# Steps in an RNA-seq analysis

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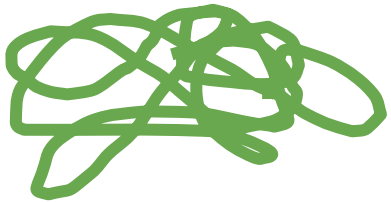
# Background on RNA-seq

**DNA** ACTGACCTAGATCAGTGTAGCGATCGTATACGAGACCGATTCATCGGCAT

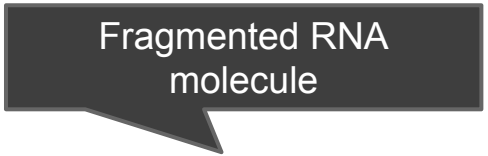
↓ transcription

**RNA** AUCAGUCGAUCACCGAU

↓ translation

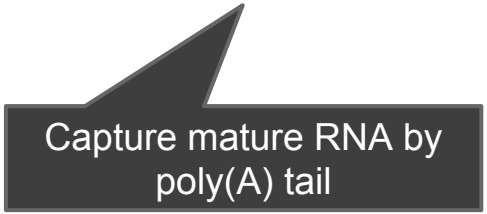


**protein**




AUGGGAAUUCACGAAUUCCUAGAAAAAAAAA

AUGGGAAUUCACGAAUUCCUAGAAAAAAAAA



AUGGGAAUUCACGAAUUCCUAGAAAAAAAA

Reverse transcribe into complementary DNA (cDNA)

ATGGGAATTCACGAATTCCTAG

AUGGGAAUUCACGAAUUCCUAGAAAAAAAA

ATGGGAATTCACGAATTCCTAG

Steps

1. Align
2. Count or quantify
3. Normalize
4. Statistical test
5. Gene set enrichment

Slide courtesy: Alyssa Frazee

Genome (DNA)

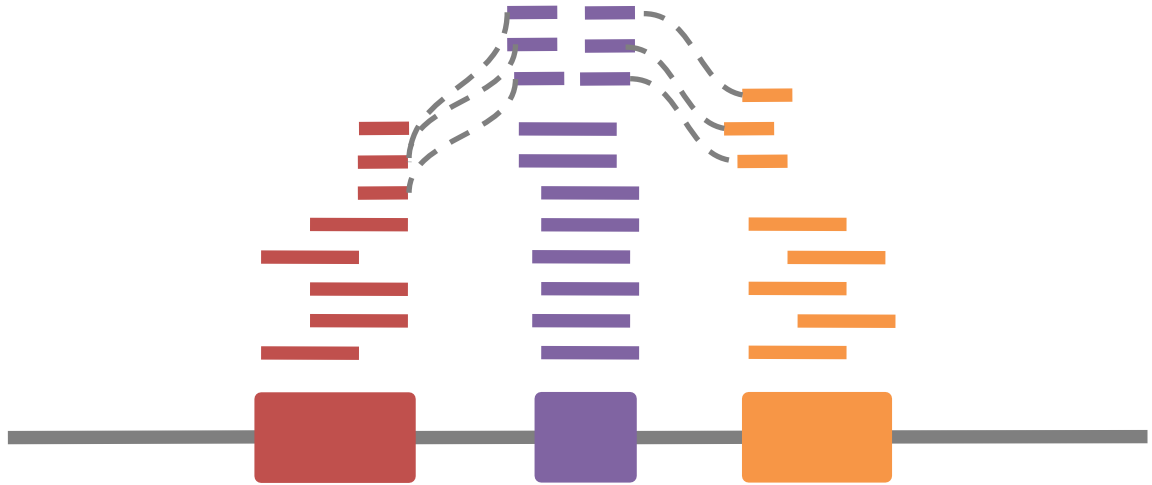
RNA transcripts (many possible variants)

# RNA-seq reads

# Step 1: Align

Software:

