Other applications of RNA-seq

A non-comprehensive list…

- Identifying the protein interaction sites on RNA molecules:

  HITS-CLIP, PAR-CLIP, iCLIP

- Identifying translation status of genes:

  Ribo-seq (Ribosomal Profiling)

- Evaluating/comparing expression of small RNA
- Understanding expression profiles of cells starting with single cells

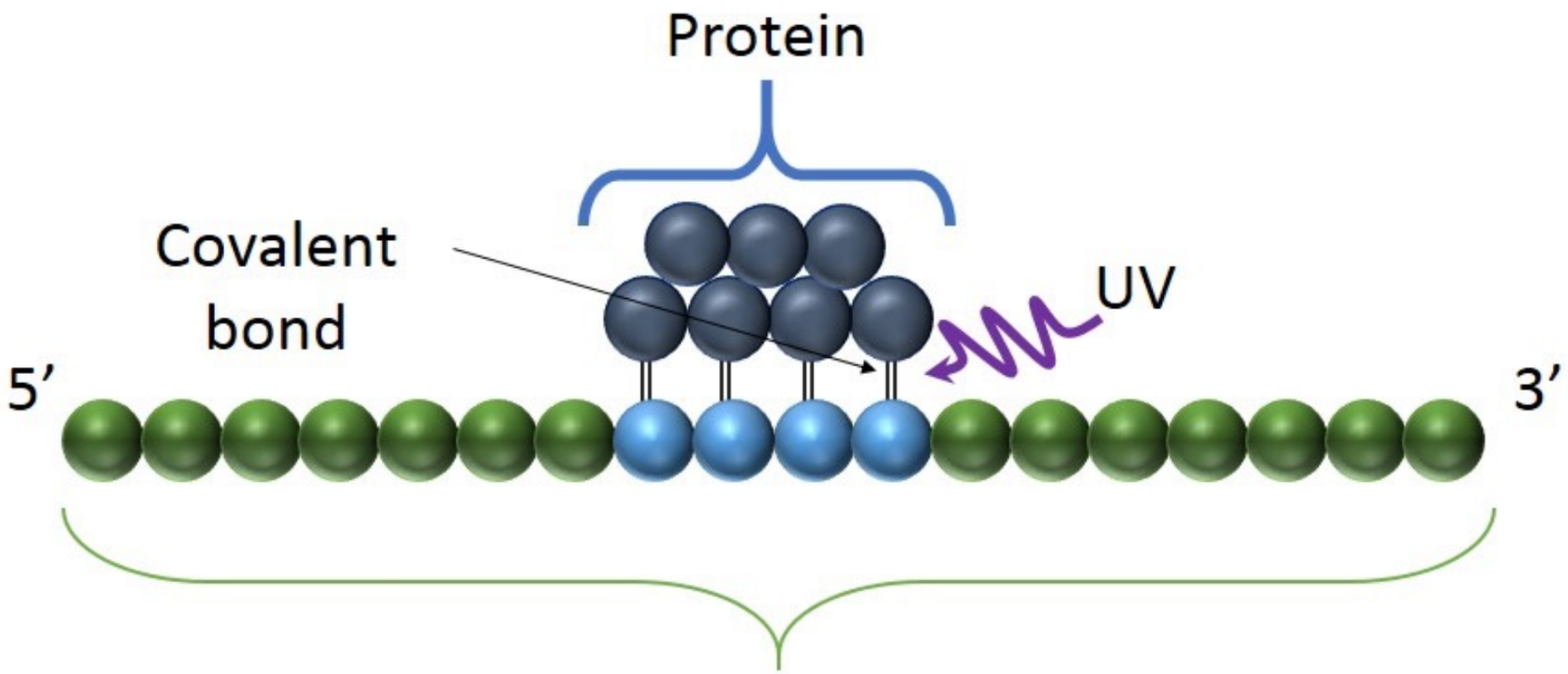
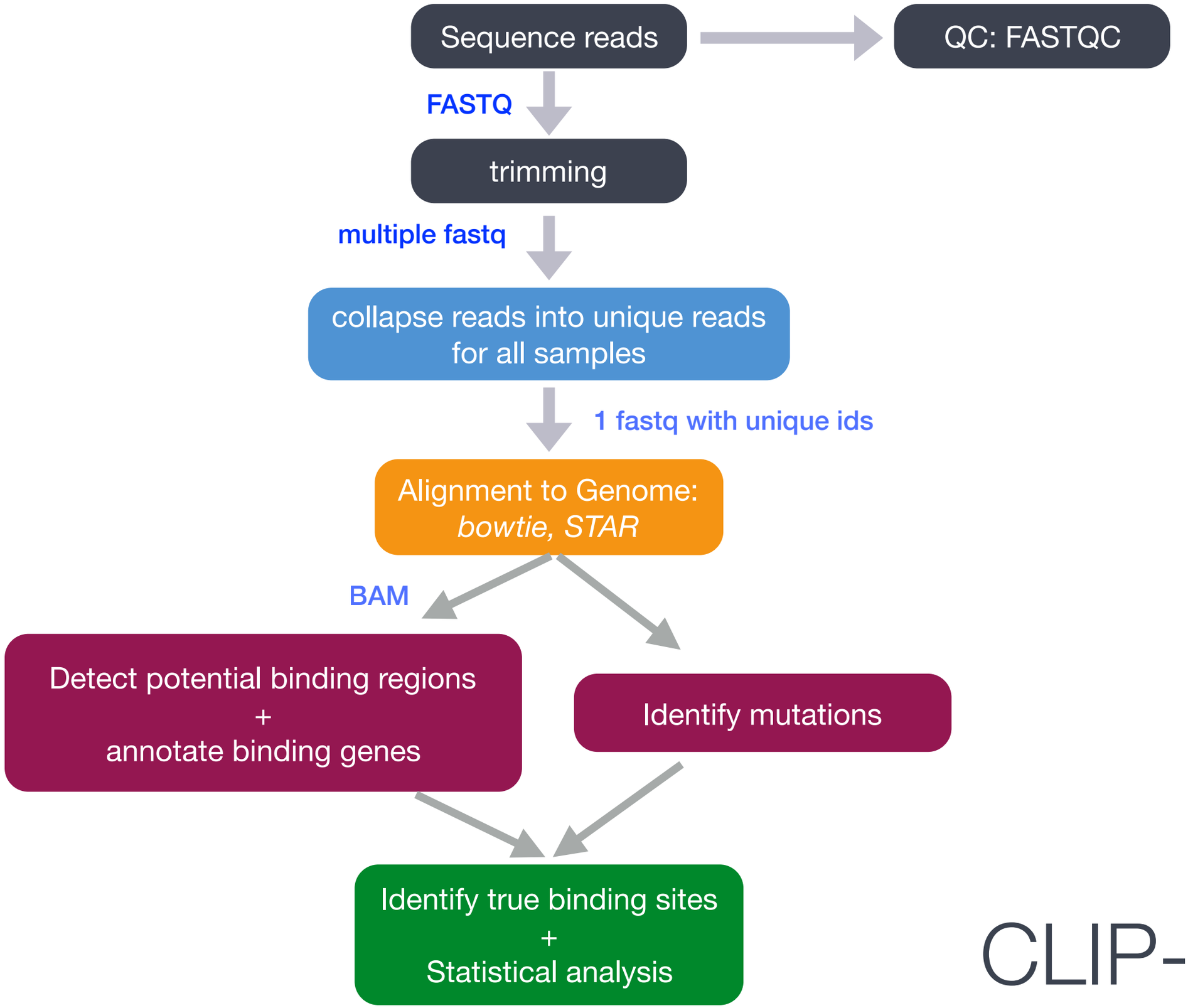


