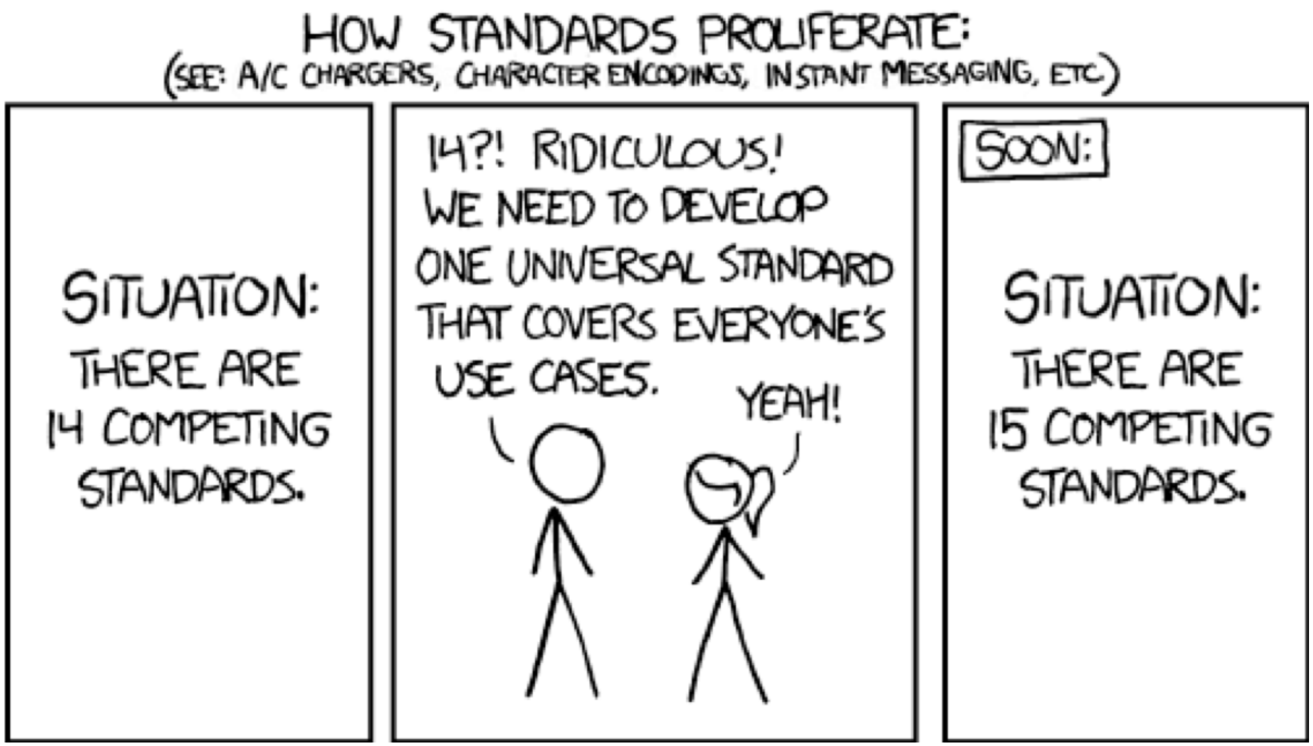
Gene Annotation Format (GTF)

In order to count genes, we need to know where they are located in the reference sequence
STAR uses a Gene Transfer Format (GTF) file for gene annotation

Chrom	Source	Feature type	Start	Stop	(Score)	Strand	Frame	Attribute
chr5	hg38_refGene	exon	138465492	138466068	.	+	.	gene_id "EGR1";
chr5	hg38_refGene	CDS	138465762	138466068	.	+	0	gene_id "EGR1";
chr5	hg38_refGene	start_codon	138465762	138465764	.	+	.	gene_id "EGR1";
chr5	hg38_refGene	CDS	138466757	138468078	.	+	2	gene_id "EGR1";
chr5	hg38_refGene	exon	138466757	138469315	.	+	.	gene_id "EGR1";
chr5	hg38_refGene	stop_codon	138468079	138468081	.	+	.	gene_id "EGR1";

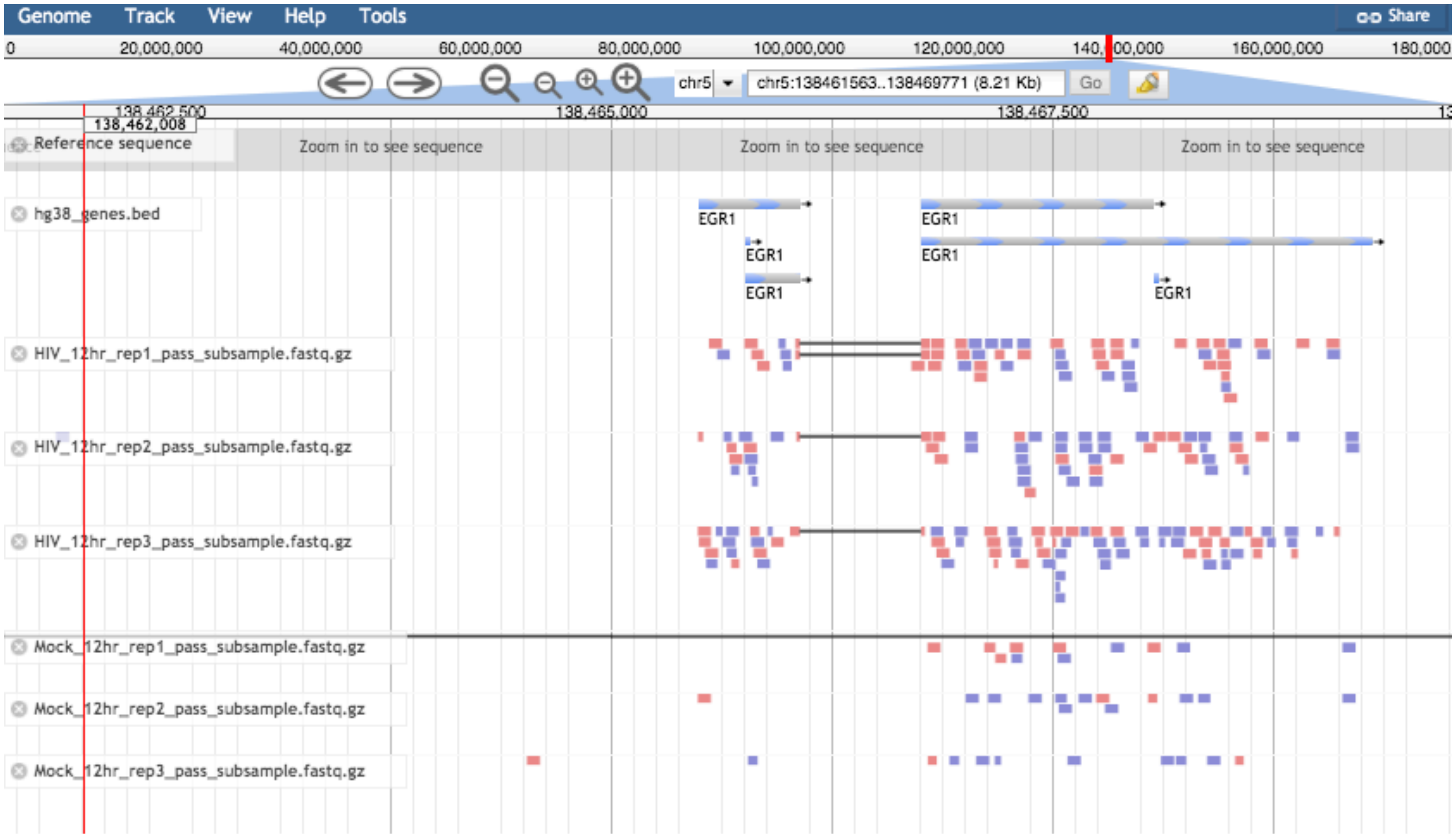
https://useast.ensembl.org/info/website/upload/gff.html

