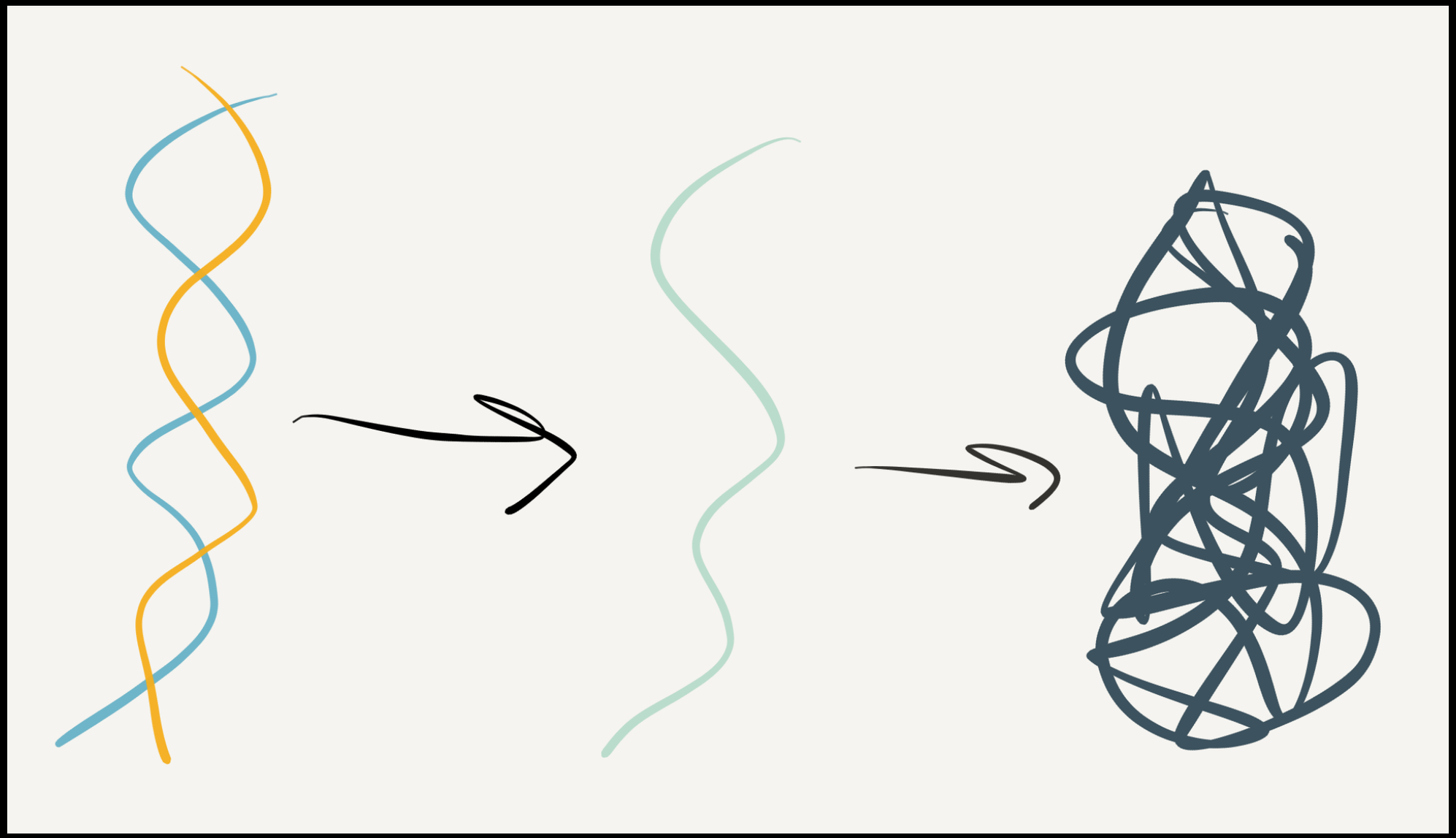
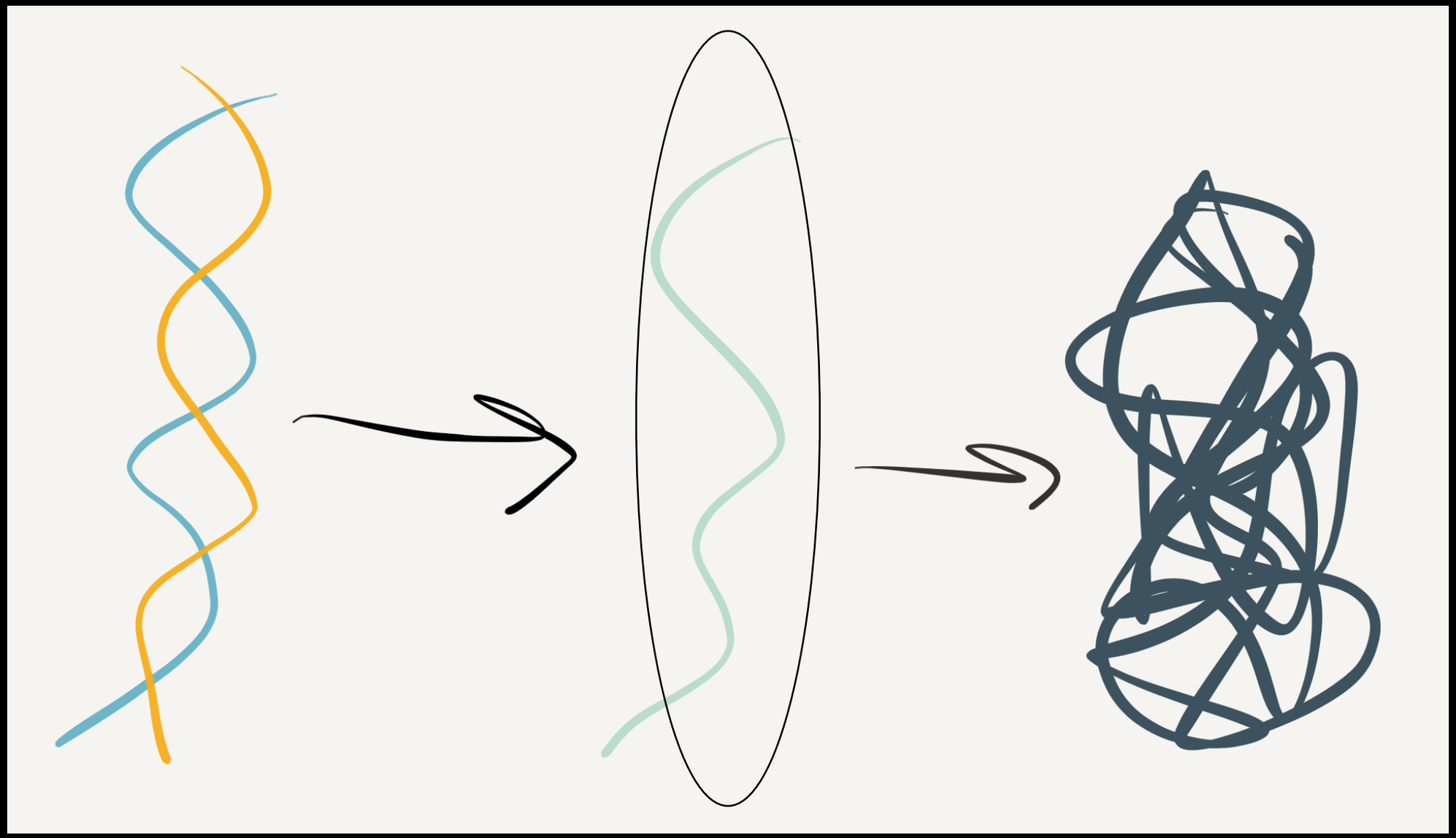
INTRO TO RNASEQ AND QUALITY CONTROL/TRIMMING

