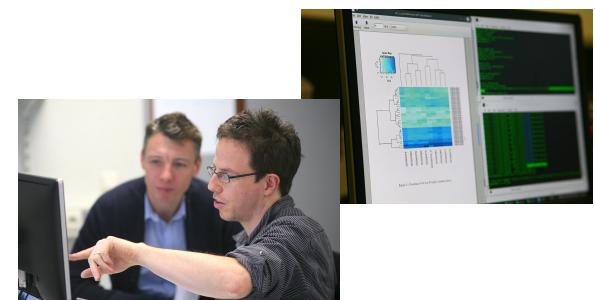
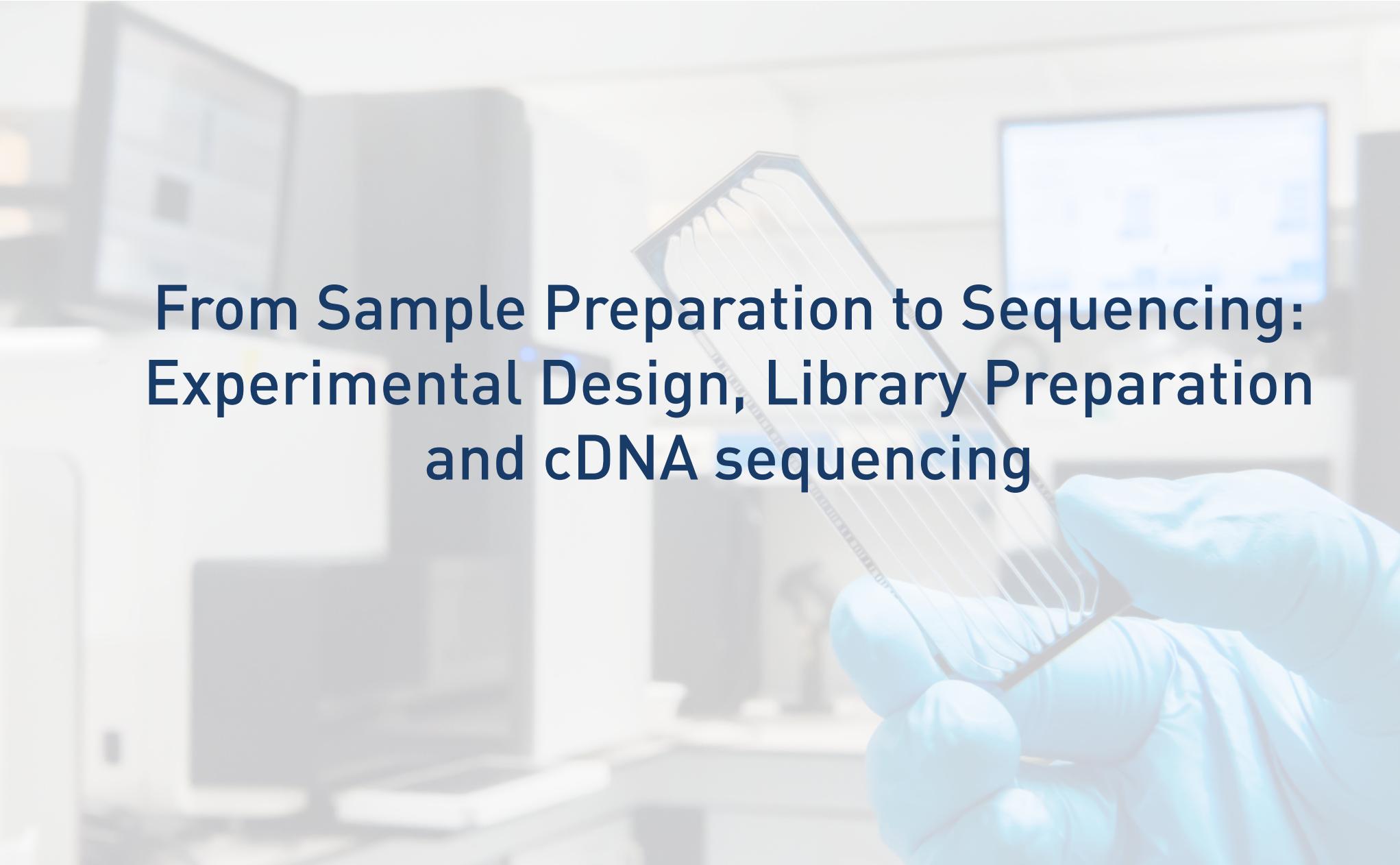