A note on standards

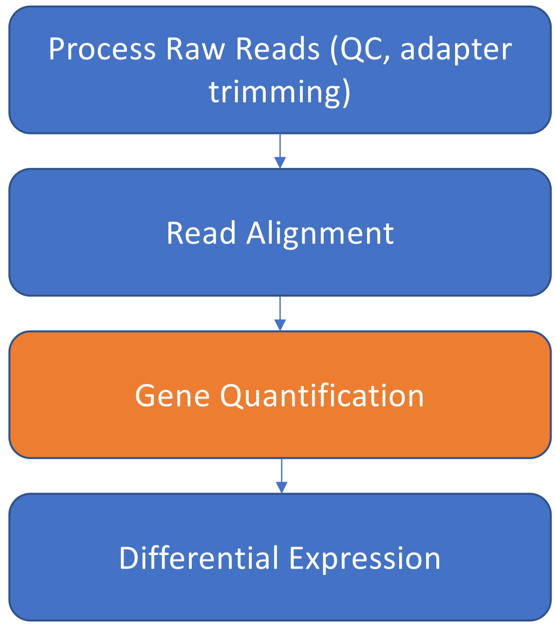


https://xkcd.com/927/

Visualizing reads with JBrowse



Workflow


Process Raw Reads (QC, adapter trimming)
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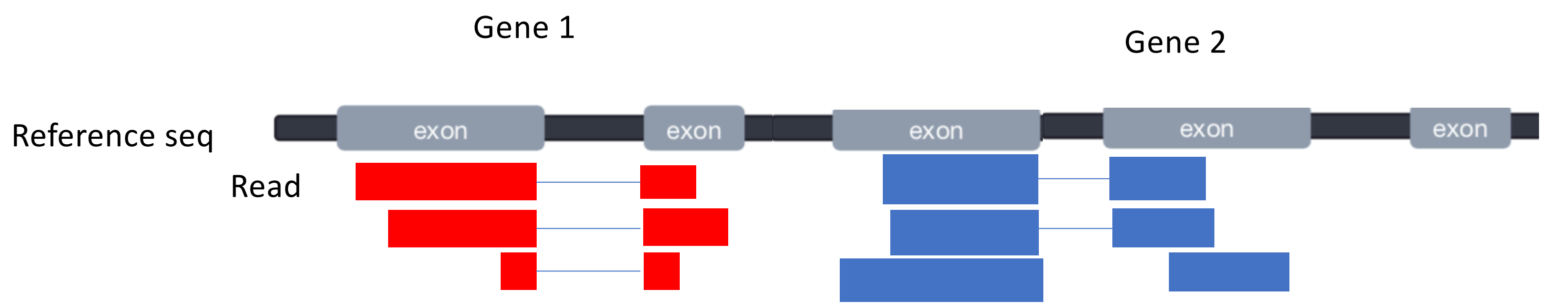
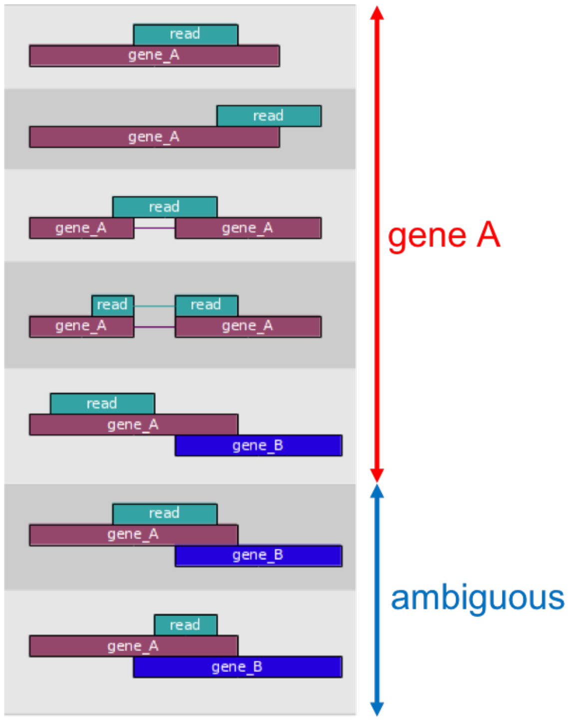
Differential Expression

Counting reads for each gene



Counting reads: featurecounts

- The mapped coordinates of each read are compared with the features in the GTF file
- Reads that overlap with a gene by >=1 bp are counted as belonging to that feature
- Ambiguous reads will be discarded
- Output will be a matrix of genes and samples

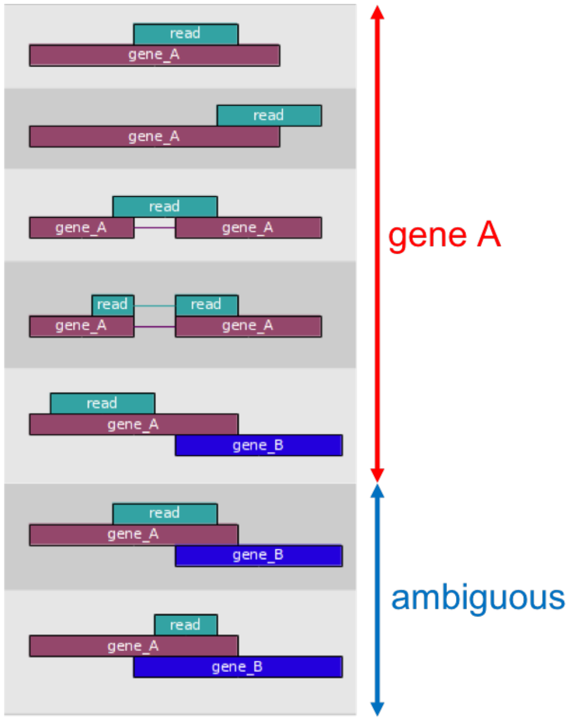


Counting reads: featurecounts

- The mapped coordinates of each read are compared with the features in the GTF file
- Reads that overlap with a gene by >=1 bp are counted as belonging to that feature
- Ambiguous reads will be discarded
- Output will be a matrix of genes and samples

Result is a gene count matrix:

Gene	Sample 1	Sample 2	Sample 3	Sample 4
A	1000	1000	100	10
B	10	1	5	6
C	10	1	10	20

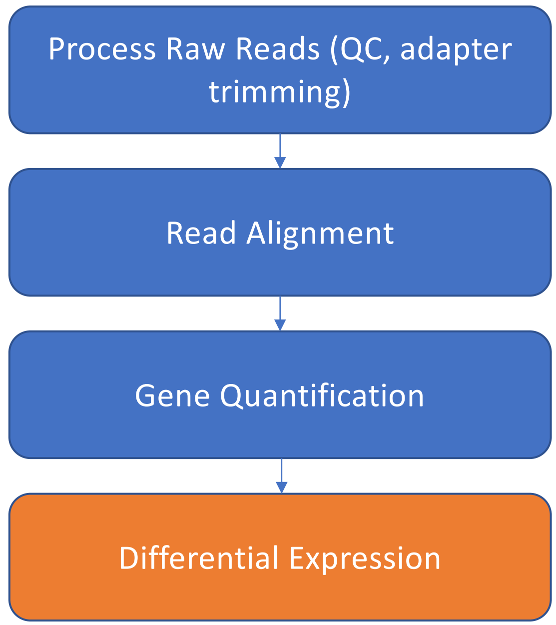


Tracking read numbers

Revisit quality control after each processing step!

Number of Reads	Source	Result
Raw reads	FastQC run 1	8 M
After Trimming	FastQC run 2	7.1 M
Aligned to genome	STAR log	6 M
Associated with genes	FeatureCounts log	5.4 M

Workflow

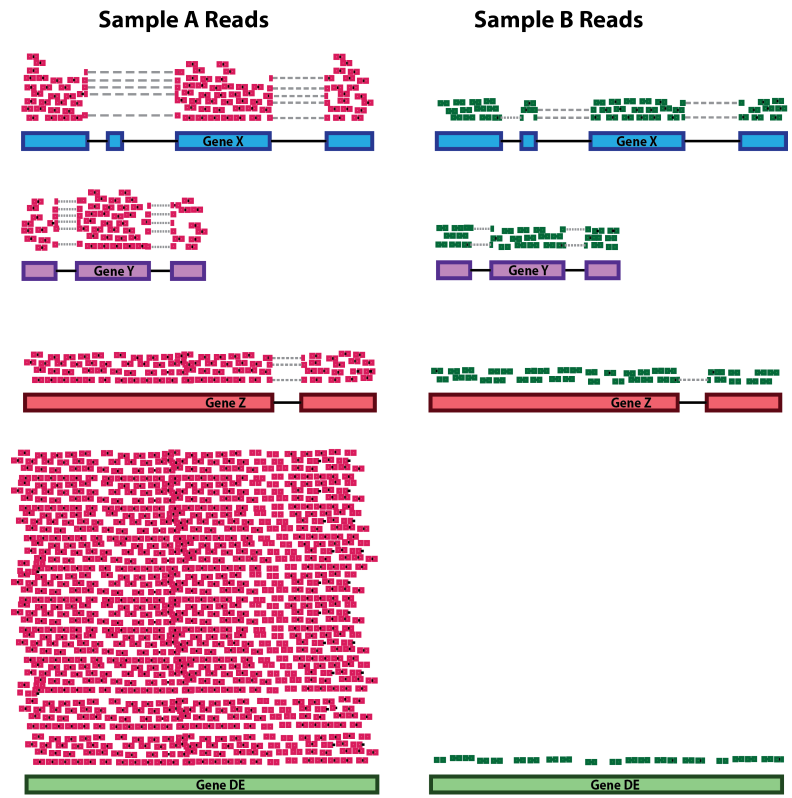

Process Raw Reads (QC, adapter trimming)
Read Alignment
Gene Quantification
Differential Expression

Testing for Differential Expression

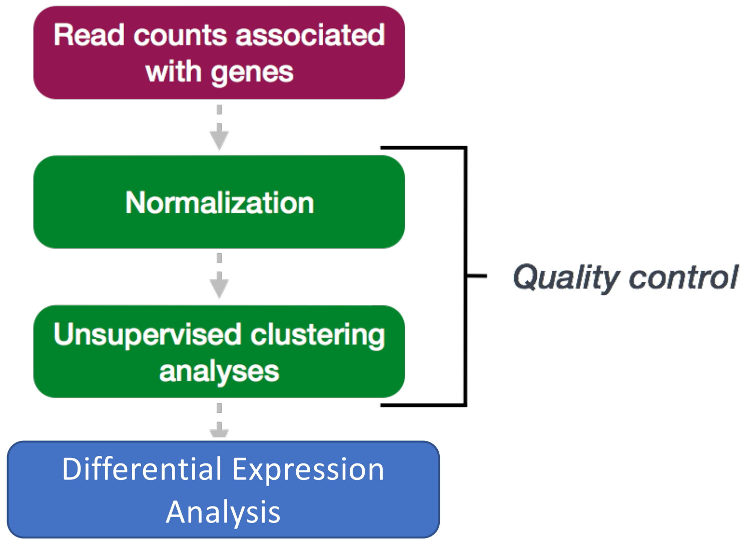
The goal of differential expression analysis (DE) is to find gene (DGE) differences between conditions, developmental stages, treatments etc.

In particular DE has two goals:

- Estimate the *magnitude* of expression differences;
- Estimate the *significance* of expression differences.