RNASEQ

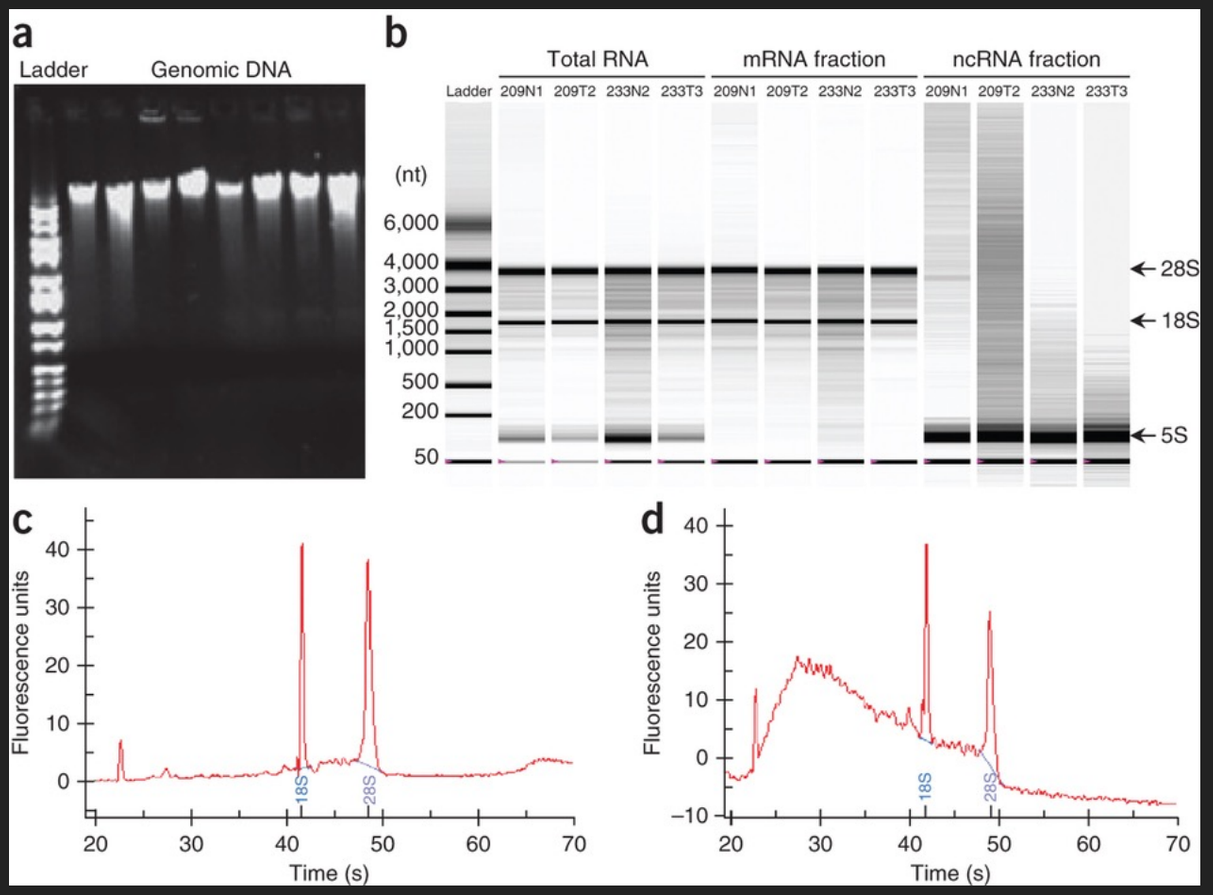


RNASEQ



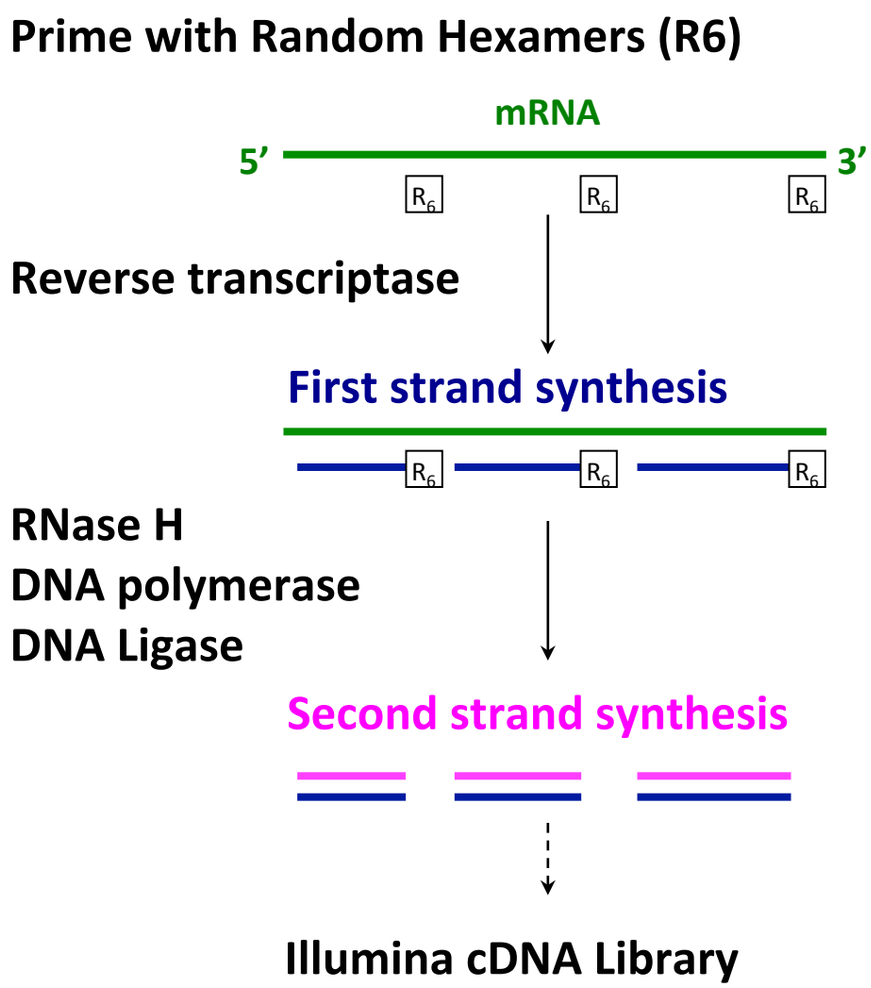
RNA EXTRACTION METHODS:

- Dependent on starting material: Tissue type, Amount
- End product: mRNA or total RNA?
- Total RNA: Trizol vs. Columns (Promega, RNeasy, ect.)
- mRNA: direct extraction or isolation from total RNA



