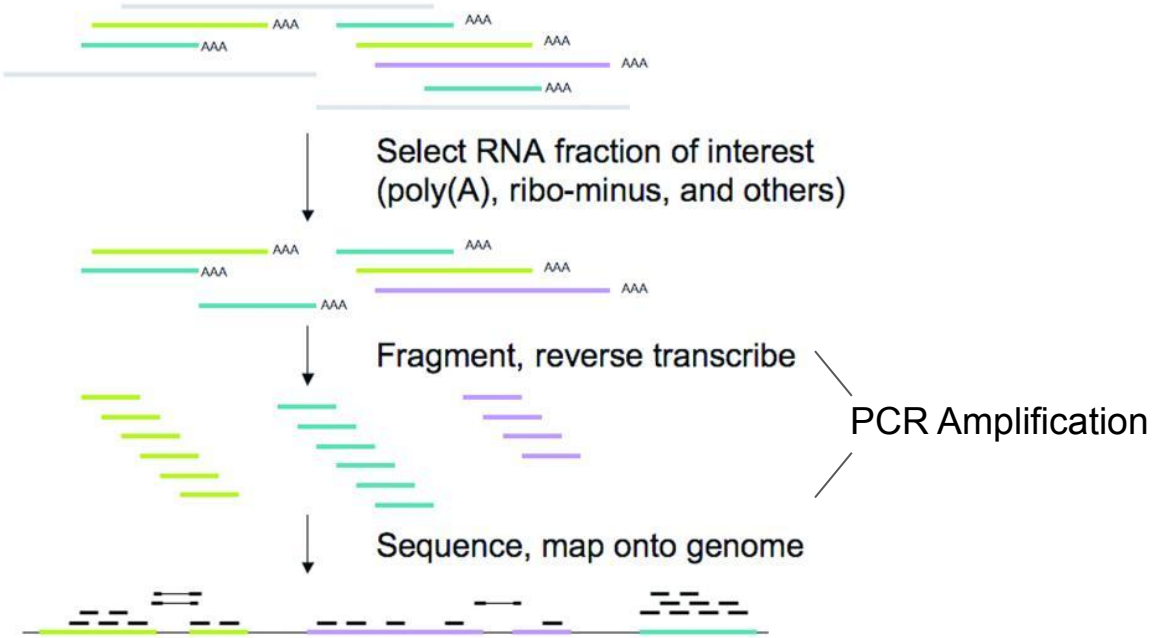
# Introduction to RNA-seq

The CCDL

*There is no optimal pipeline for the variety of different applications and analysis scenarios in which RNA-seq can be used. Scientists plan experiments and adopt different analysis strategies depending on the organism being studied and their research goals.*