Differential Expression with DESeq2



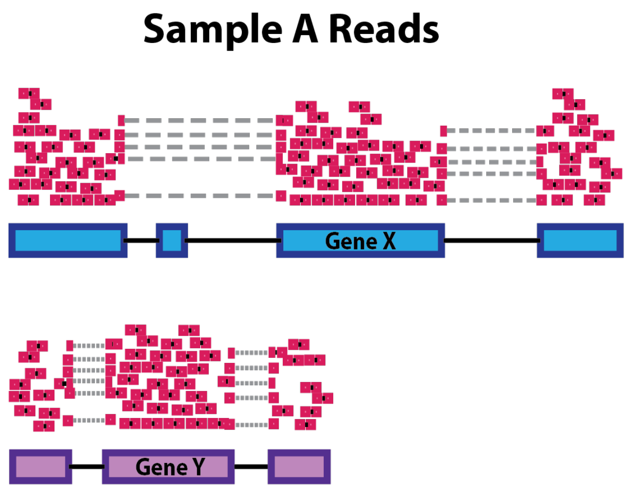
All steps are done with one click in Galaxy!

https://hbctraining.github.io/DGE_workshop

Normalization

The number of sequenced reads mapped to a gene depends on

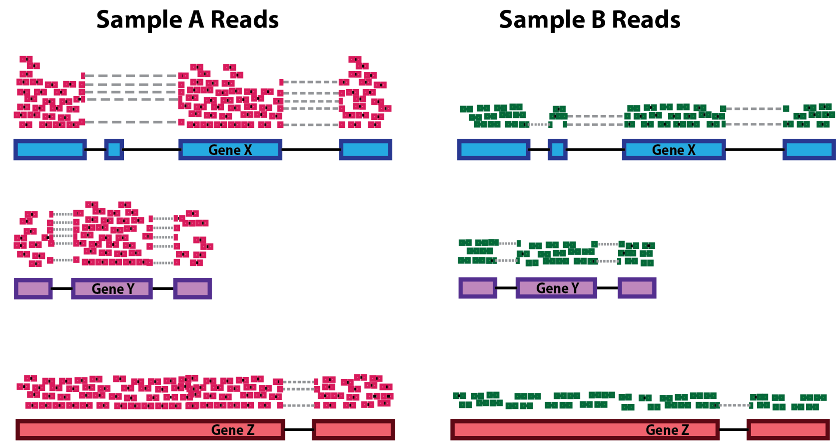
- Gene Length



Normalization

The number of sequenced reads mapped to a gene depends on

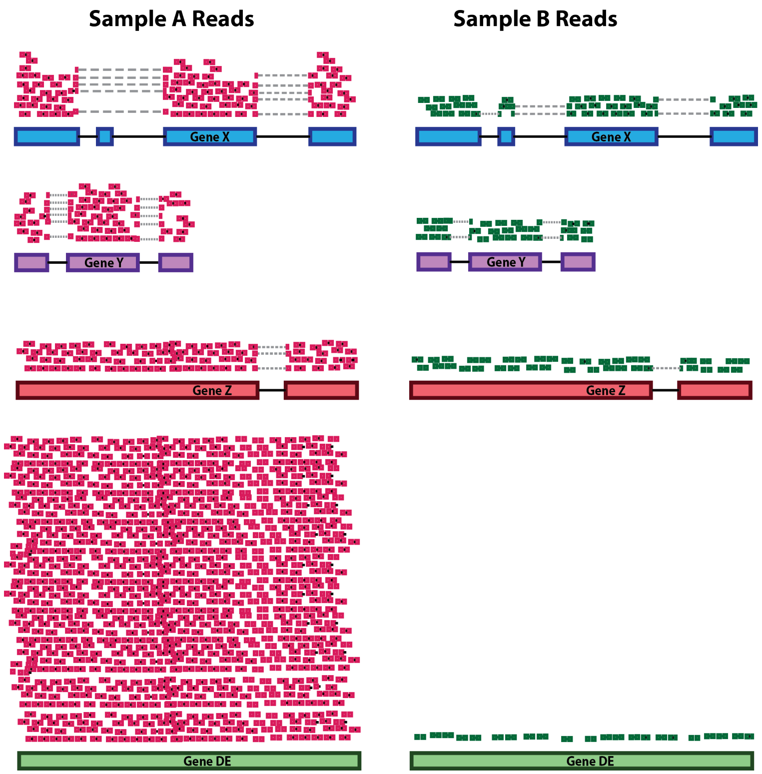
- Gene Length
- Sequencing depth



Normalization

The number of sequenced reads mapped to a gene depends on

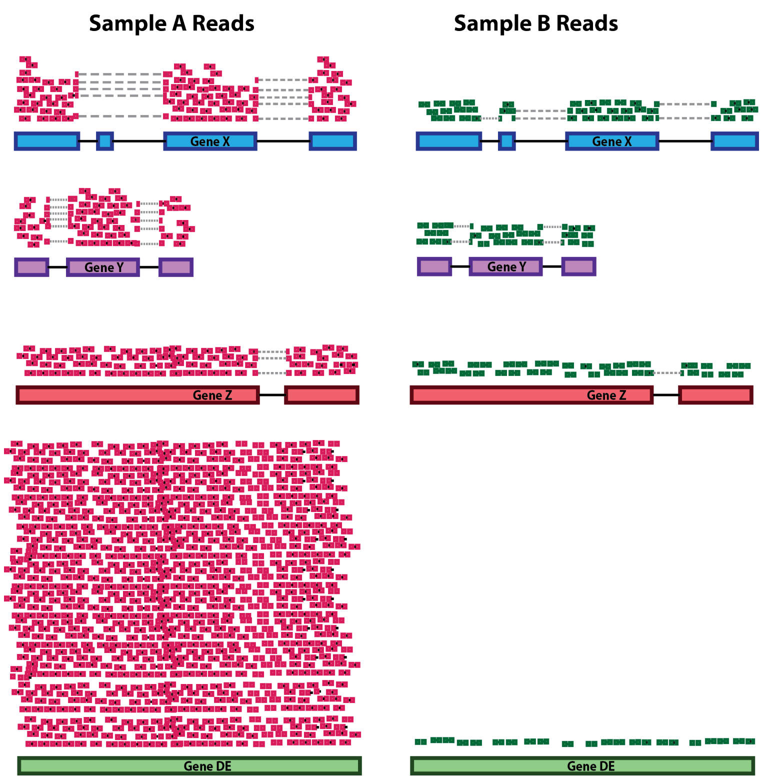
- Gene Length
- Sequencing depth
- The expression level of other genes in the sample



Normalization

The number of sequenced reads mapped to a gene depends on

- Gene Length
- Sequencing depth
- The expression level of other genes in the sample
- **It's own expression level**



Normalization eliminates the factors that are not of interest!

Normalization methods

Normalization method	Description	Accounted factors	For Differential Expression?
CPM (counts per million)	counts scaled by total number of reads in a sample	sequencing depth	NO
TPM (transcripts per kilobase million)	counts per length of transcript (kb) per million reads mapped	sequencing depth and gene length	NO
RPKM/FPKM (reads/fragments per kilobase of exon per million reads/fragments mapped)	similar to TPM	sequencing depth and gene length	NO
DESeq2's **median of ratios** [1]	counts divided by sample-specific size factors determined by median ratio of gene counts relative to geometric mean per gene	sequencing depth and RNA composition	YES

https://hbctraining.github.io/DGE_workshop

Normalization: DESeq2 Median of Ratios

Accounts for both sequencing depth and composition

Step 1: creates a pseudo-reference sample (row-wise geometric mean)

For each gene, a pseudo-reference sample is created that is equal to the geometric mean across all samples.

gene	sampleA	sampleB	pseudo-reference sample
1	1000	1000	$\sqrt{(1000 * 1000)}$ = **1000**
2	10	1	$\sqrt{(10 * 1)}$ = **3.16**
...	...	...	...

Normalization: DESeq2 Median of Ratios

Step 2: calculates ratio of each sample to the reference

Calculate the ratio of each sample to the pseudo-reference. Since most genes aren't differentially expressed, ratios should be similar.

gene	sampleA	sampleB	pseudo-reference sample	ratio of sampleA/ref	ratio of sampleB/ref
1	1000	1000	1000	1000/1000 = **1.00**	1000/1000 = **1.00**
2	10	1	3.16	10/3.16 = **3.16**	1/3.16 = **0.32**
...	...	...	...		

Normalization: DESeq2 Median of Ratios

Step 2: calculates ratio of each sample to the reference

Calculate the ratio of each sample to the pseudo-reference.

gene	sampleA	sampleB	pseudo-reference sample	ratio of sampleA/ref	ratio of sampleB/ref
1	1000	1000	1000	1000/1000 = **1.00**	1000/1000 = **1.00**
2	10	1	3.16	10/3.16 = **3.16**	1/3.16 = **0.32**
...	...	...	...	...	...
				Median = 2.08	Median = 0.66

Step 3: calculate the normalization factor for each sample (size factor)

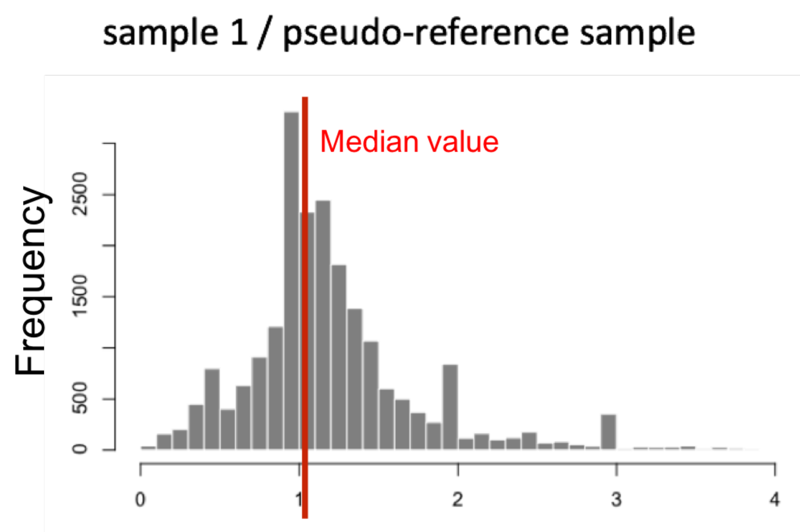
The median value of all ratios for a given sample is taken as the normalization factor (size factor) for that sample:

Normalization: DESeq2 Median of Ratios

Visualization of normalization factor for a sample:

- Median should be ~1 for each sample, otherwise data should be examined for the presence of large outliers
- This method is robust to imbalance in up-/down- regulation and large numbers of differentially expressed genes

Assumptions of this method:
Not all genes are differentially expressed



Normalization: DESeq2 Median of Ratios

Step 4: calculate the normalized count values using the normalization factor

This is performed by dividing each raw count value in a given sample by that sample's size factor to generate normalized count values.