http://www.nature.com/nprot/journal/v8/n11/full/nprot.2013.141.html

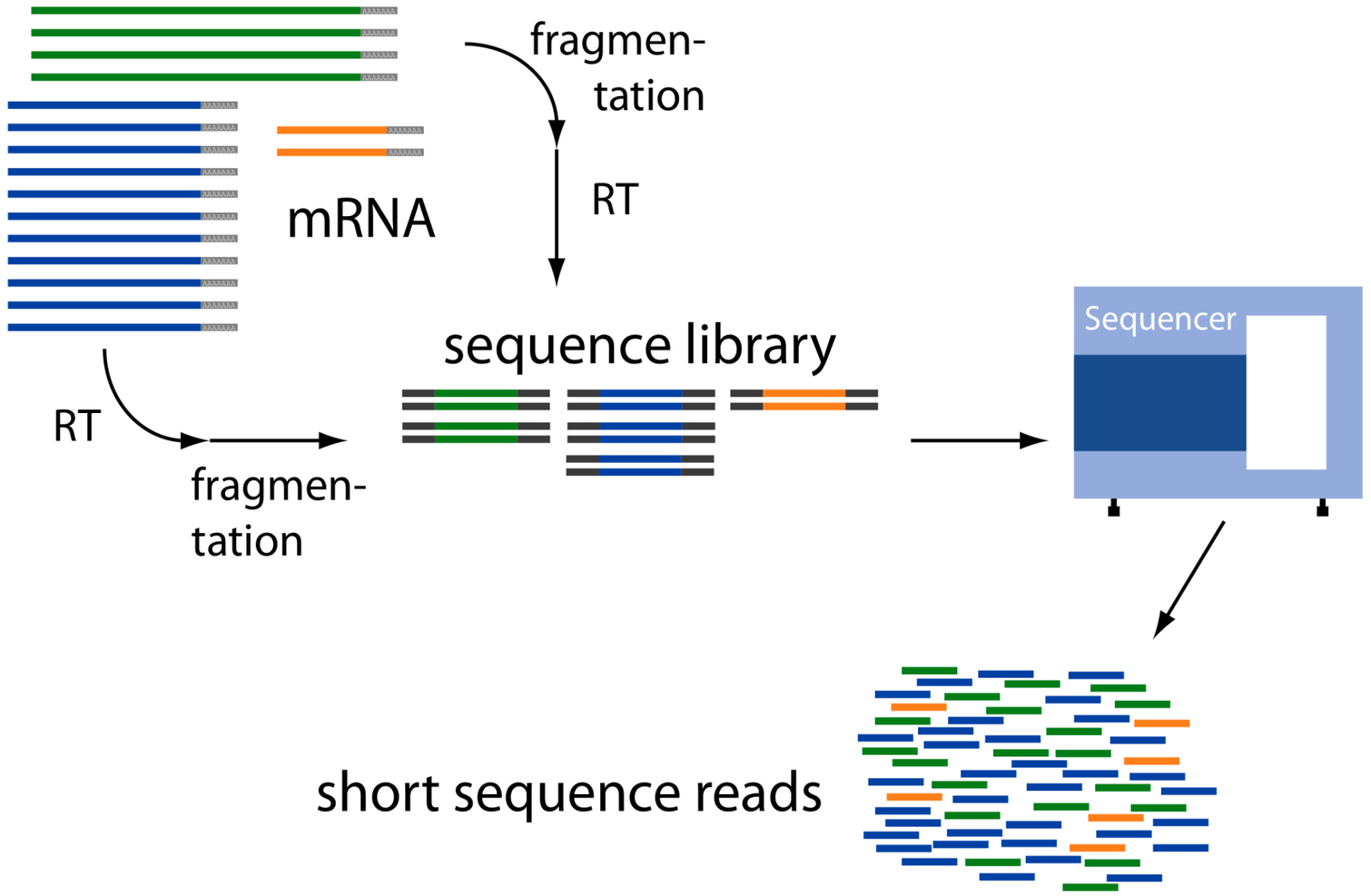
RNA-Seq: How do we make cDNA?



Slide courtesy of Joshua Levin, Broad Institute.

Slide from: https://github.com/trinityrnaseq/RNASeq_Trinity_Tuxedo_Workshop/blob/master/docs/rnaseq_workshop_slides.pdf

Overview of RNA-Seq



From: http://www2.fml.tuebingen.mpg.de/raetsch/members/research/transcriptomics.html

Slide from: https://github.com/trinityrnaseq/RNASeq_Trinity_Tuxedo_Workshop/blob/master/docs/rnaseq_workshop_slides.pdf

Common Data Formats for RNA-Seq

FASTA format:

```
>61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
```

FASTQ format:

```
@61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
+
ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBC?CCCCCCCCC@@CACCCCCA
```

Read

Quality values

AsciiEncodedQual(x) = -10 * log10(Pwrong(x)) + 33

AsciiEncodedQual (‘C’) = **64**

So, Pwrong(‘C’) = 10^((**64**-33/ (-10)) = 10^-3.4 = **0.0004**

Slide from: https://github.com/trinityrnaseq/RNASeq_Trinity_Tuxedo_Workshop/blob/master/docs/rnaseq_workshop_slides.pdf

Paired-end Sequences

Two FastQ files, read name indicates
left (/1) or right (/2) read of paired-end

```
@61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
+
ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBC?CCCCCCCCC@@CACCCCCA
```

```
@61DFRAAXX100204:1:100:10494:3070/2
CTCAAATGGTTAATTCTCAGGCTGCAAATATTCGTTCAGGATGGAAGAACA
+
C<CCCCCCCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBCCCC
```