- Conesa *et al.* 2016



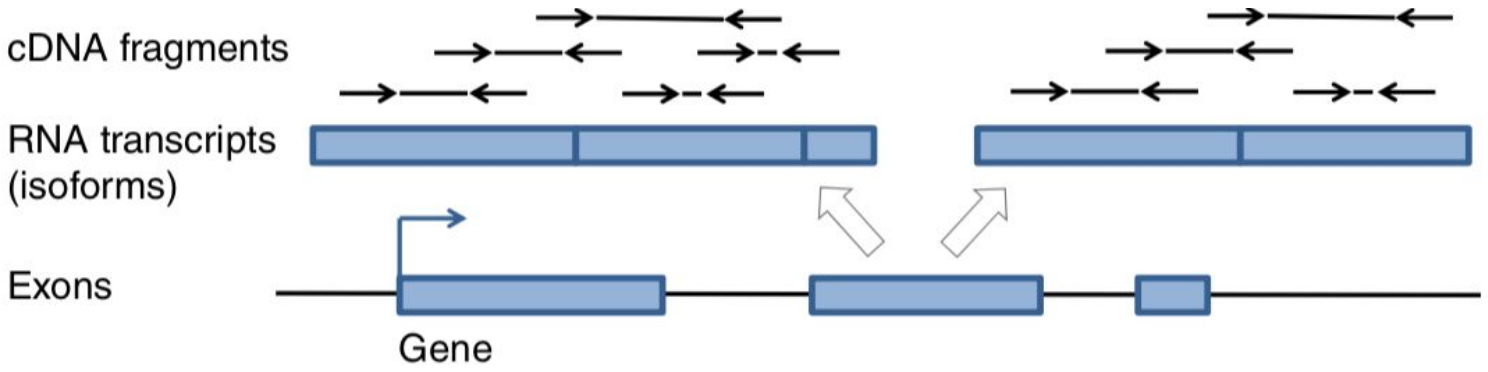

Pepke *et al.* 2009

# Biases to be aware of

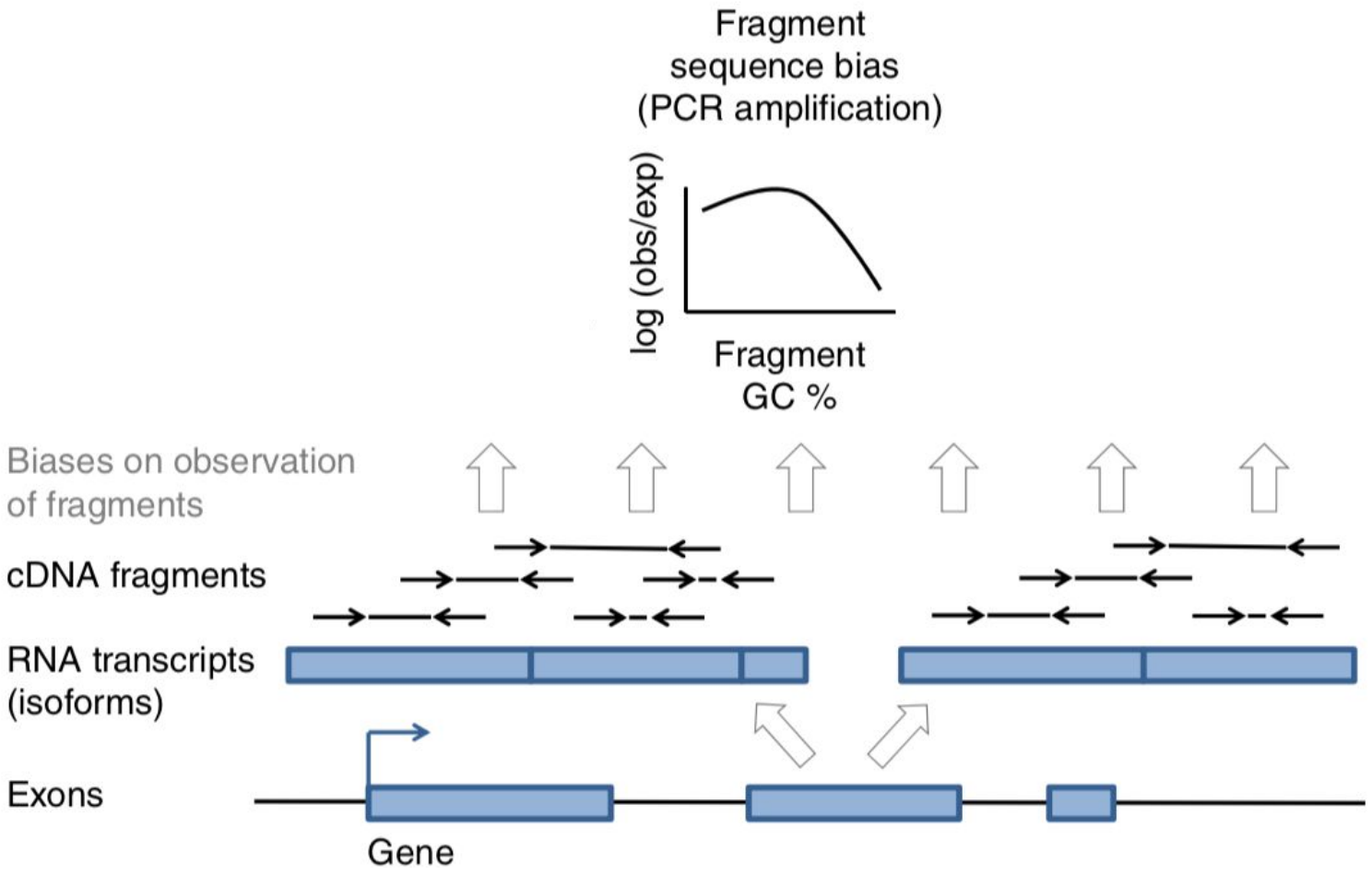
- Library size or sequencing depth - the total number of reads is not always equivalent between samples
- Gene length - longer genes are more likely to be observed