**G GENOMICS
CORELEUVEN**





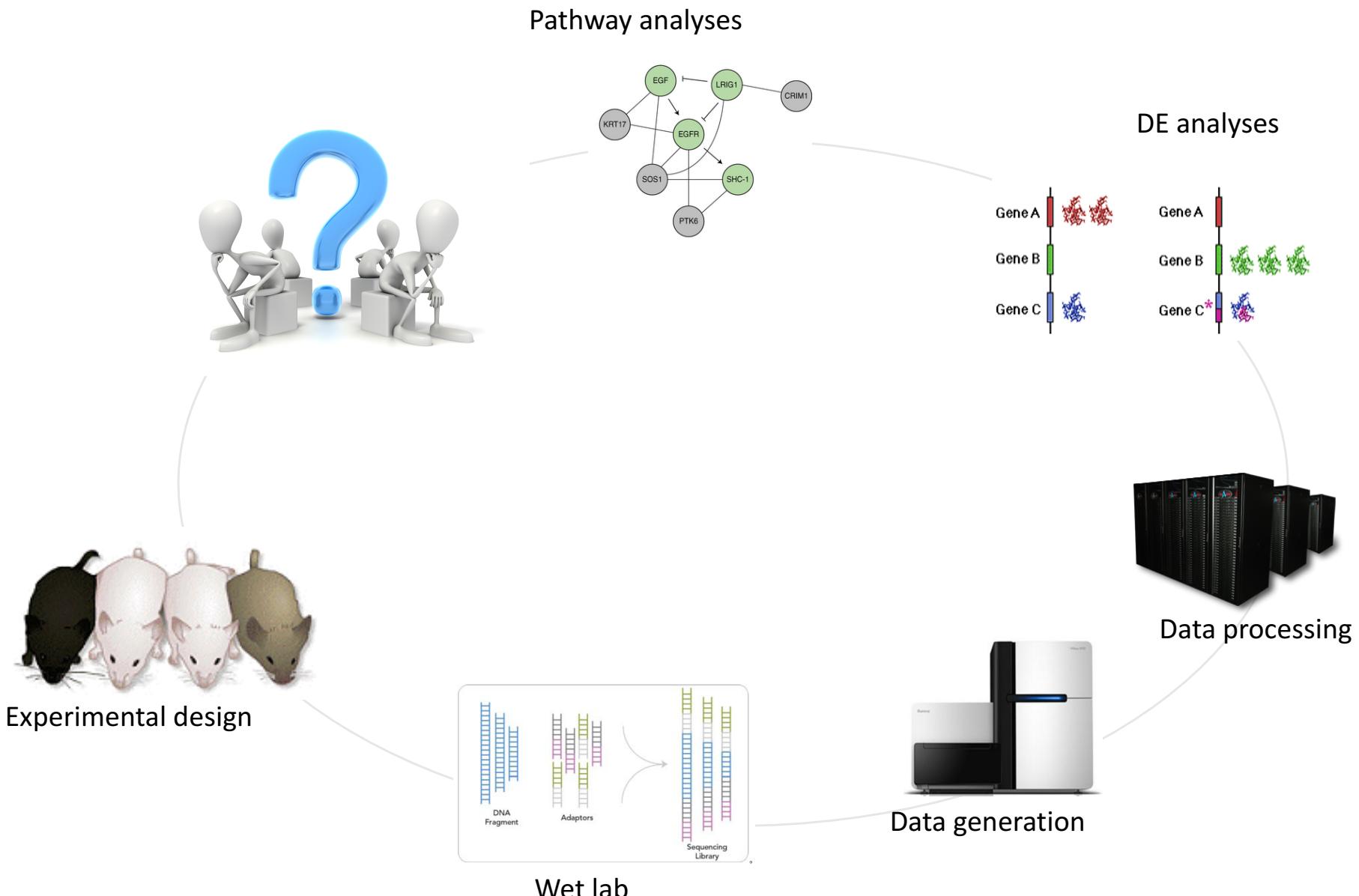


From Sample Preparation to Sequencing: Experimental Design, Library Preparation and cDNA sequencing



Genomics Core
RNA Seq workshop 12/01/2018
Céline Helsmoortel

RNA-Seq - Workflow



Experimental Design

- What biological question am I trying to answer?
- What types of samples ?
- How much sequence do I need?
- Length of read?
- Platform?
- Single-end or paired-end?
- Barcoding?
- Pooling?
- Biological replicates: how many?
- Technical replicates: how many?
- Protocol considerations?

RNA-Seq Applications

- Gene expression profiling
- Detection of
 - isoform / splice variants
 - novel transcripts
 - gene fusions
- Identification of Transcription Start / End Sites (TTS & TES)
- Identification & quantification
 - rare transcripts
 - common transcripts
- Derive strand information
- ...

RNA-Seq Applications

- Detection of
 - isoform / splice variants
 - novel transcripts
 - gene fusions
- Identification & quantification
 - rare transcripts
 - common transcripts
- Gene expression profiling

Sequence Whole Transcript

- Identification of Transcription Start / End Sites (TTS & TES)

Sequence Specific Position

RNA-Seq Applications

- Detection of
 - isoform / splice variants
 - novel transcripts
 - gene fusions
- Identification & quantification
 - rare transcripts
 - common transcripts
- Gene expression profiling

Sequence Whole Transcript

- Identification of Transcription Start / End Sites (TTS & TES)

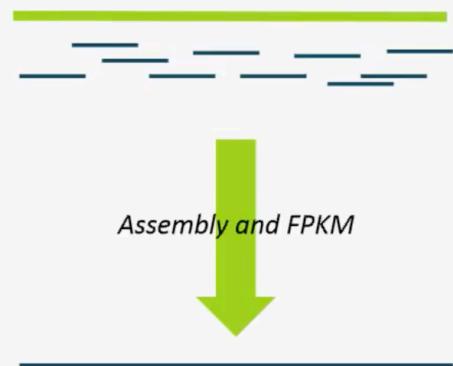
Sequence Specific Position

2 major types of RNA-Seq

Whole Transcriptome Sequencing

mRNA or total RNA-Seq

- *de novo* assembly of transcripts
- Isoform detection
- Expression profiling (RPKM needed)



Many reads for 1 transcript are needed

Expression Profiling Sequencing

mRNA

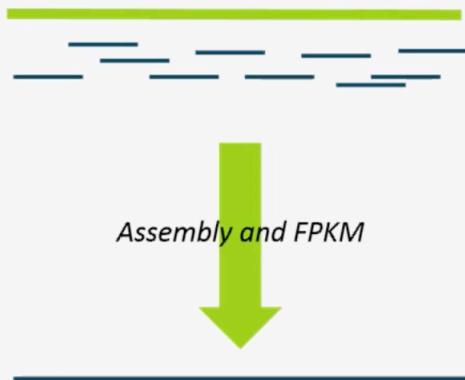
- Counting the number of sequences
- Sequencing one fragment per transcript



Only 1 read for 1 transcript is enough
If we can sequence the same positions of all different transcripts

Which RNA-Seq type to choose?

Whole Transcriptome Sequencing

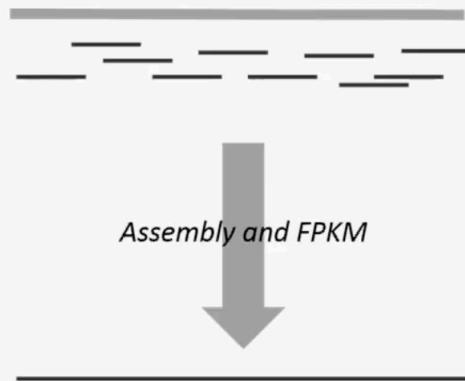


Expression Profiling Sequencing



Which RNA-Seq type to choose?