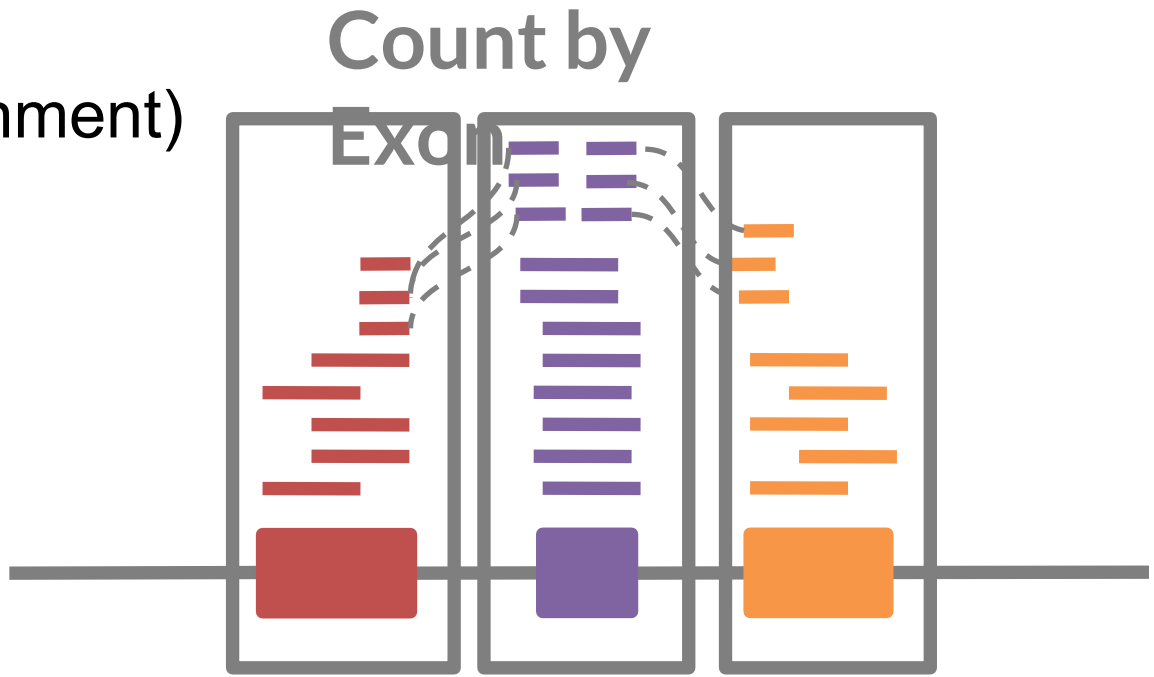
- HiSat
- Rail
- Star
- Tophat2



# Step 2: Count

Software:

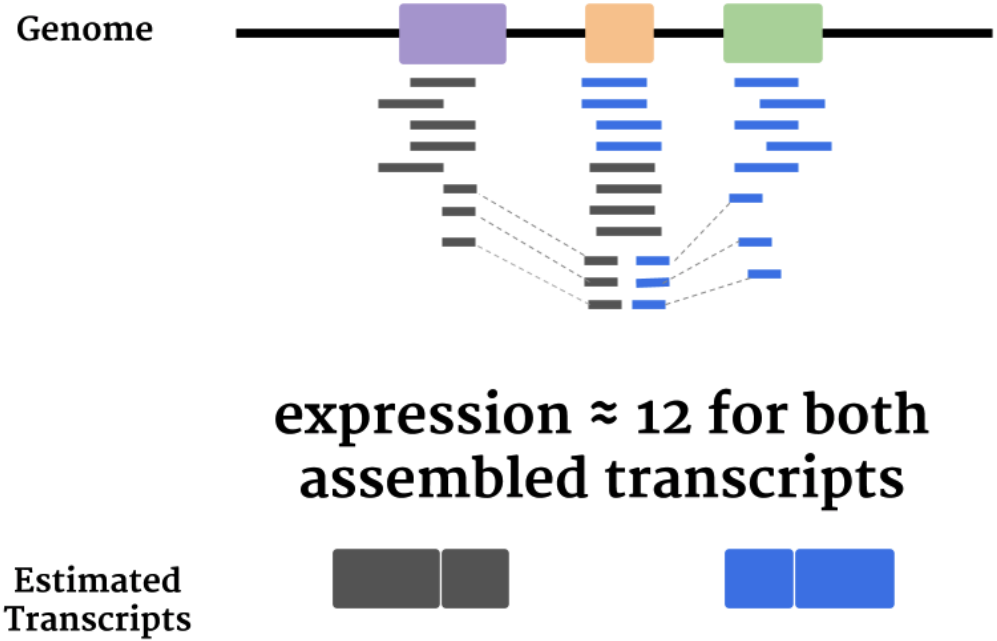
- HTSeq
- featureCounts
- kallisto (no alignment)
- derfinder