SampleA normalization factor = 2.08

SampleB normalization factor = 0.66

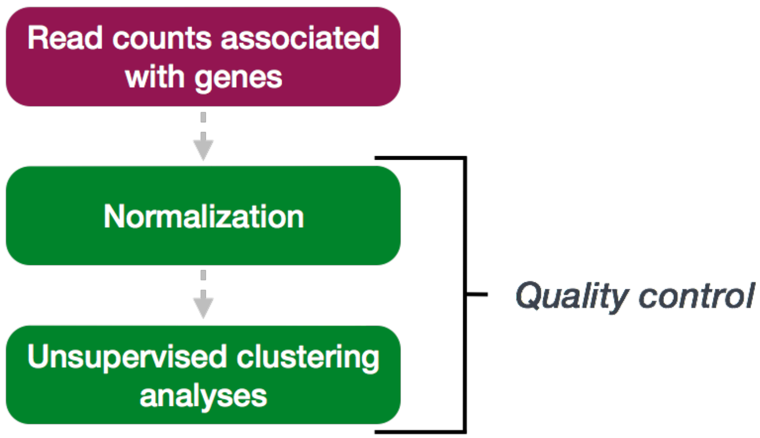
Raw Counts

gene	sampleA	sampleB
1	1000	1000
2	10	1

Normalized Counts

gene	sampleA	sampleB
1	1000/2.08 = **480.77**	1000 / 0.66 = **1515.16**
2	10/2.08 = **4.81**	1 / 0.66 = **1.52**

Unsupervised Clustering

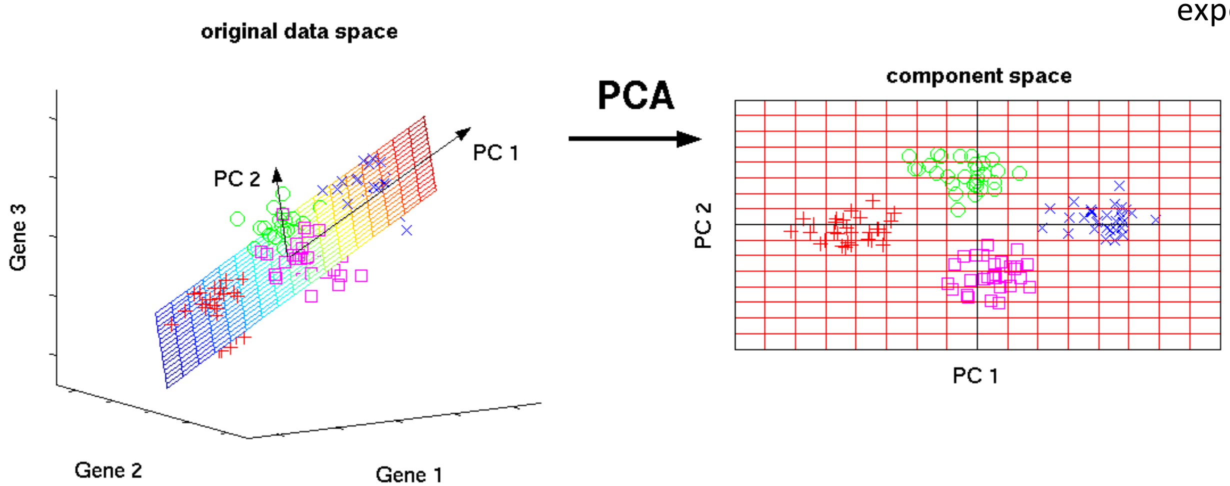


Principle Component Analysis

Here is an example with three genes measured in many samples:

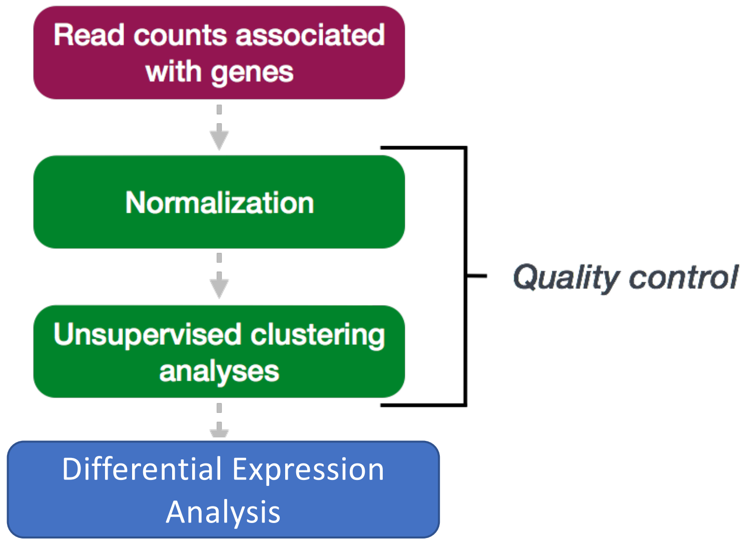
Gene	Sample 1	Sample 2	Sample 3	Sample 4
Gene 1	1000	1000	100	10
Gene 2	10	1	5	6
Gene 3	10	1	10	20

…



- Each gene that we measure is a "dimension" and we can visualize up to 3
- PCA can help us visualize relationships in out data in a lower number of dimensions
- PCA is an important QC step! Do your samples cluster as expected?

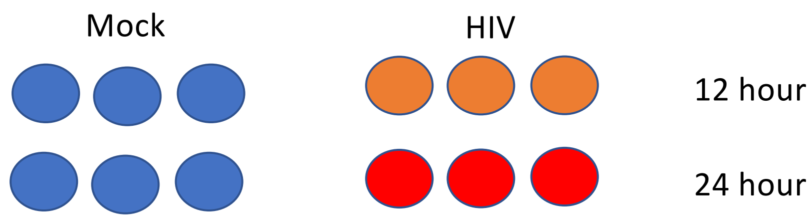
Differential Expression with DESeq2



https://hbctraining.github.io/DGE_workshop

Multi-factor design

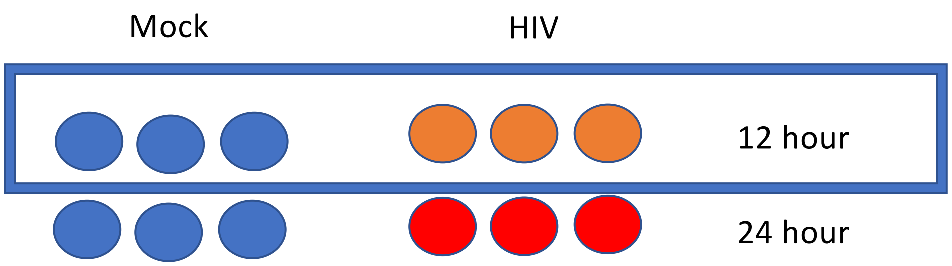
CD4+ T cell infected with either Mock or HIV



Sample	Condition	Time
1	Mock	12
2	Mock	12
3	Mock	12
4	Mock	24
5	Mock	24
6	Mock	24
7	HIV	12
8	HIV	12
9	HIV	12
10	HIV	24
11	HIV	24
12	HIV	24