Whole Transcriptome Sequencing



Multiple fragments per transcript

- **Waste of sequence reads** (intended for entire regions of transcripts)
- **Consumption of computational resources** (Assembly; RPKM, FPKM)

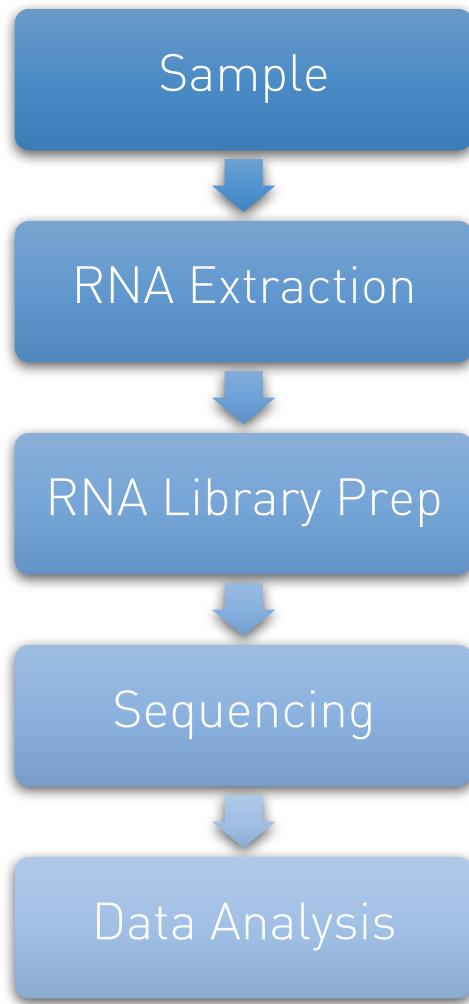
Expression Profiling Sequencing



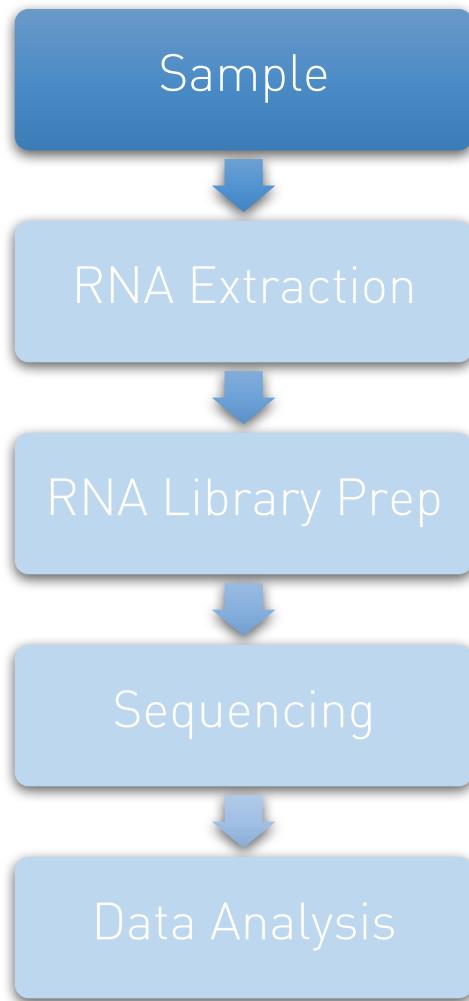
One fragment per transcript

- **Only 1/10 of sequence reads** needed
- Higher number of **multiplexing** possible
- Significantly **less amount of computational resources** required