Abundance measures like TPM (Transcripts Per Million) take this into account

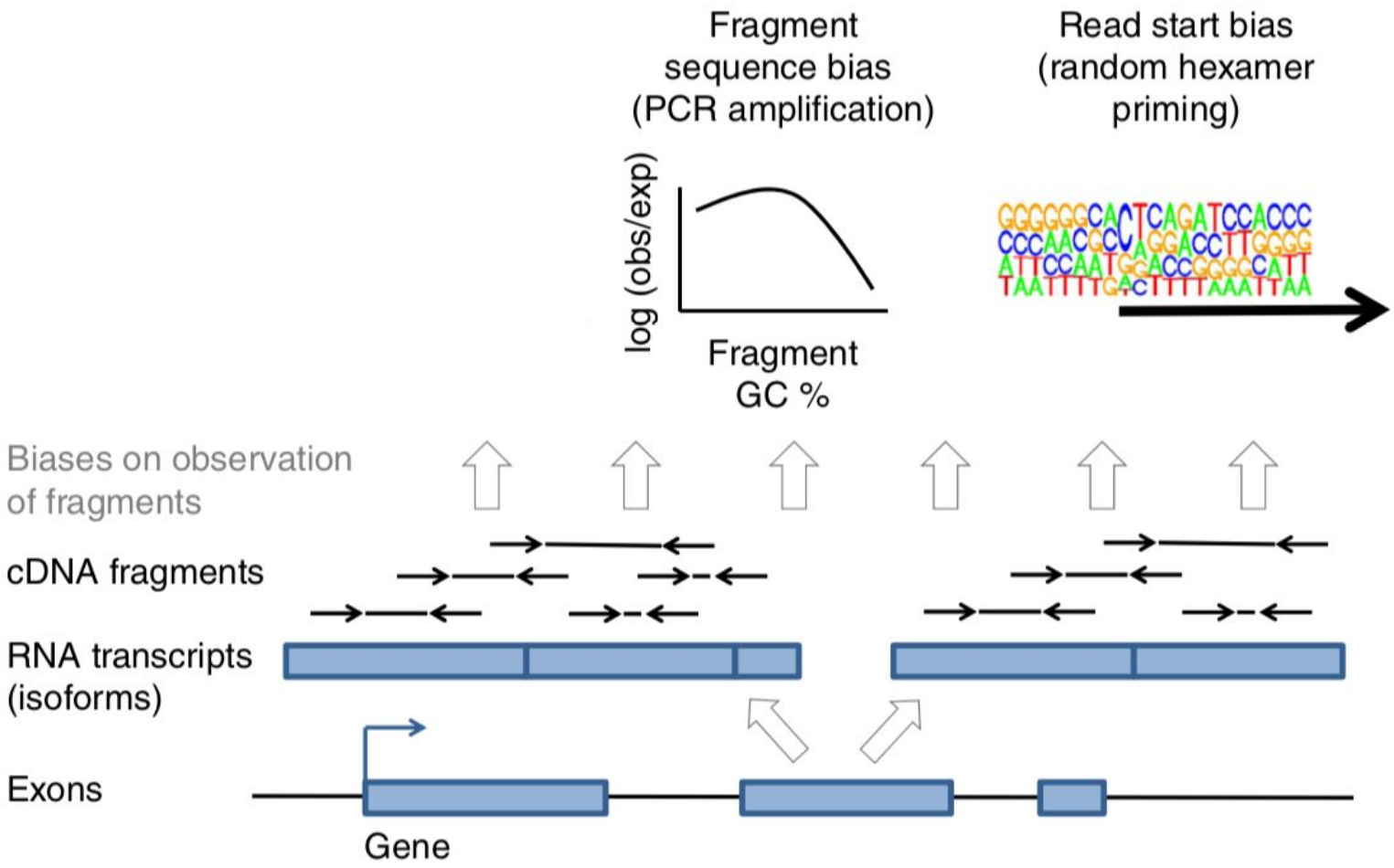
StatsQuest. RPKM, FPKM and TPM, clearly explained.