Multi-factor design

CD4+ T cell infected with either Mock or HIV

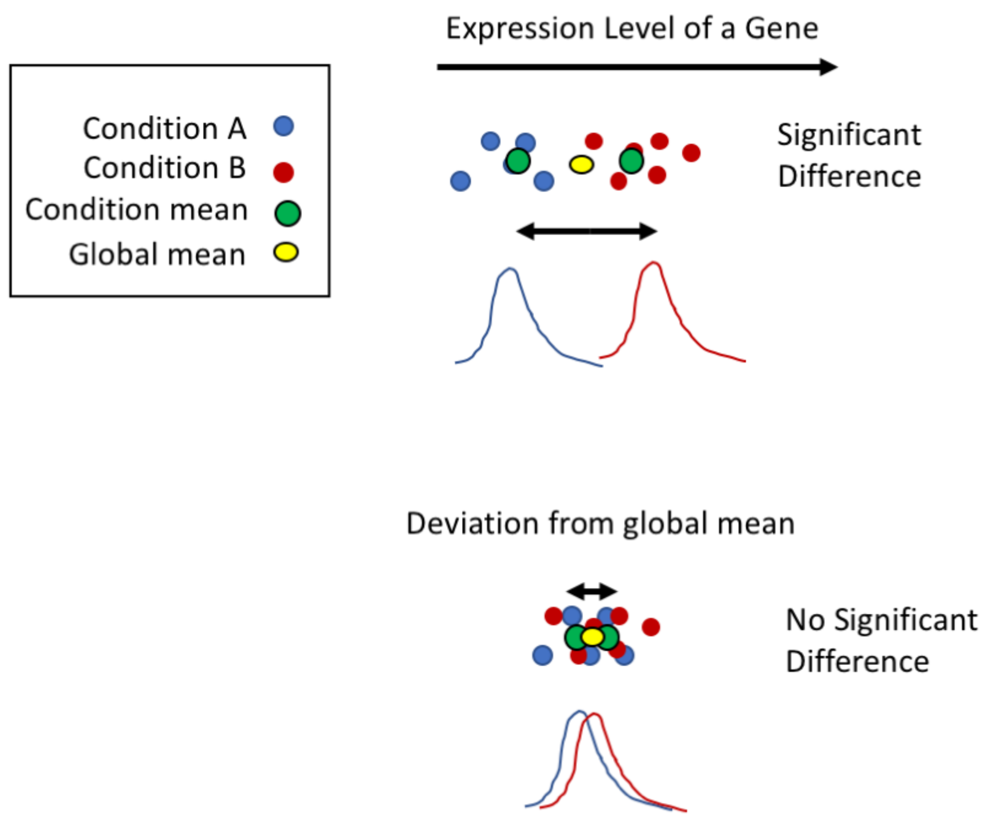


Sample	Condition	Time
1	Mock	12
2	Mock	12
3	Mock	12
4	Mock	24
5	Mock	24
6	Mock	24
7	HIV	12
8	HIV	12
9	HIV	12
10	HIV	24
11	HIV	24
12	HIV	24

We choose a primary "factor" for comparison, but can optionally include other factors to be controlled for.

DESeq2 Test for Differential Expression

- Fit a probability distribution to each gene we measured
- Perform a statistical test (Wald test) to determine whether there is a difference between conditions



DESeq2 Outputs

- Tables:
  - Results
  - Normalized Counts

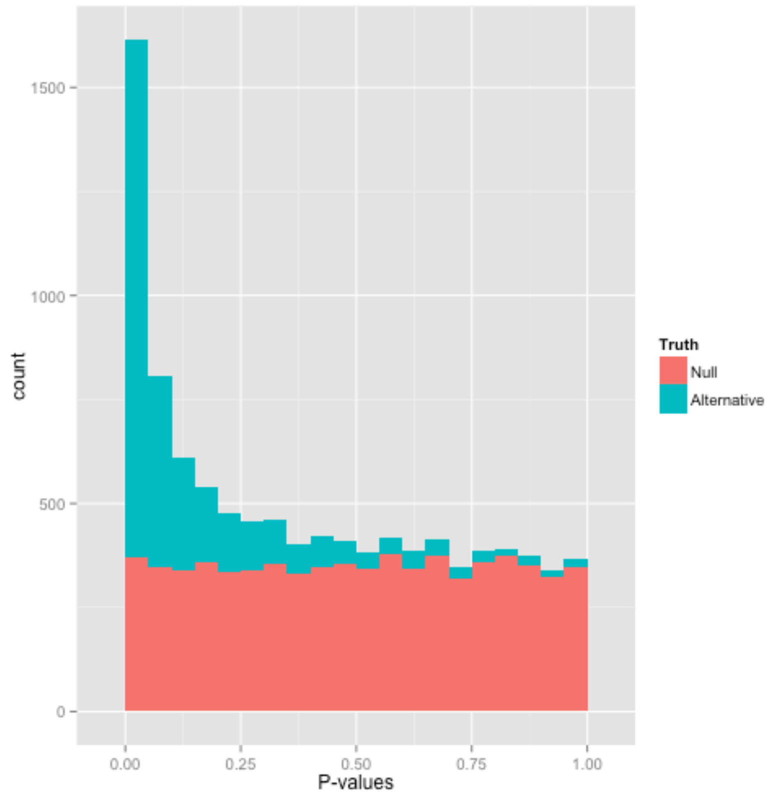
- Plots:
  - PCA
  - P-value Histogram
  - MA

Results table

GeneID	Base mean	log2(FC)	StdErr	Wald-Stats	P-value	P-adj
EGR1	1273.65	-2.22	0.12	-18.65	1.25E-77	1.44E-73
MYC	5226.12	1.41	0.11	12.53	4.95E-36	2.87E-32
OPRK1	78.35	-1.83	0.17	-10.57	4.11E-26	1.59E-22
CCNI2	7427.12	0.93	0.10	9.43	4.27E-21	1.24E-17
STRA6	785.78	0.97	0.11	8.61	7.29E-18	1.69E-14

- Mean of normalized counts – averaged over all samples from two conditions
- Log of the fold change between two conditions
- Standard Error of Log FC estimate – will reflect the ”noisiness” of the gene
- P-value – the probability that the log2FoldChange is not zero
- Adjusted P value – accounting for multiple testing correction

P-value histogram



- Plot of raw p-values

- P-value: Probability of getting a logFC as extreme as observed if the true logFC = 0 for that gene (null hypothesis)

How to interpret:

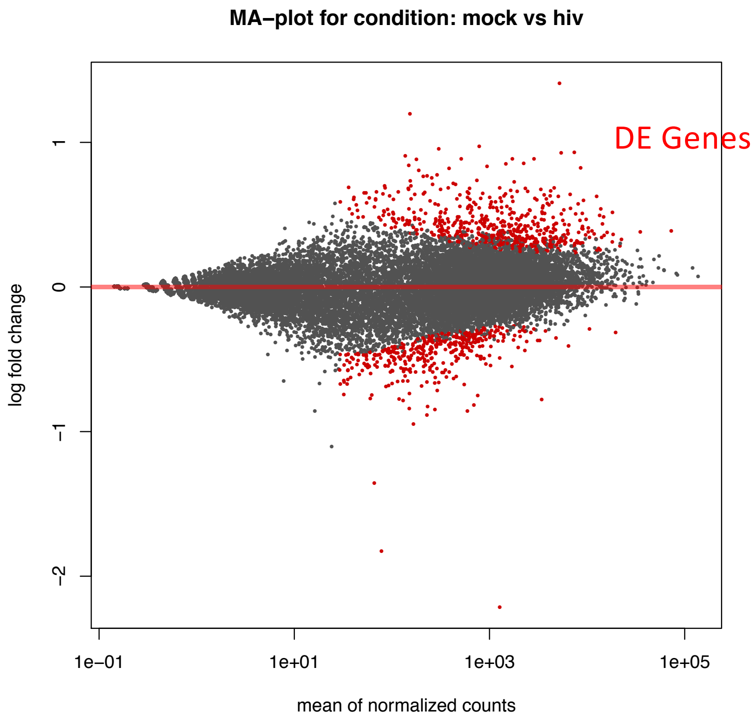
- Random P-values are expected to be uniform, if you have true positives you should see a peak close to zero

http://varianceexplained.org/statistics/interpreting-pvalue-histogram/

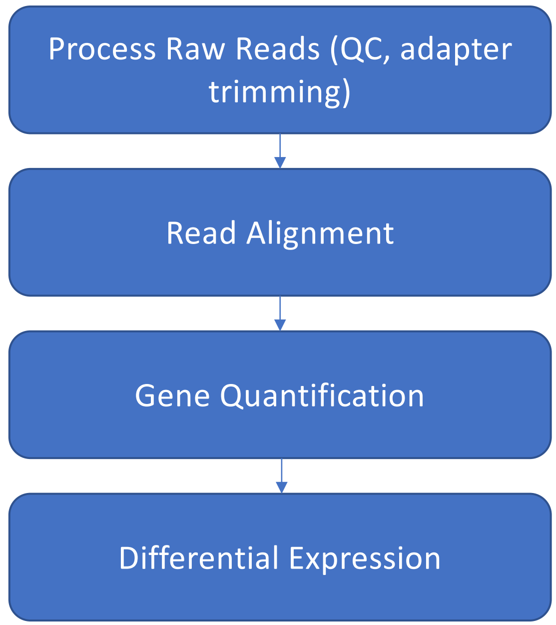
MA plot

Shows the relationship between

- M: The difference in expression Log(HIV) – Log(Mock) = Log(HIV/Mock)
- A: Average expression strength Average(Mock, HIV)
- Genes with adjusted *p*-value < 0.1 are in red
- Can be used as an overview or to diagnose problems