# Step 2: Assemble and quantify

Software:

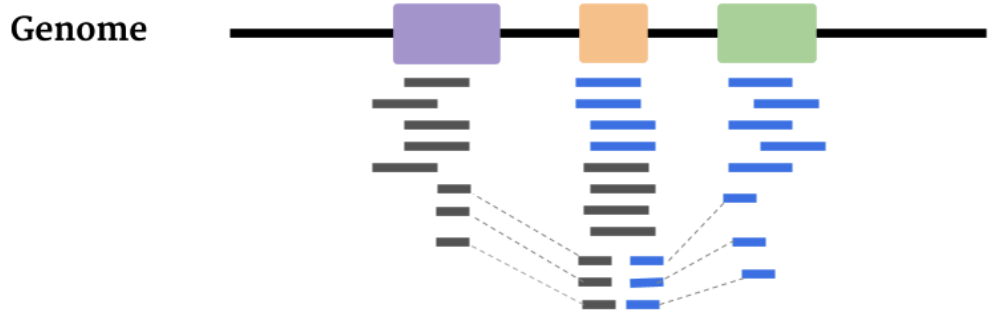
- StringTie
- Cufflinks
- Trinity
- RSEM



# Step 3: Normalize

Software Normalize:

- EDAseq
- cqn
- DESeq2/edgeR
- Ballgown
- derfinder



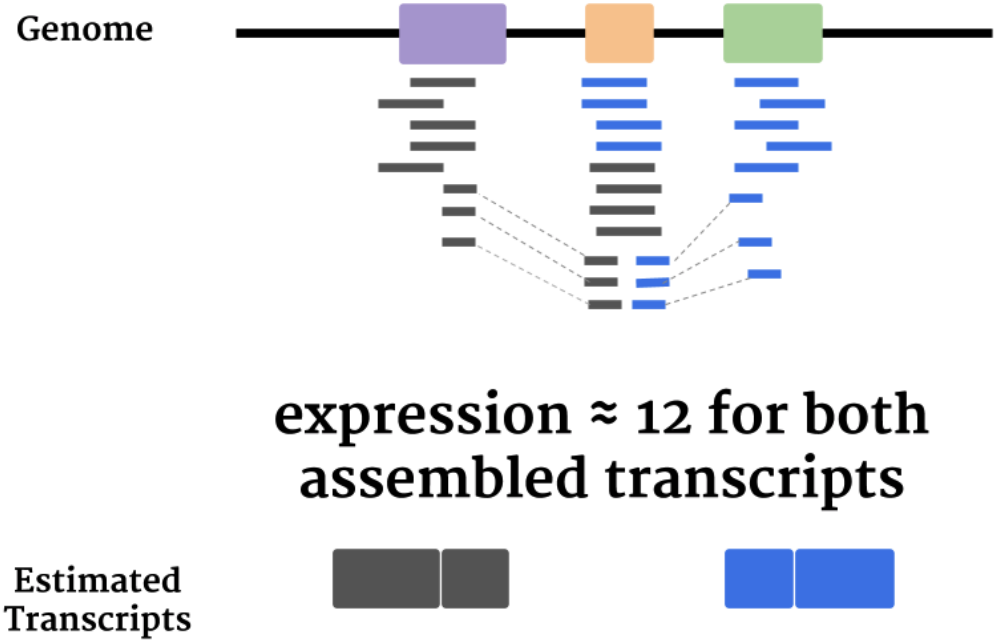

Software Batch Effects:

- sva
- RUVseq

# Step 4: Statistical tests

Software:

- DESeq2/edgeR
- Ballgown
- derfinder



# Step 5: Gene set enrichment

Software:

- goseq
- SeqGSEA