RNA-Seq experiment

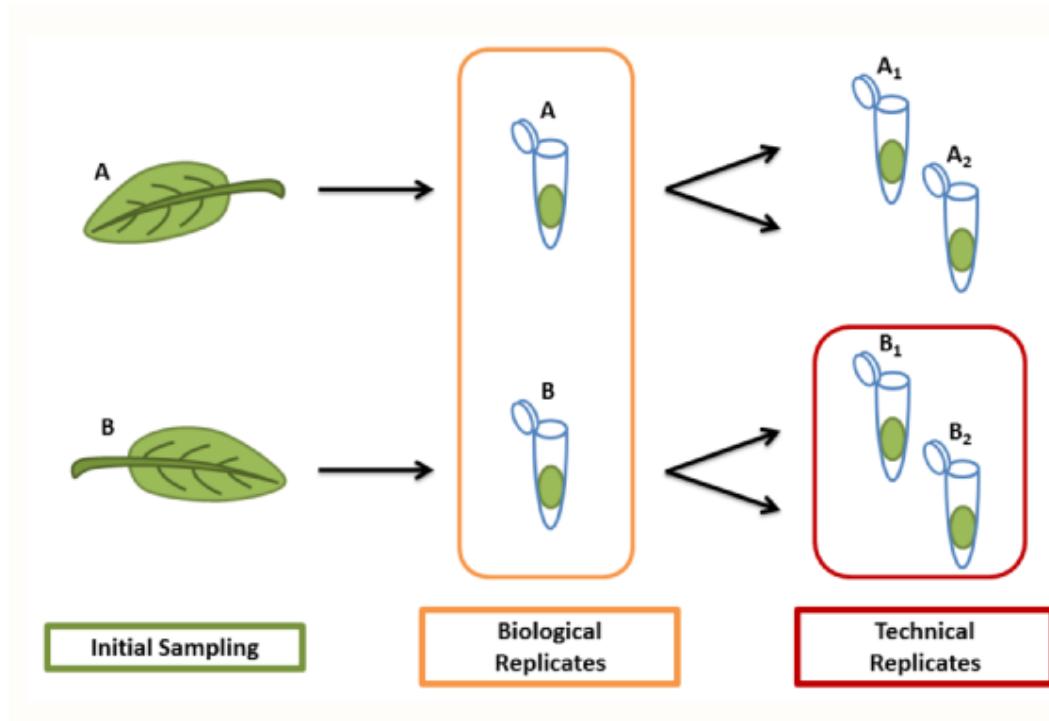


RNA-Seq experiment



Replicates

- #Samples - #Replicates?
 - biological
 - technical



Biological Coefficient of Variation

Cell Line



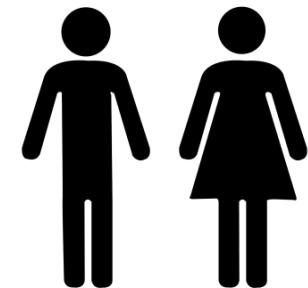
Experimental:
0.05 - 0.09
Example: 0.1

Inbred
Model Organisms



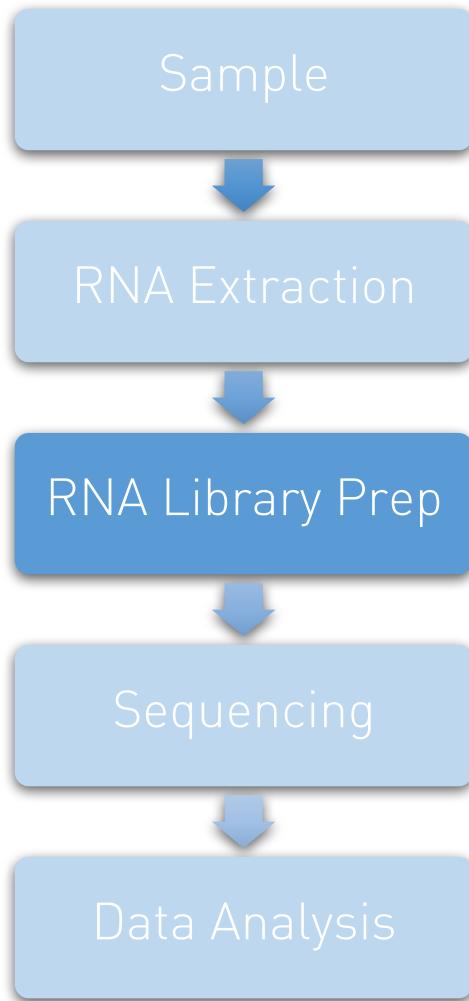