Conclusions



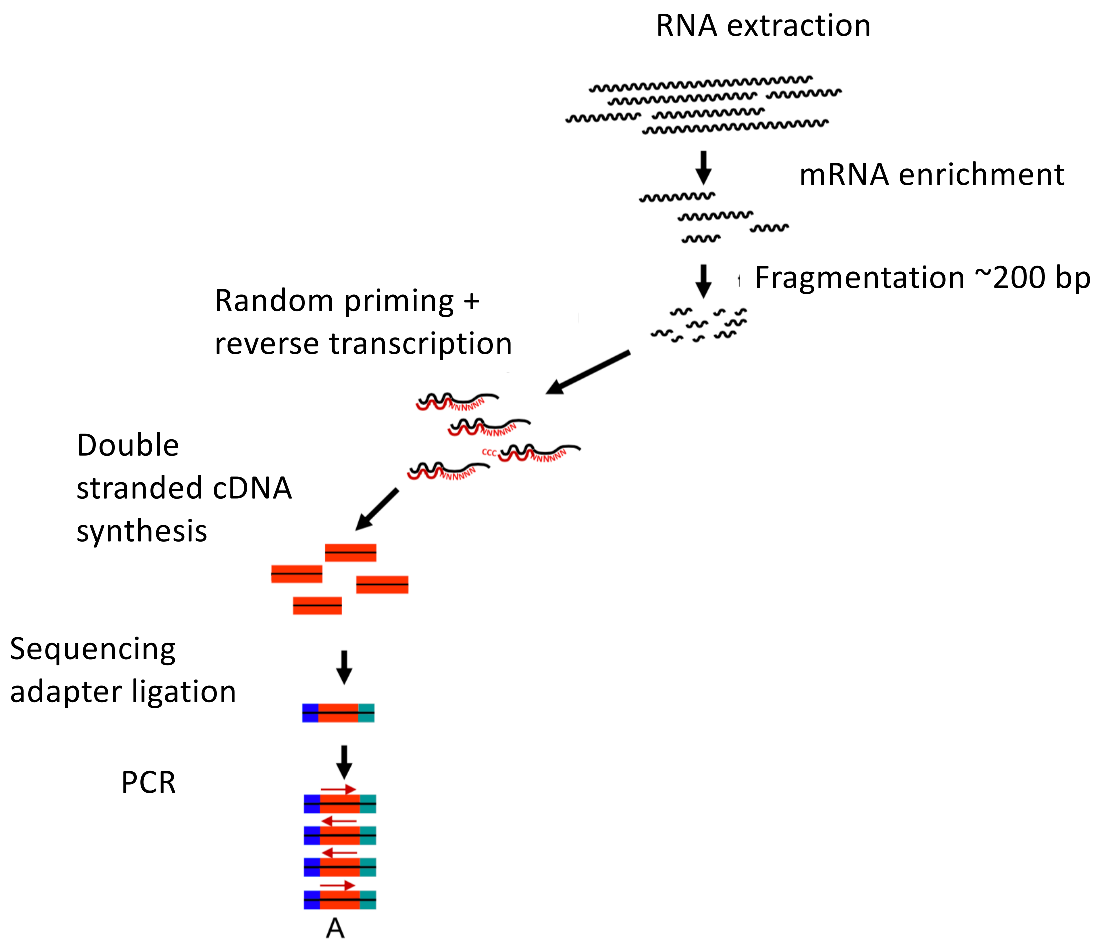
References

https://www.bioconductor.org/packages/3.3/bioc/vignettes/DESeq2/inst/doc/DESeq2.pdf

https://hbctraining.github.io/DGE_workshop

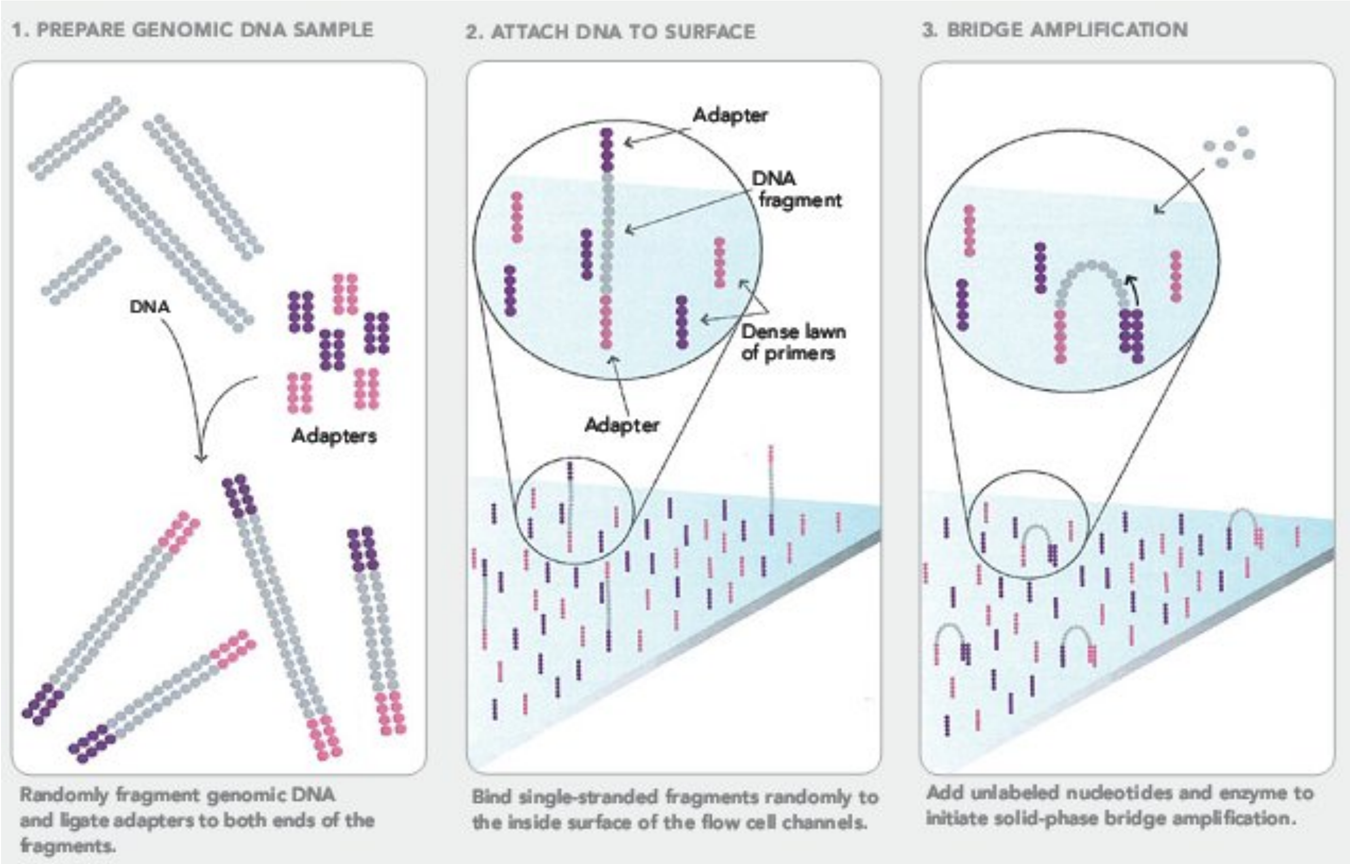
https://galaxyproject.org/tutorials/rb_rnaseq/

Extra Slides



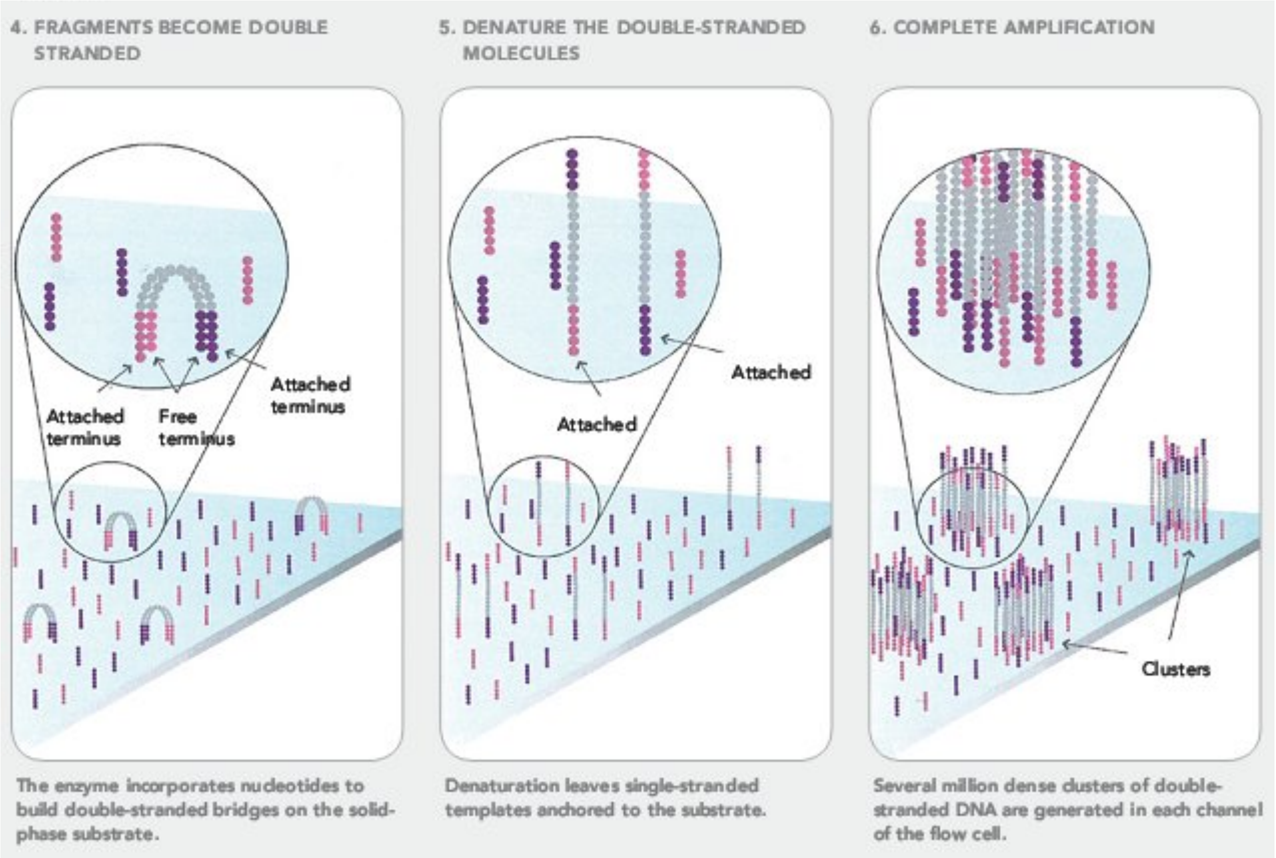
Good resource: [Griffiths et al Plos Comp Bio 2015]

Next Generation Sequencing (NGS)



https://sites.google.com/site/himbcorelab/illumina_sequencing

Next Generation Sequencing (NGS)