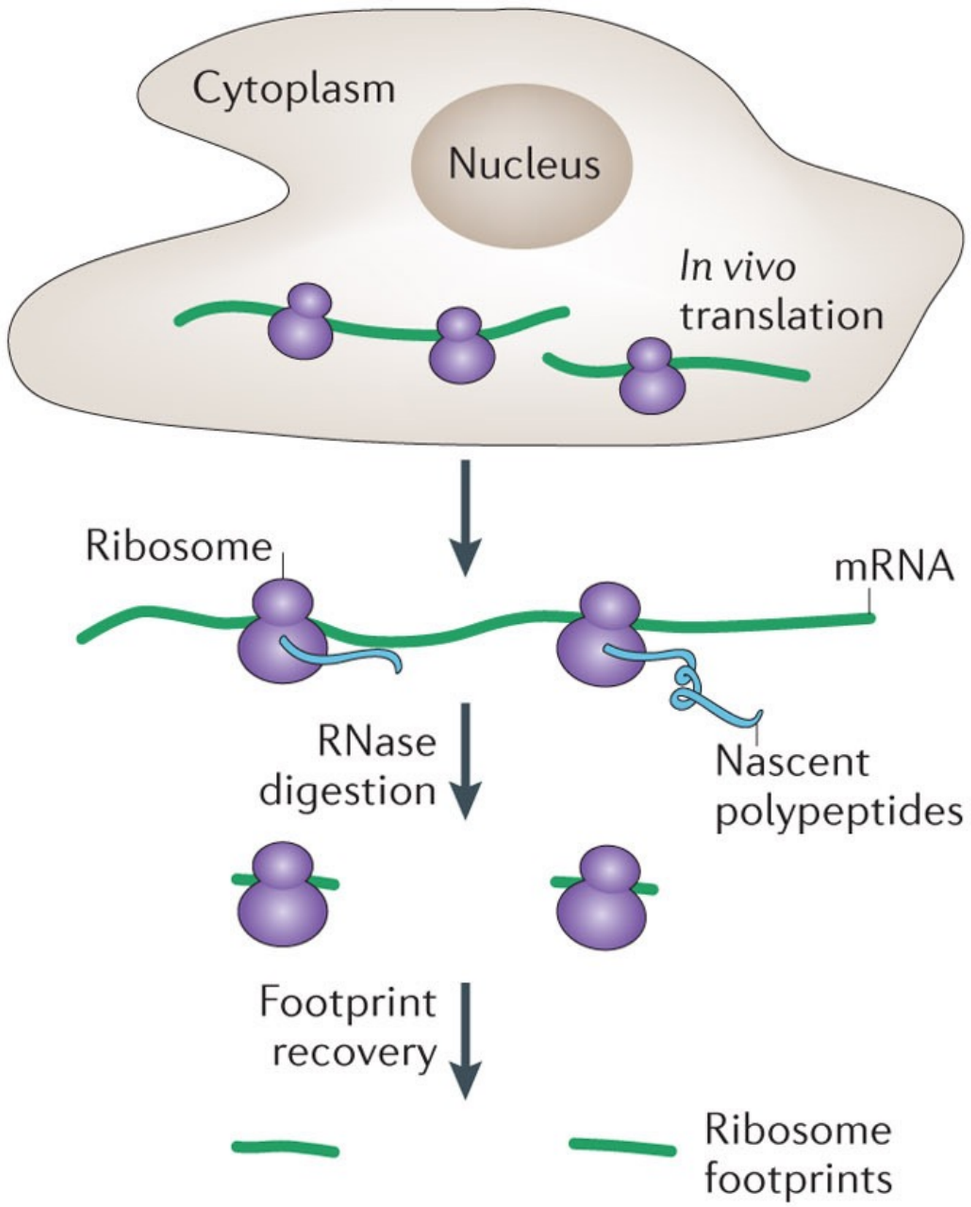
Figure 1: Basic principle of CLIP. Covalent bonds are formed between proximal proteins and RNA upon exposure to ultraviolet light. These bonds only occur at the sites of direct contact and preserve RNA-protein interactions.