Experimental:
0.13-0.31
Example: 0.2

Population

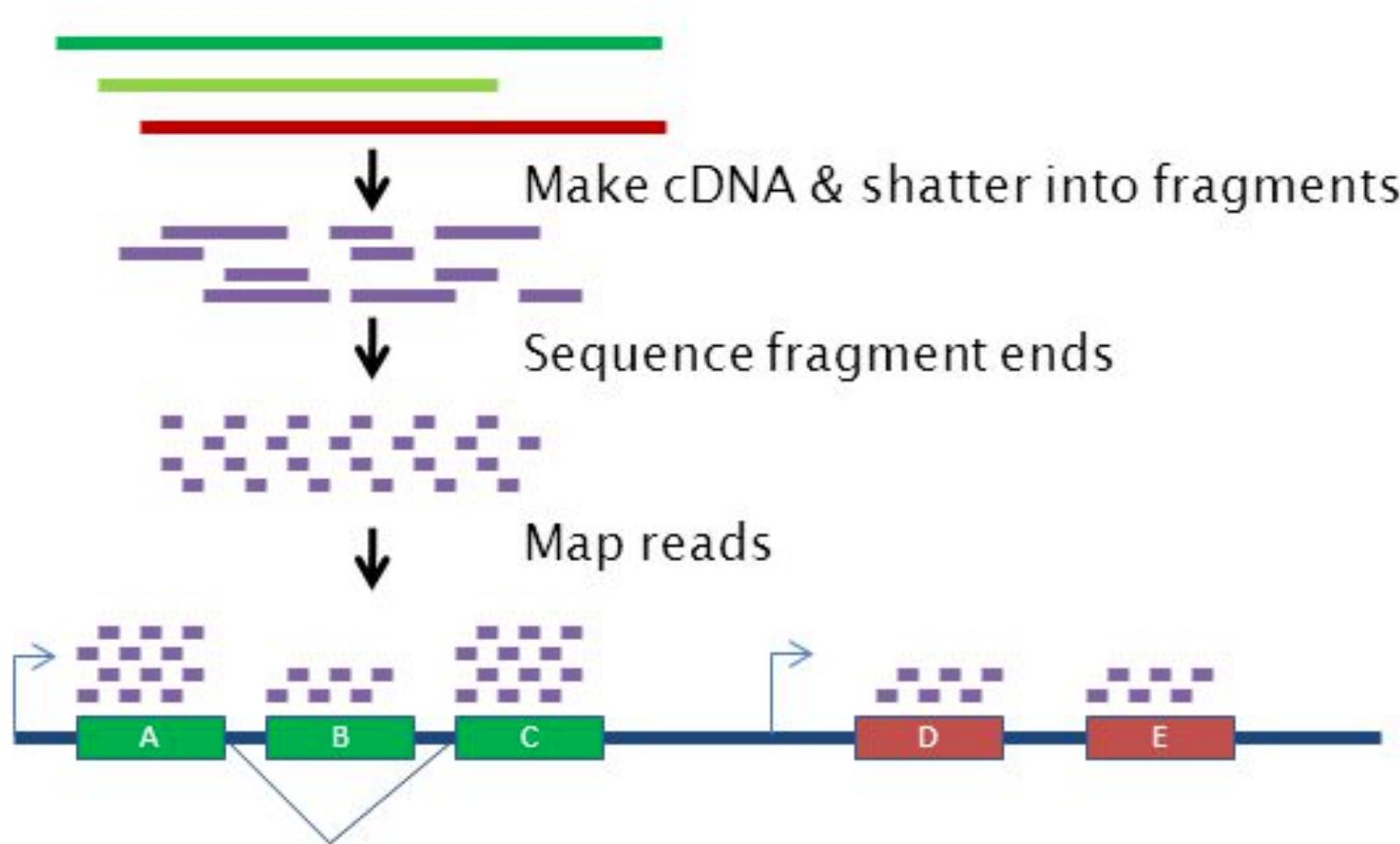


Experimental:
0.24-0.79
Example: 0.5

RNA-Seq experiment



RNA-Seq



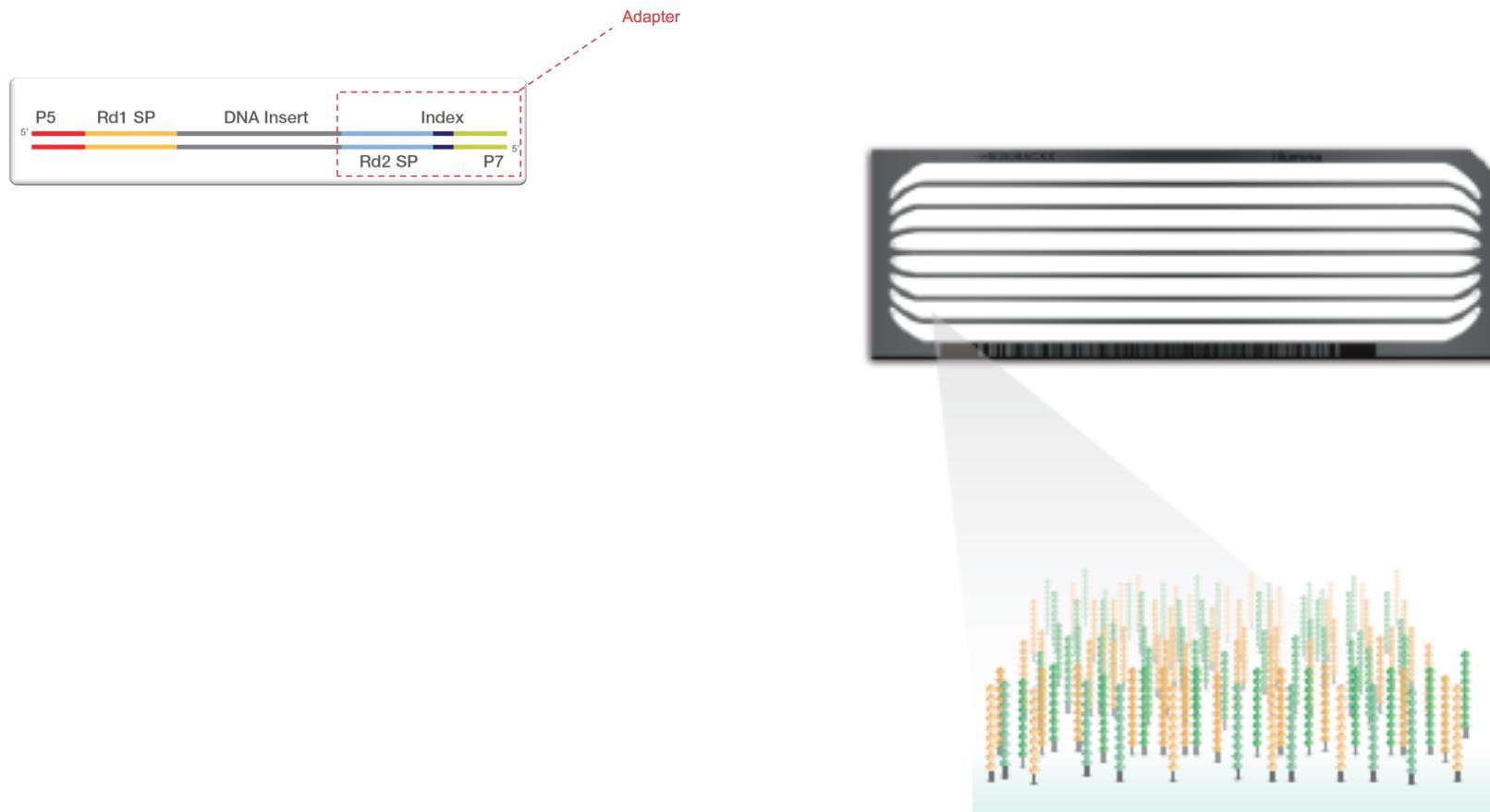
Illumina sequencing library



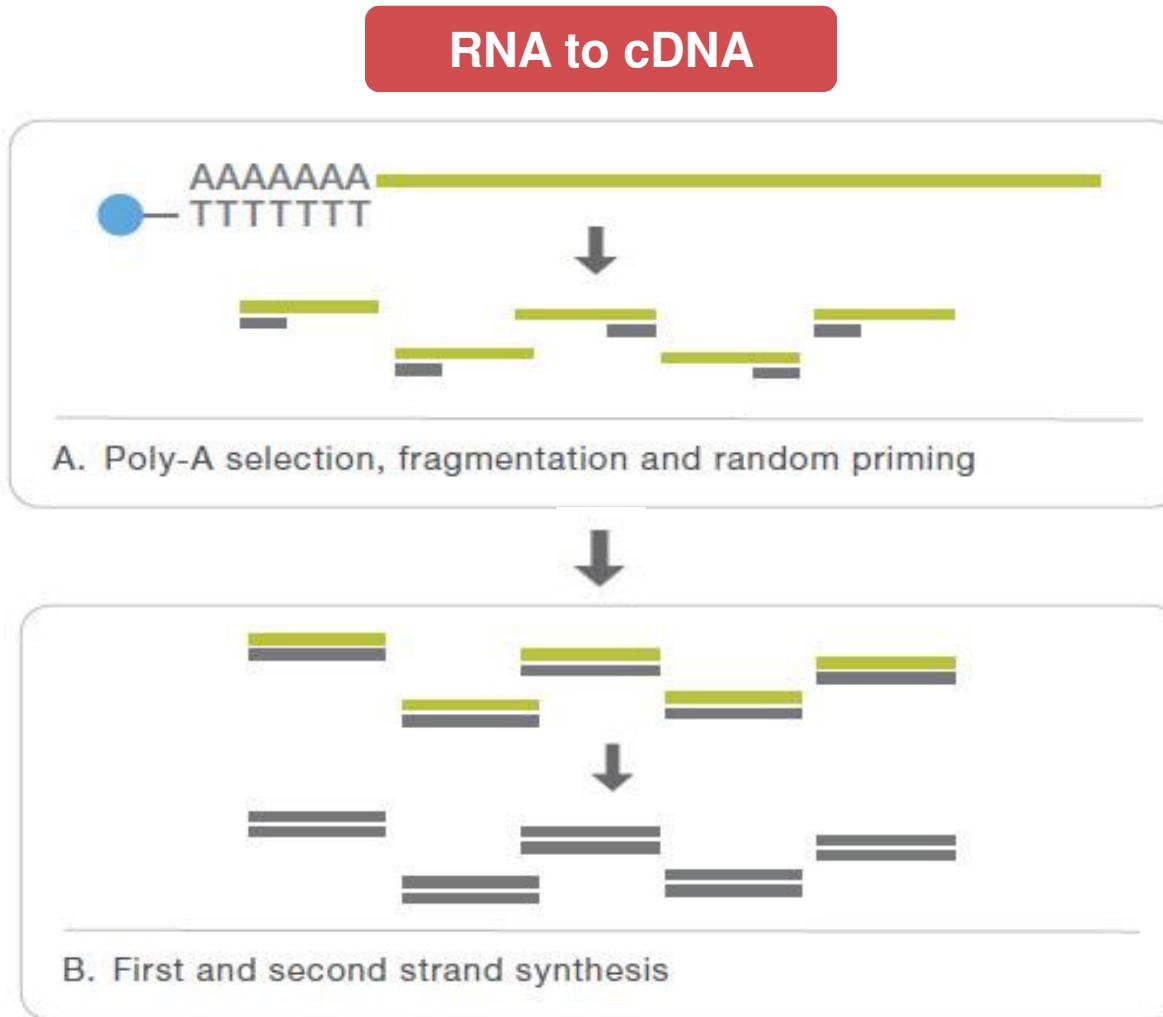
Indexing



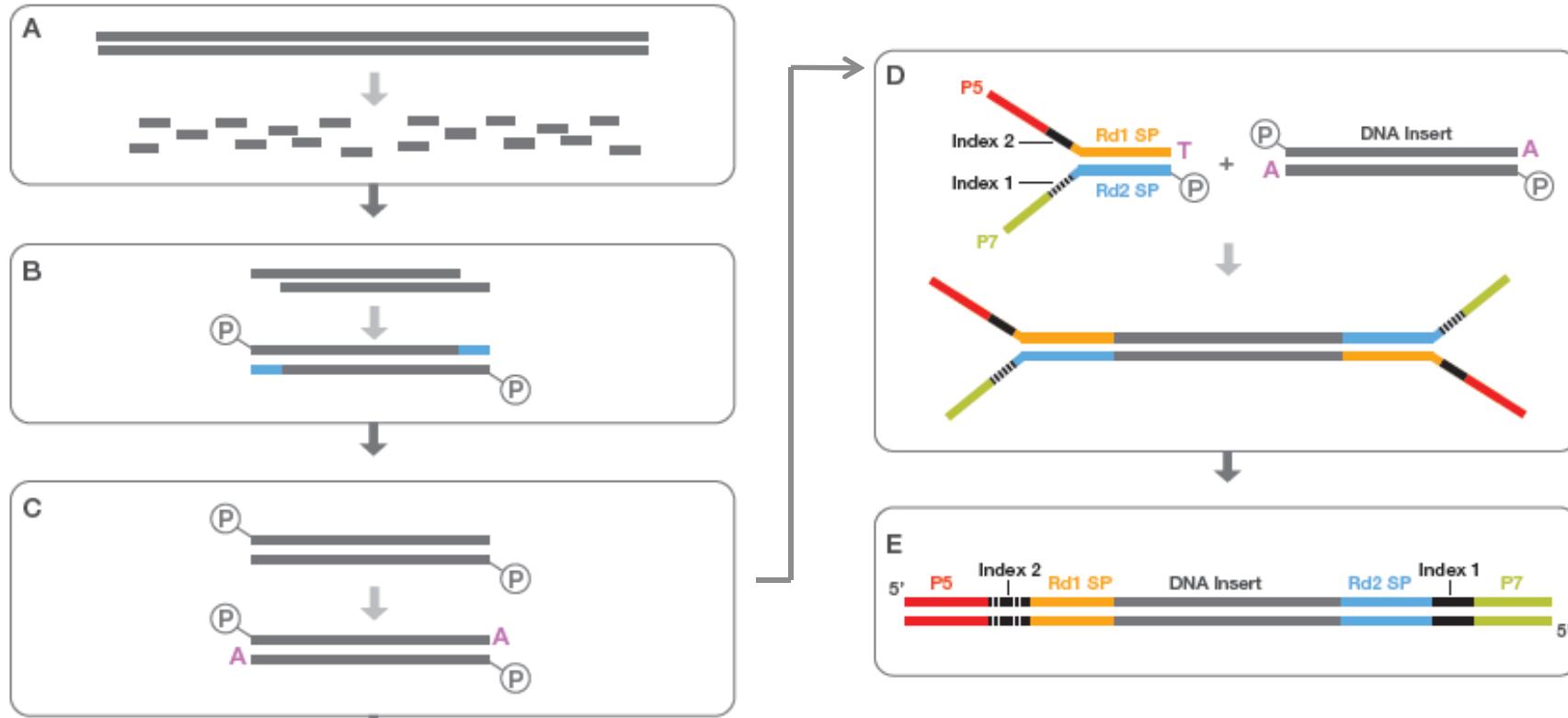
Binding to the flowcell