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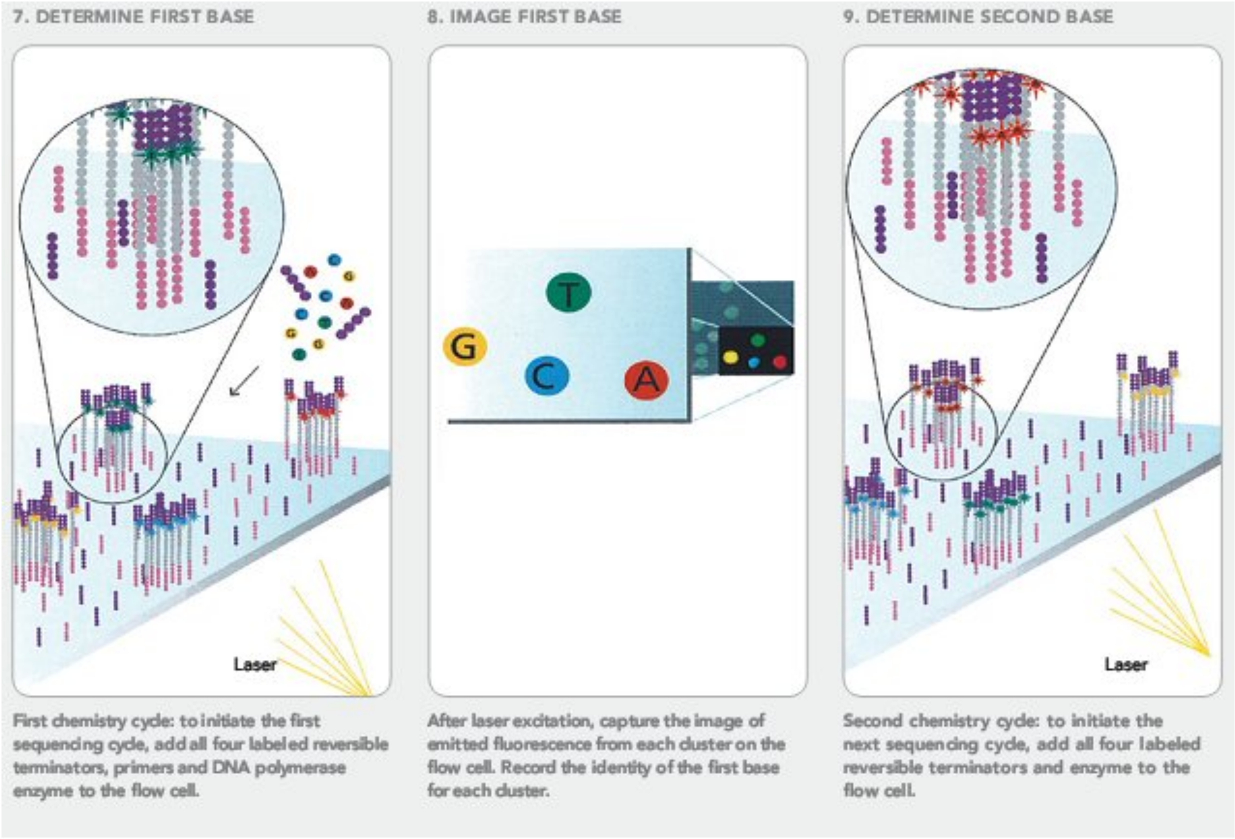
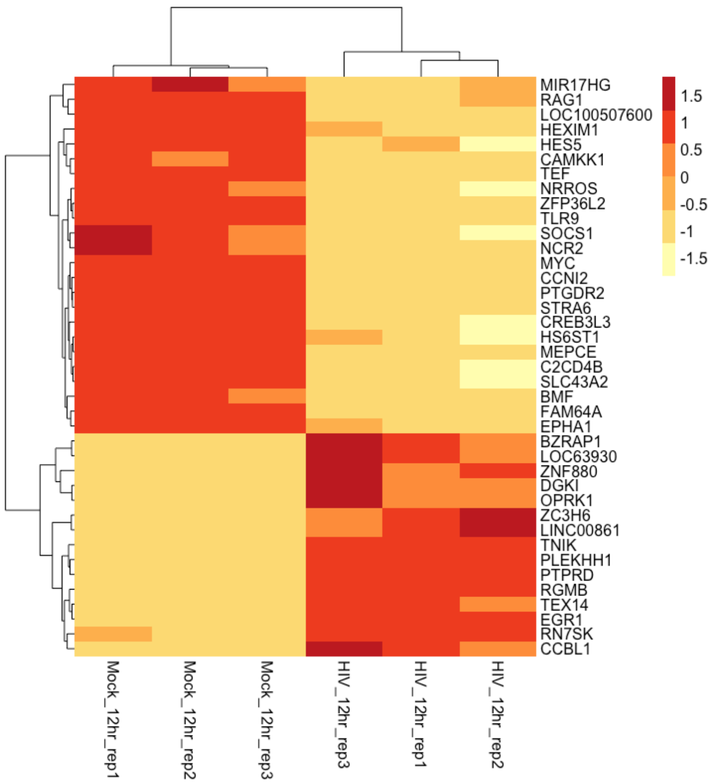


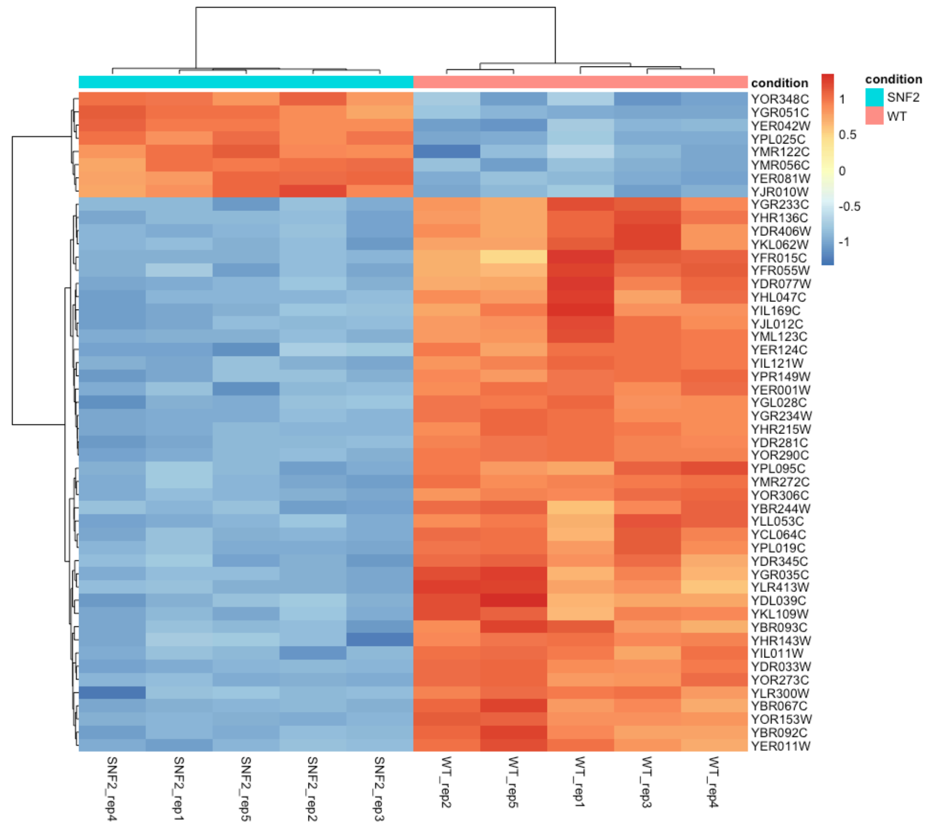
https://sites.google.com/site/himbcorelab/illumina_sequencing

Final Heatmap – not part of DESeq2 output



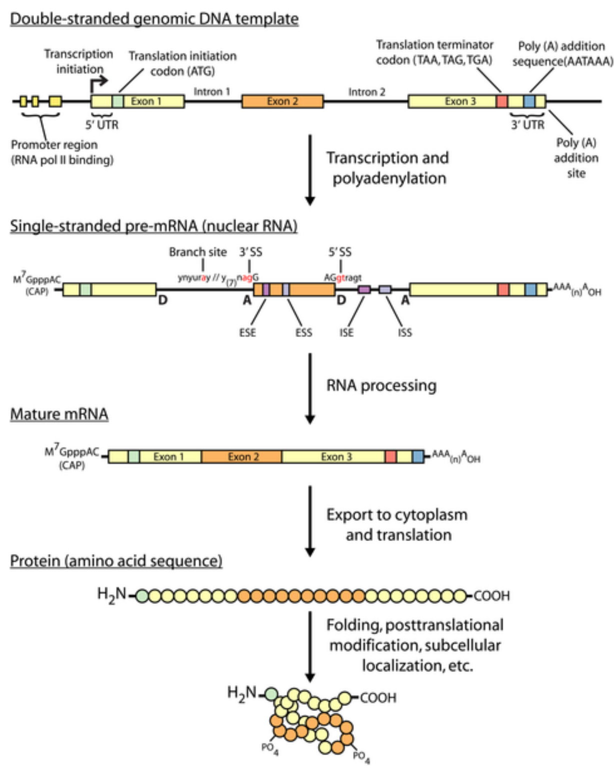
Common RNAseq analysis goals

- Novel transcript discovery
- Transcriptome assembly
- Single cell analysis
- Quantify alternative splicing
- **Differential Expression**



Replace with actual heatmap

Fig 1. An overview of the central dogma of molecular biology.