https://en.wikipedia.org/wiki/CLIP#/media/File:Basic_Principle_of_CLIP.jpg

CLIP-seq

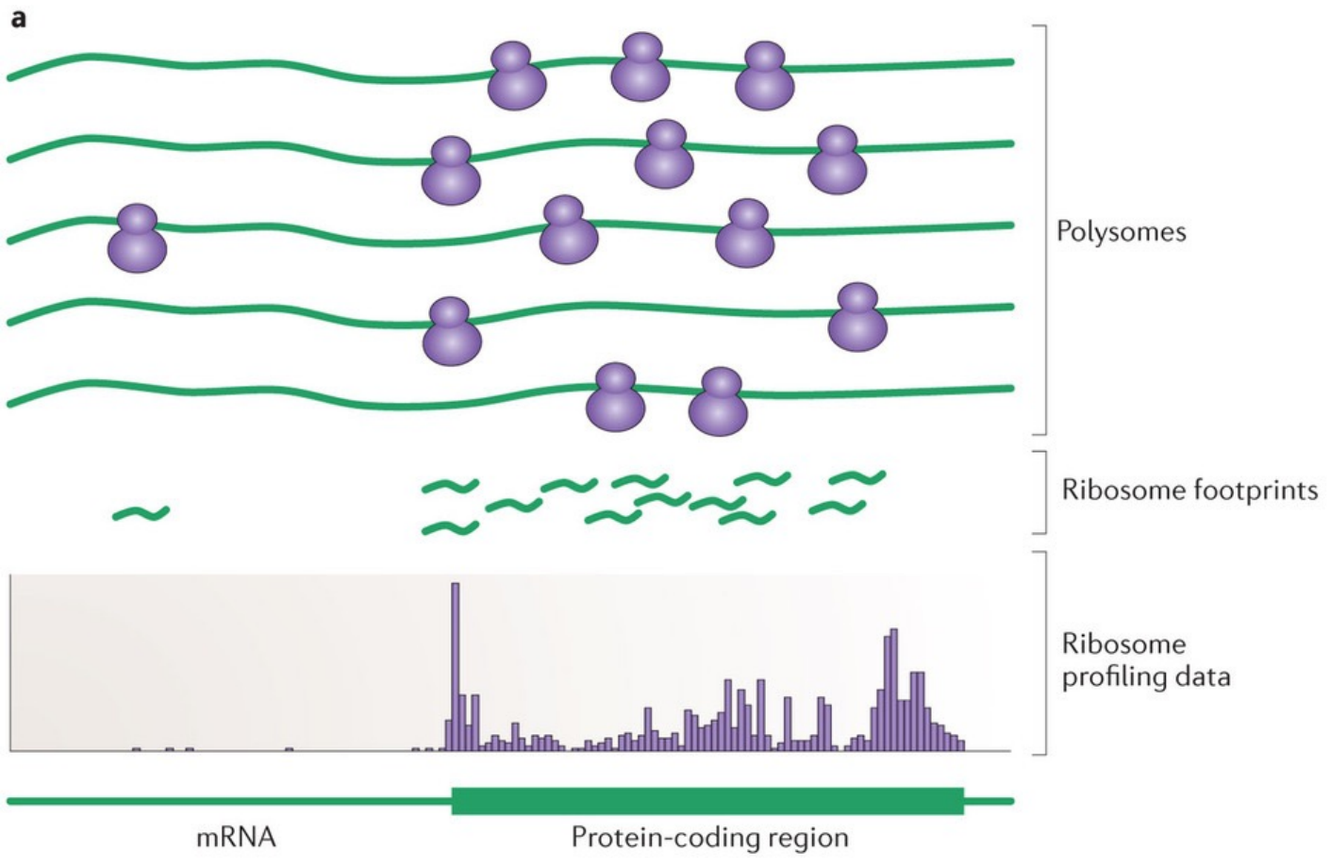

Sequence reads
QC: FASTQC
FASTQ
trimming
multiple fastq
collapse reads into unique reads
for all samples
1 fastq with unique ids
Alignment to Genome:
bowtie, STAR
BAM
Detect potential binding regions
+
annotate binding genes
Identify mutations
Identify true binding sites
+
Statistical analysis