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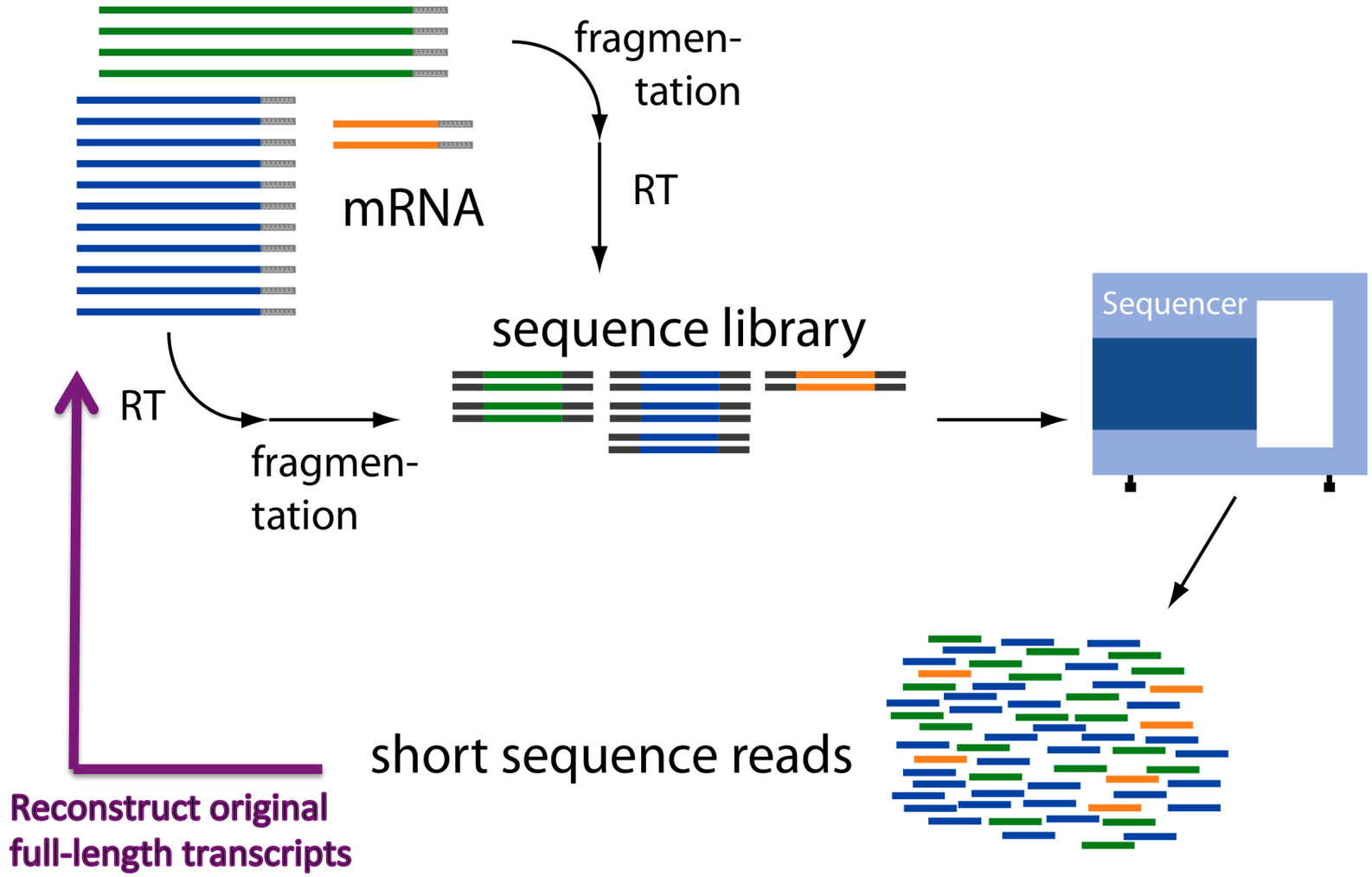
Overview of RNA-Seq



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COMMON RNASEQ APPLICATIONS

- Raw genetic data
  - Population genomics
  - Phylogenomics
- Experimental biology
  - Differential expression analysis

Journal of Ornithology (2018) 159:599–629
https://doi.org/10.1007/s10336-018-1532-5

REVIEW

Avian transcriptomics: opportunities and challenges

Elinor Jax[1,2] · Michael Wink[3] · Robert H. S. Kraus[1,2]

