# RNA-seq introduction

## RNA-seq goals

### Functional studies

#### Differential gene expression

### SNP analysis

### Transcriptome assembly

### Novel gene finding

### Splice variant analysis

## Background - sequencing

### Illumina

### Library prep

### Paired vs single end

## Sequencing files

### Fastq

# RNA-seq workflow

## Introduction

### Type of sequencing

### Experimental design

#### block design

#### coverage considerations

### Eukaryote vs prokaryote

#### Tools for eukaryote

##### Hisat2 - string tie - ballgown

##### STAR - DESeq2

#### Prokaryote

##### Bowtie2 - HTseq - DESeq2

## basic steps

### QC

#### QC trimming? trimmomatic

#### Adaptor trimming

#### use python script for example

### Mapping

#### Alignment output

##### SAM and BAM

##### Show reads mapped in IGV

##### Introduction to samtools

##### Flagstats

### Counting - use python script

## DESeq2 introduction

### Distribution assumptions

### Count based

### Statistics used

## DESeq2 workshop

### Importing experiment design

### Importing read matrix

### Exploratory

#### PCA

#### Count matrix heatmaps

### Differential expression

#### Extract significant up and down

### Example showing how to separate out all the plasmids

### Examples on how to add annotation

### Briefly introduce pathway analysis, GO terms.  Mariam will go over this later