CLIP-seq



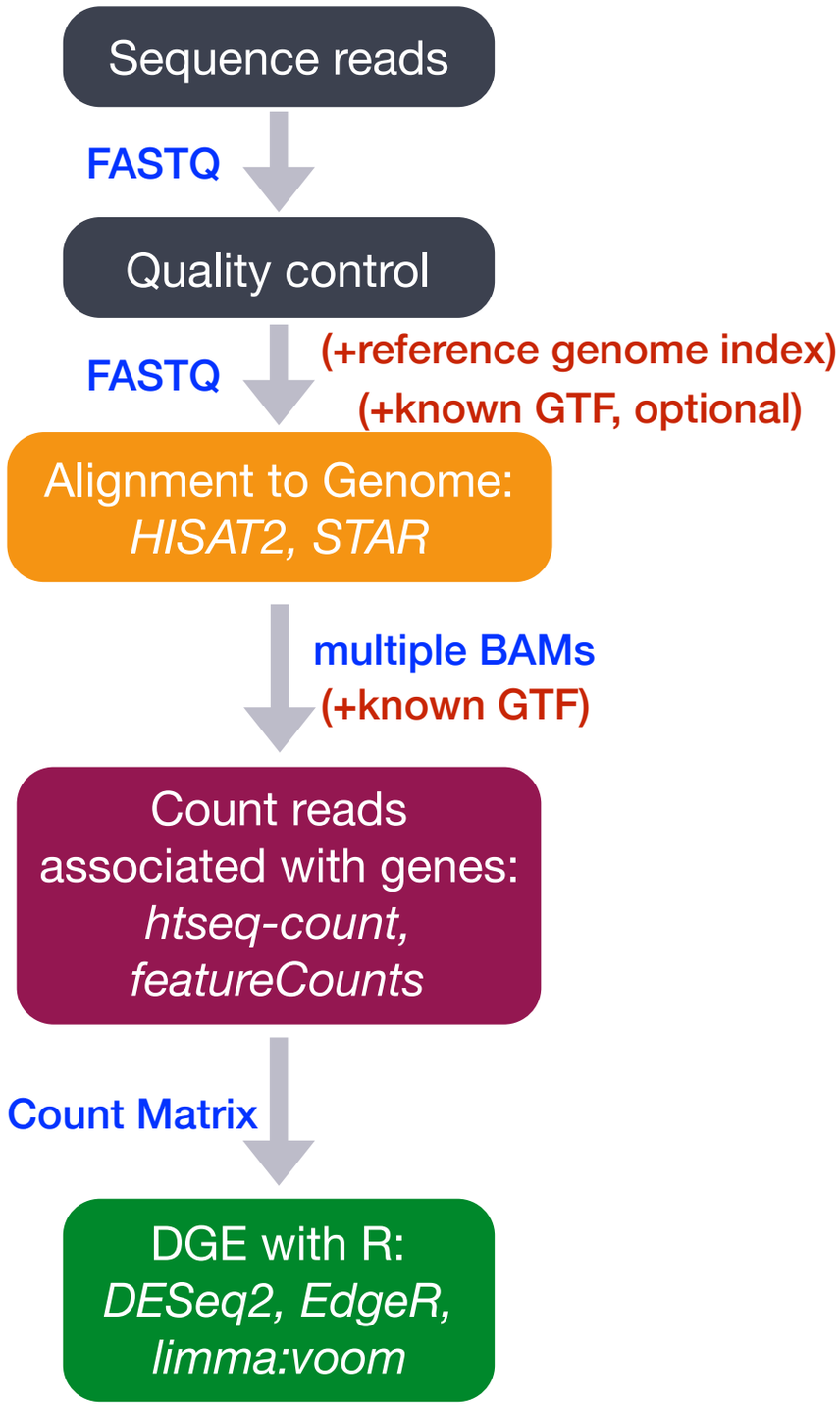
Ribo-seq

Nature Reviews Genetics 15, 205–213 (2014) doi:10.1038/nrg3645

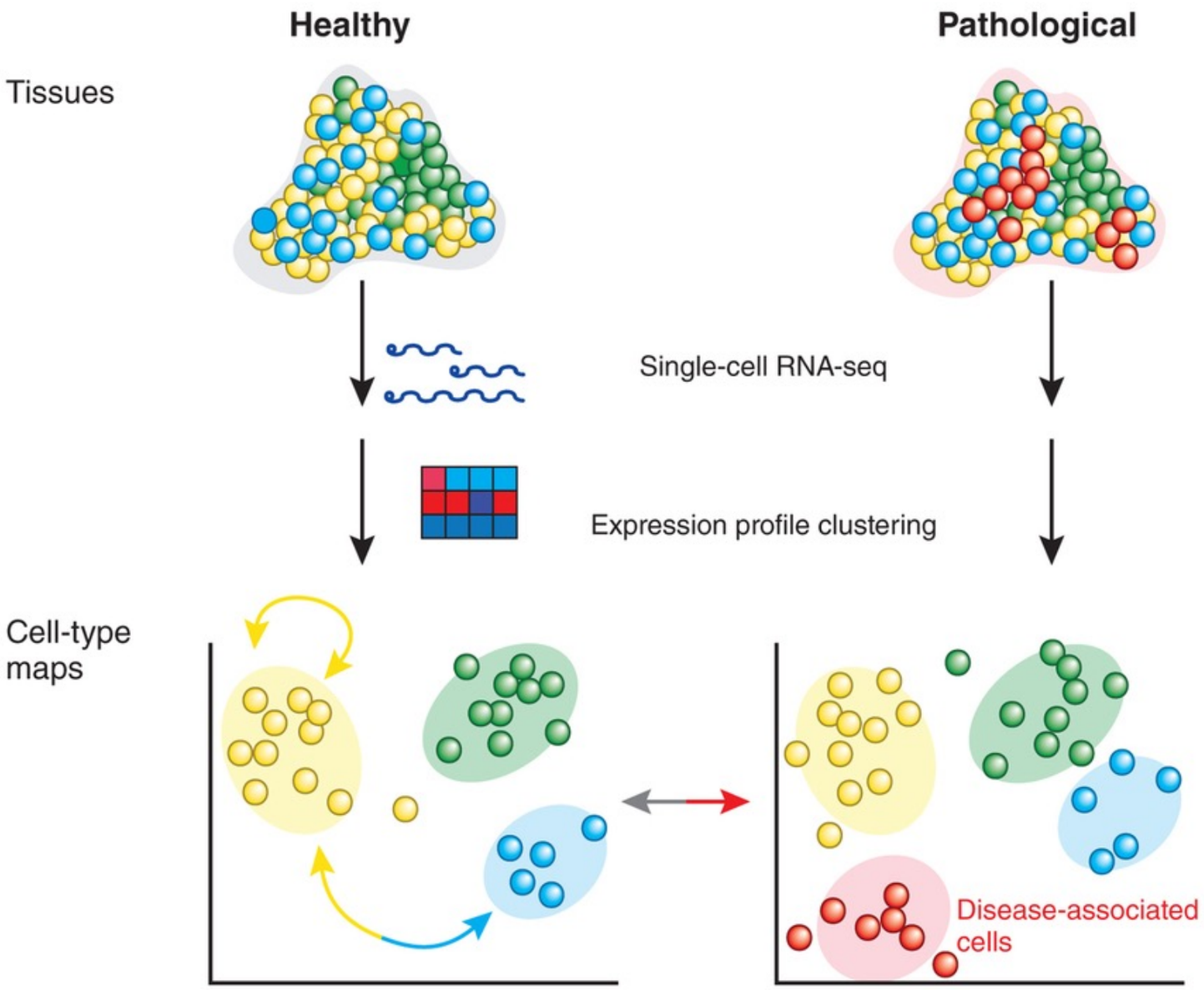


Ribo-seq

Nature Reviews Genetics 15, 205–213 (2014) doi:10.1038/nrg3645


Sequence reads
FASTQ
Quality control
FASTQ
(+reference genome index)
(+known GTF, optional)
Alignment to Genome:
HISAT2, STAR
multiple BAMs
(+known GTF)
Count reads
associated with genes:
htseq-count,
featureCounts
Count Matrix
DGE with R:
DESeq2, EdgeR,
limma:voom