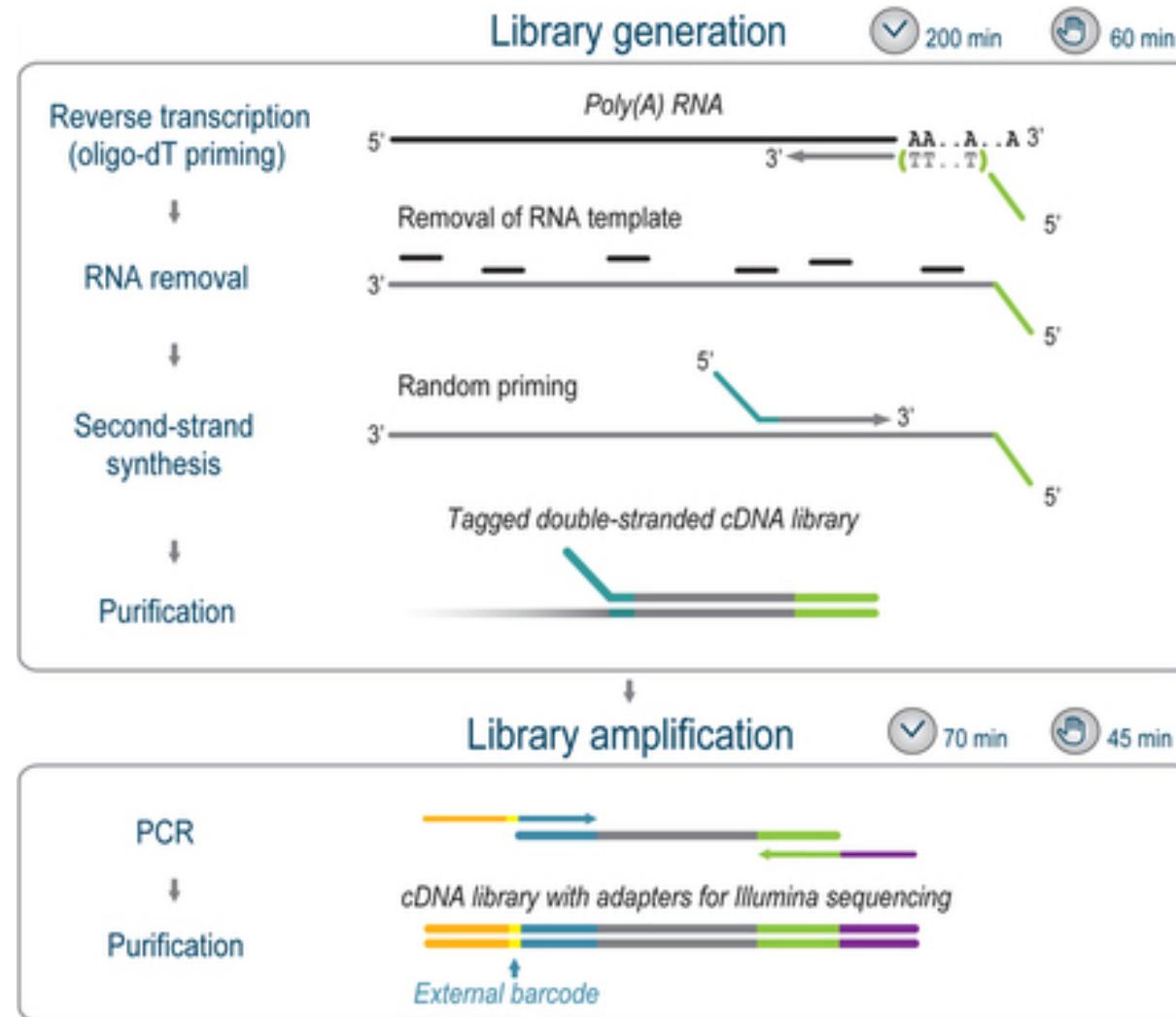
Illumina TruSeq stranded mRNA prep



Illumina TruSeq stranded mRNA prep



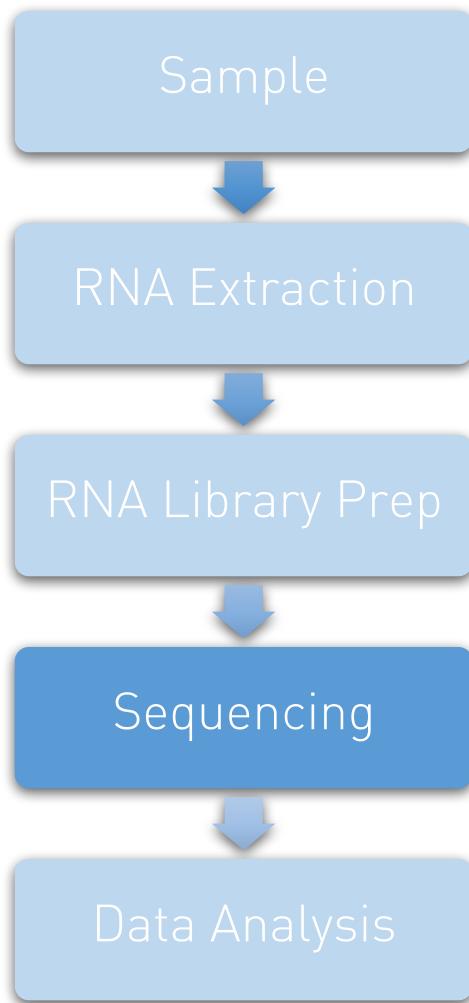
LexoGen QuantSeq prep



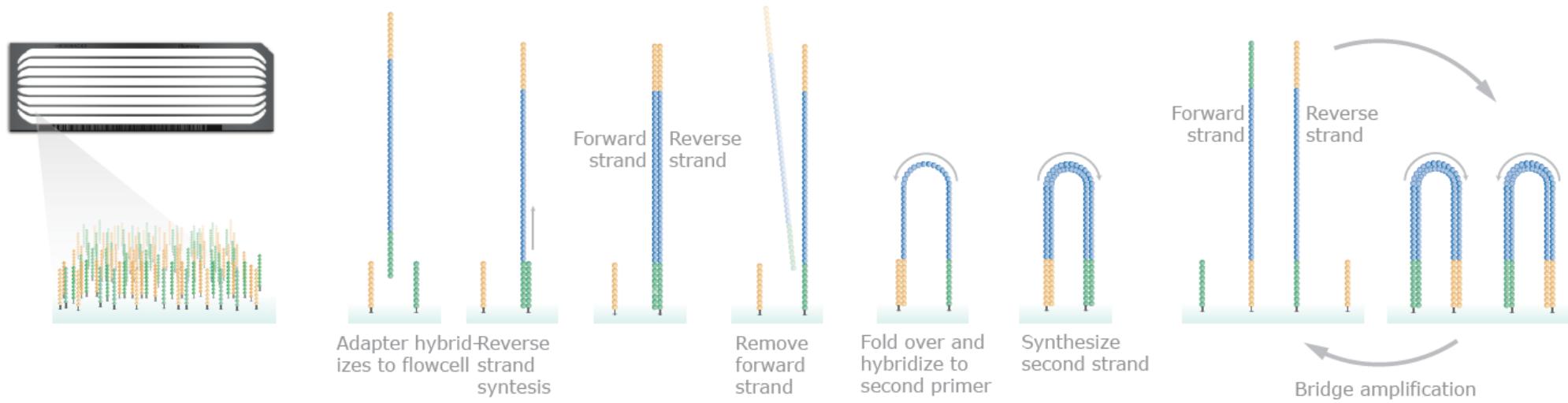
Library preparation

	Illumina TruSeq Stranded mRNA	Lexogen QuantSeq
Applications	Differential Expression (DE) RNA-seq SNP calling Differential Splicing Gene Fusion	Differential Expression (DE)
Number of Replicates (for DE)	Depends of the BCV	Depends of the BCV
Sequencing Depth (for DE)	10-20M	1-5M
Input volume	0.1 - 4µg total RNA	50ng - 4µg total RNA
Input quality	High, optimal all samples have the same quality (RIN value)	
Number of Samples on a Lane (for DE)	10-16	96