Griffith M, Walker JR, Spies NC, Ainscough BJ, Griffith OL (2015) Informatics for RNA Sequencing: A Web Resource for Analysis on the Cloud. PLOS Computational Biology 11(8): e1004393. https://doi.org/10.1371/journal.pcbi.1004393
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

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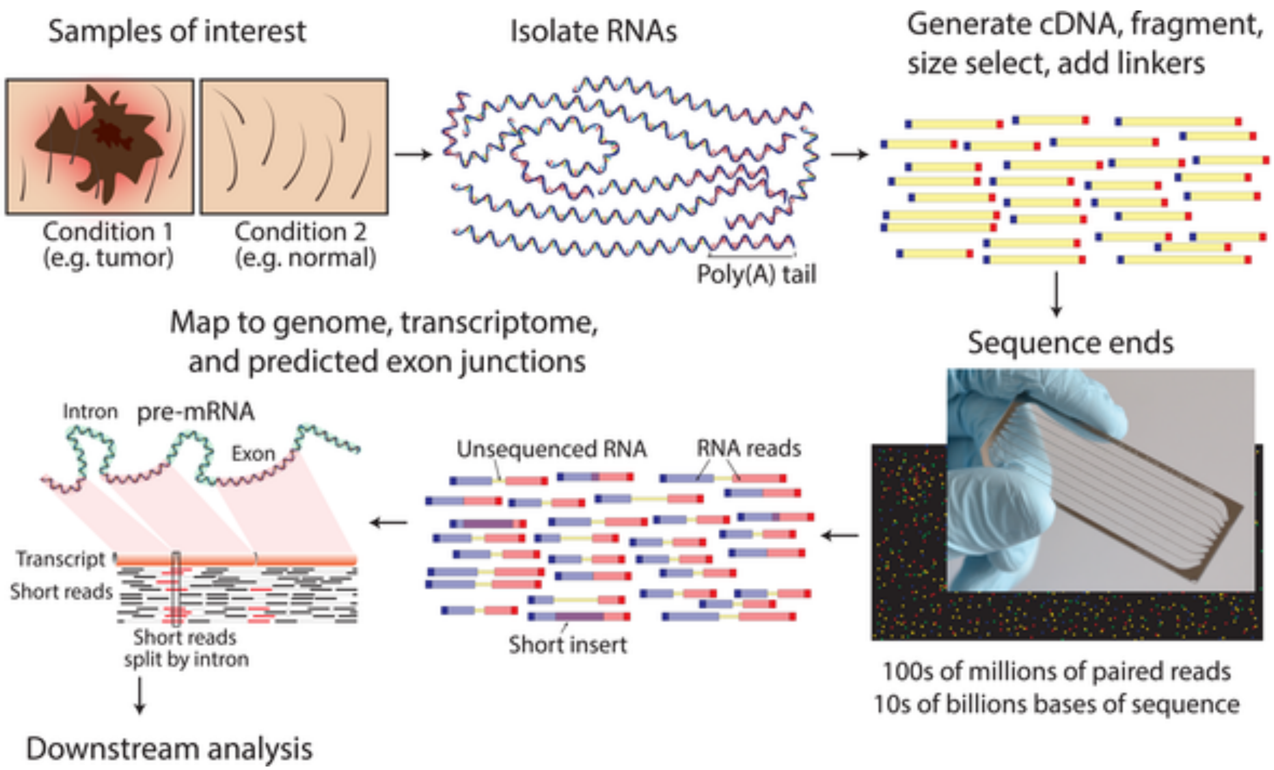
Fig 2. RNA-seq data generation.



Griffith M, Walker JR, Spies NC, Ainscough BJ, Griffith OL (2015) Informatics for RNA Sequencing: A Web Resource for Analysis on the Cloud. PLOS Computational Biology 11(8): e1004393. https://doi.org/10.1371/journal.pcbi.1004393
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

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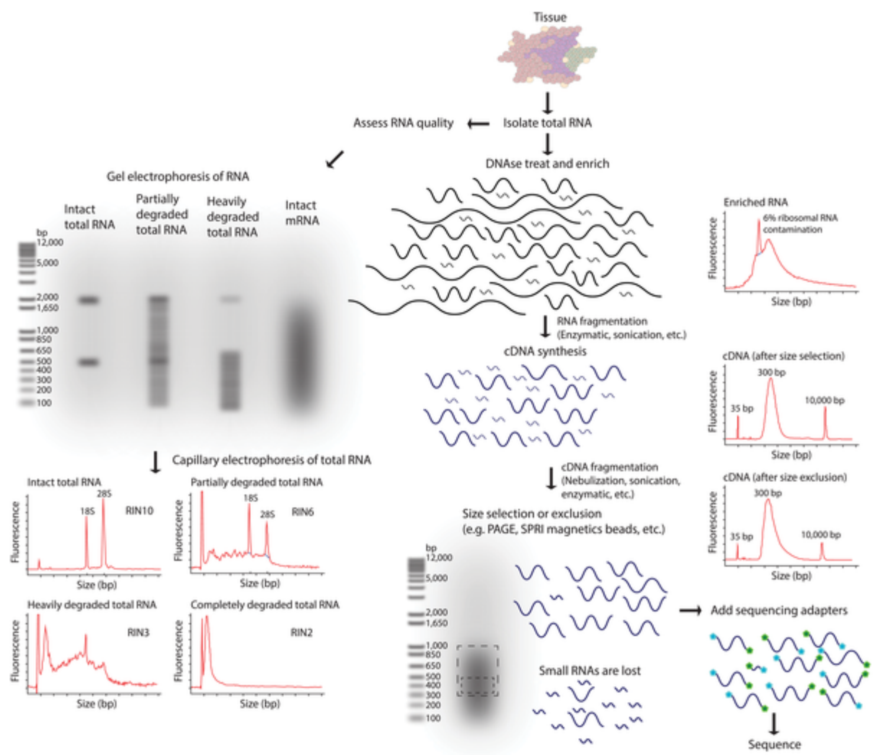
Fig 3. RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



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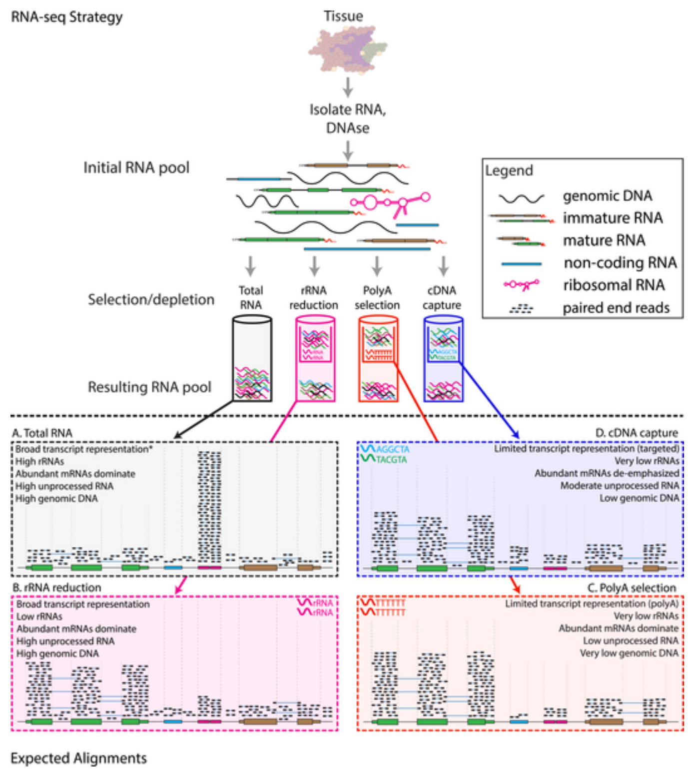
PLOS Computational Biology 11(8): e1004393. https://doi.org/10.1371/journal.pcbi.1004393
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

Fig 4. RNA-seq library enrichment strategies that influence interpretation and analysis.



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PLOS Computational Biology 11(8): e1004393. https://doi.org/10.1371/journal.pcbi.1004393
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

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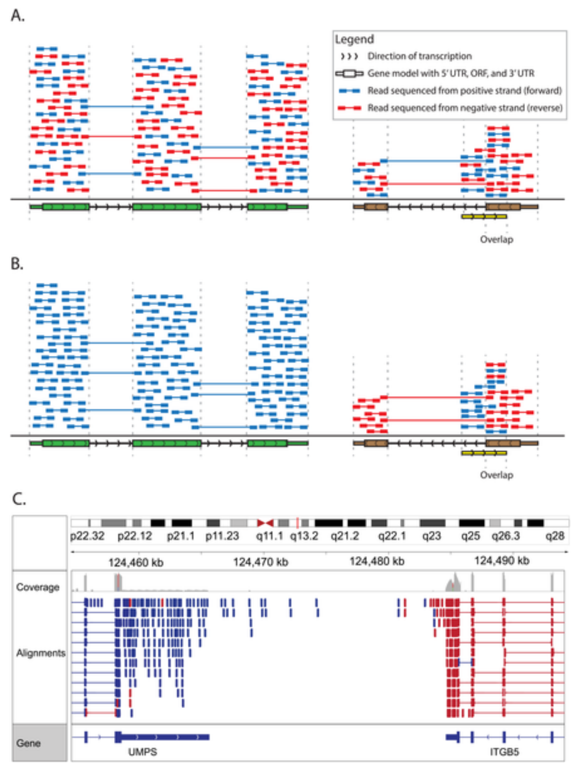
Fig 6. Comparison of stranded and unstranded RNA-seq library methods and their influence on interpretation and analysis.



Griffith M, Walker JR, Spies NC, Ainscough BJ, Griffith OL (2015) Informatics for RNA Sequencing: A Web Resource for Analysis on the Cloud. PLOS Computational Biology 11(8): e1004393. https://doi.org/10.1371/journal.pcbi.1004393
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

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Test for Differential Expression

DESeq2 will seek to fit a probability distribution to each gene we measured and perform a statistical test to determine whether there is a difference between conditions