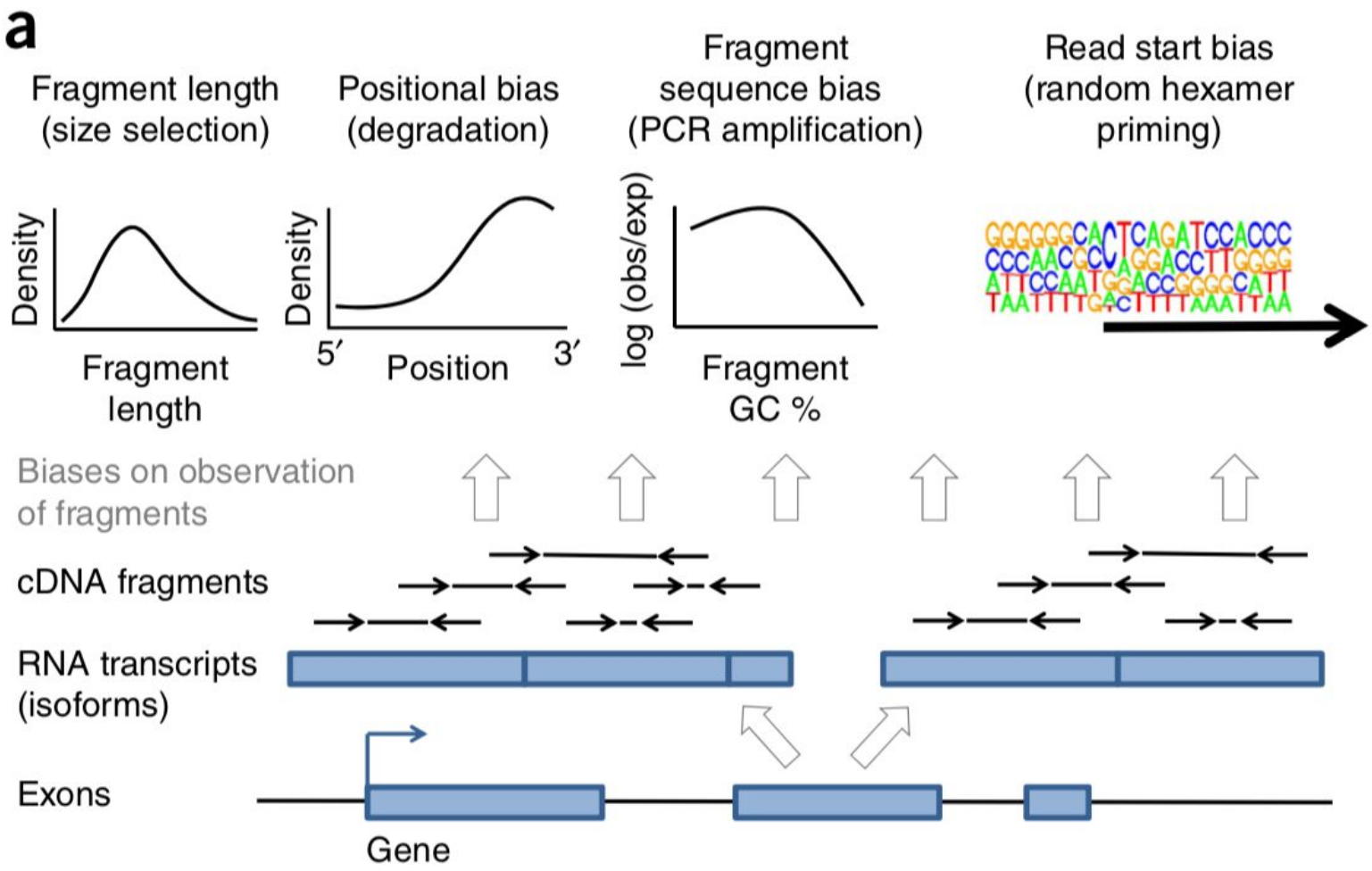

Love *et al.* 2016




Love *et al.* 2016

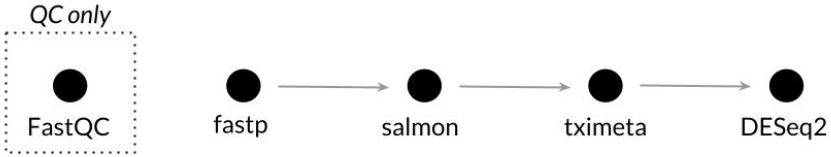



Love *et al.* 2016




Love *et al.* 2016

# Overview of pipeline

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|---|---|---|---|---|
| **TOOL** | **fastp** | **Salmon** | **tximeta** | **DESeq2** |
| **PURPOSE** | Adapter trimming, quality filtering, length filtering | Quantification of transcripts via lightweight mapping to *transcriptome*, GC-bias correction | Import of transcript (tx) abundances and counts from Salmon and summary to the gene-level for more robust statistics, accounts for gene length changes across samples due to differential isoform usage | Library size and composition normalization, transformation for visualization and clustering, testing for differential gene expression |
| **INPUT FILES** | FASTQ | FASTQ that have been preprocessed with fastp, transcriptome to map against | Estimated counts and abundances from Salmon | SummarizedExperiment R object which contains unnormalized counts and length information |

# What you'll learn to do in this module

- Perform quality control checks with FastQC (Andrews)
- Perform FASTQ preprocessing with fastp (Chen *et al.* 2018)
- Quantify transcripts with Salmon (Patro *et al.* 2017)
- Import quantification estimates with tximeta and summarize to the gene level (Love *et al.* 2020; Soneson *et al.* 2015)
- Perform exploratory data analysis with DESeq2 (Love *et al.* 2014)
- Perform differential expression analysis with DESeq2
- Make fancy volcano plots and fancy heatmaps (Blighe *et al.*; Gu 2016)

# Tool-specific tutorials

Getting Started with Salmon

Importing transcript abundance datasets with tximport

Analyzing RNA-seq data with DESeq2

# Links to follow-up information

StatQuest Video: A Gentle Guide to RNA-seq

StatQuest Video: RPKM, FPKM, and TPM

StatQuest Video: DESeq2, part 1, Library Normalization

Hansen et al. Biases in Illumina transcriptome sequencing caused by random hexamer priming. *Nucleic Acid Research*. 2010.

Michigan State University Research Technology Support Facility “FastQC Tutorial & FAQ”