Ribo-seq



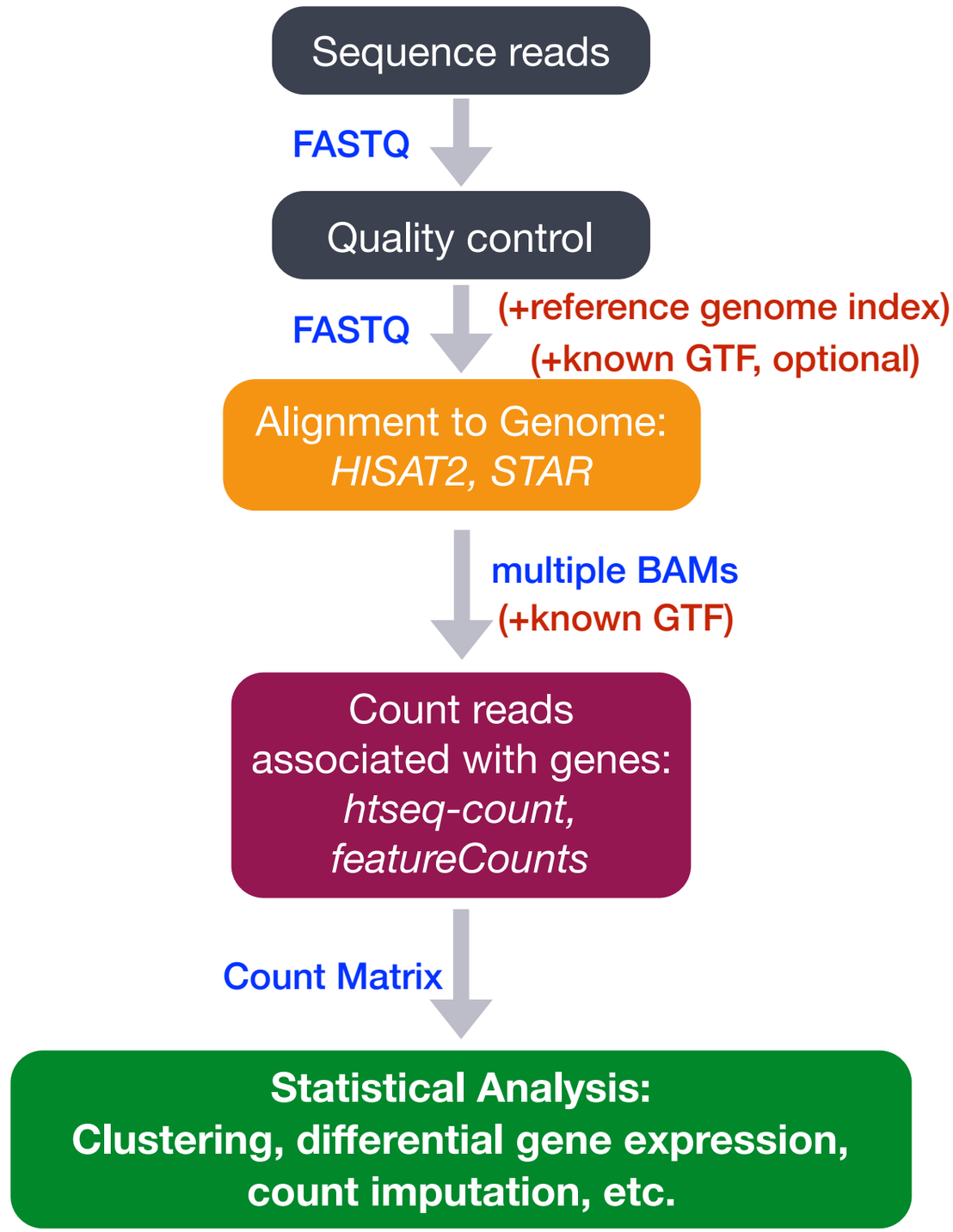
Types of analyses

Within cell type
- Stochasticity, variability of transcription
- Regulatory network inference
- Allelic expression patterns
- Scaling laws of transcription

Between cell types
- Identify biomarkers
- (Post)-transcriptional differences

Between tissues
- Cell-type compositions
- Altered transcription in matched cell types

scRNA-seq


Sequence reads
FASTQ
Quality control
FASTQ
(+reference genome index)
(+known GTF, optional)
Alignment to Genome:
HISAT2, STAR
multiple BAMs
(+known GTF)
Count reads
associated with genes:
htseq-count,
featureCounts
Count Matrix
Statistical Analysis:
Clustering, differential gene expression,
count imputation, etc.

scRNA-seq

Sequence reads

QC: FASTQC

FASTQ

trimming

multiple fastq

(+known reference:
miRBase, tRNAs …)

collapse reads into unique reads
for all samples

1 fastq with unique ids

Alignment to Genome:
bowtie, STAR

novel miRNA:
miRDeep2

Count reads on known
small RNAs:
seqbuster, tdrmapper

BAM

Detect clusters over
the genome:
seqcluster

Count Matrix

(+known GTF, optional)

Count Matrix

DGE with R:
DESeq2, EdgeR,
limma:voom

small RNA-seq