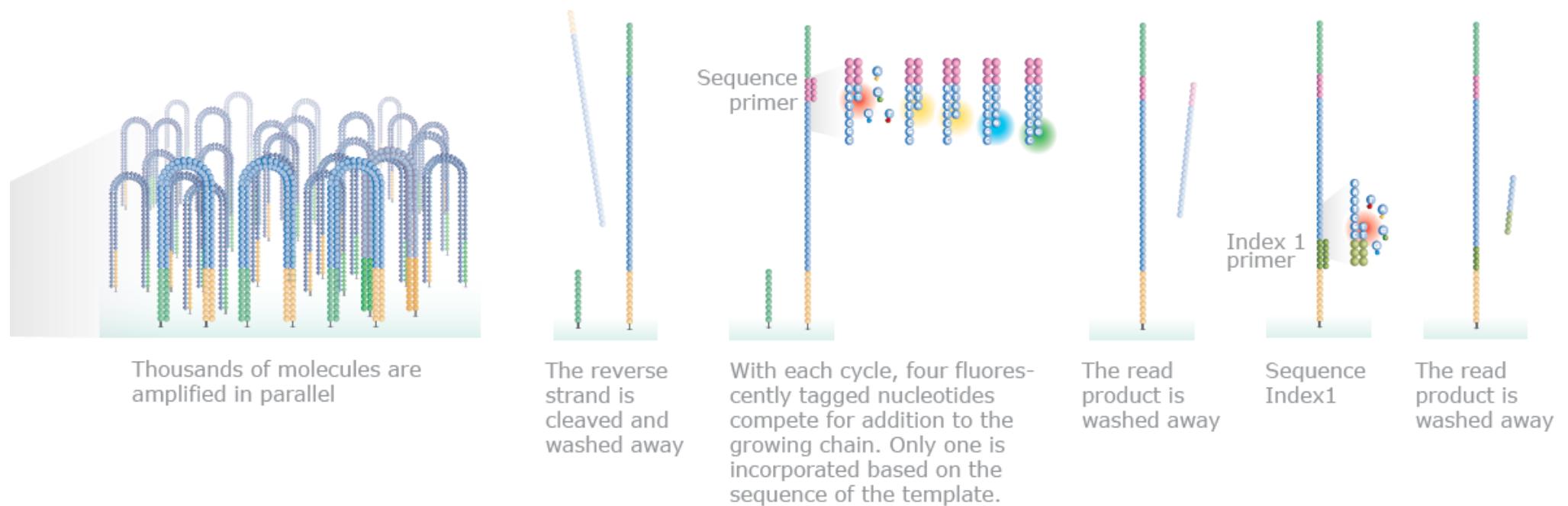
RNA-Seq experiment



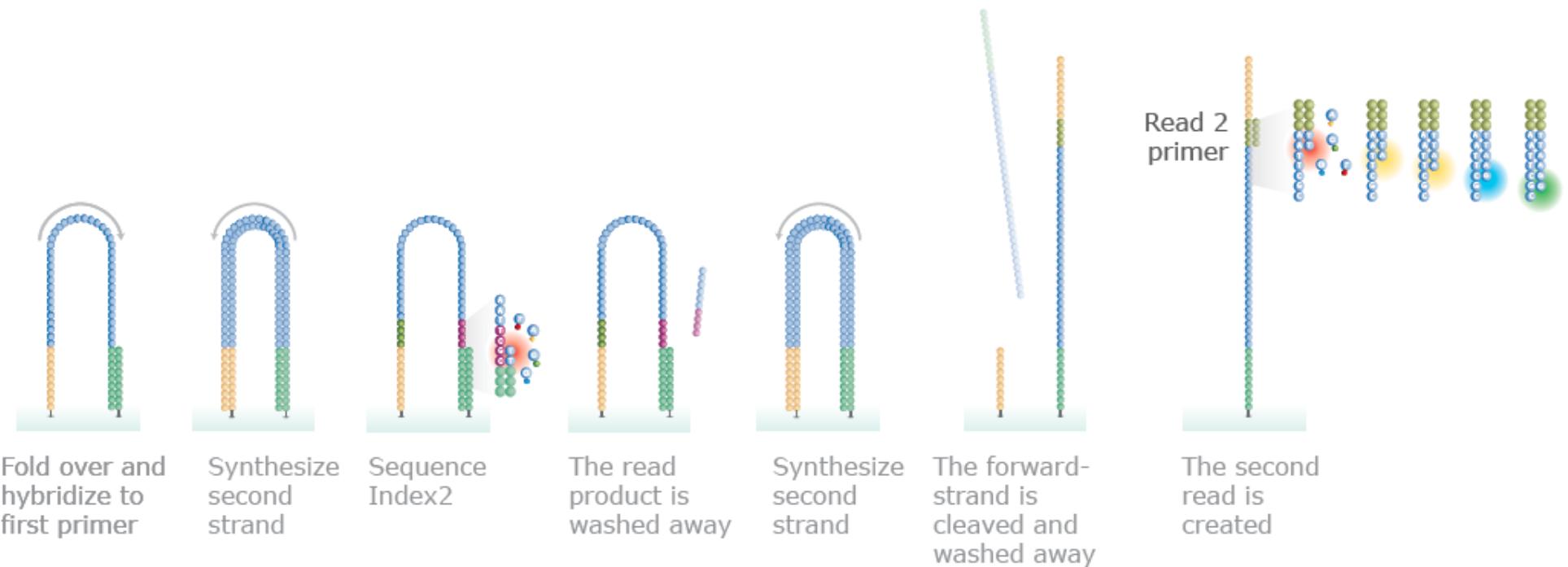
Illumina Sequencing



Illumina Sequencing



Illumina Sequencing



Sequencing depth

Depends on the application and used protocol

- Differential Expression (using Illumina TruSeq RNA stranded) 10-20M
- Differential Expression (using Lexogen Quantseq) 1-5M
- SNP calling >20M
- Differential Splicing >20M
- Gene Fusion >50M



Questions?



Genomics Core
RNA Seq workshop 12/01/2018
Céline Helsmoortel