Received: 29 September 2017 / Revised: 27 December 2017 / Accepted: 15 January 2018 / Published online: 5 February 2018

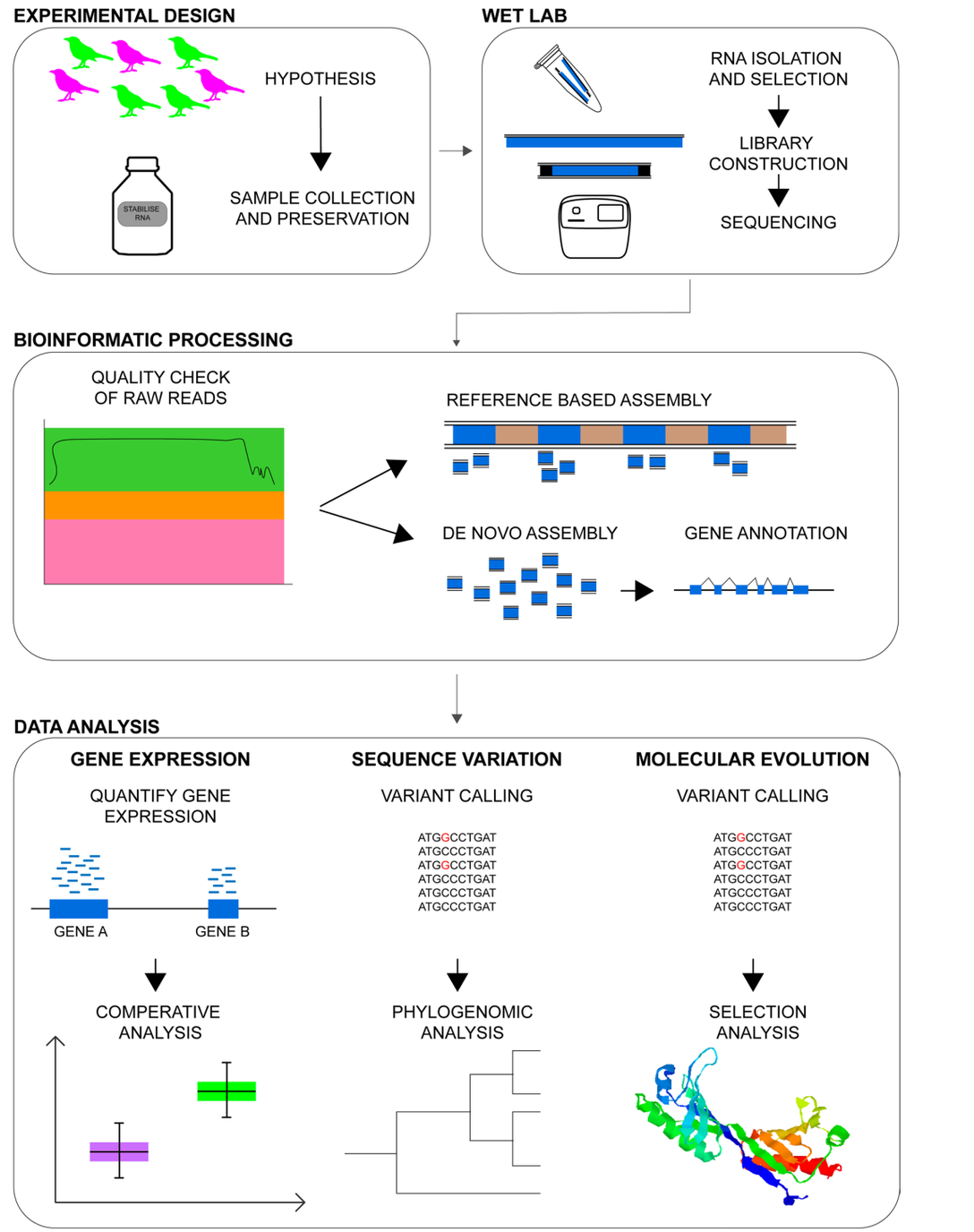

Fig. 2 Overview of a typical RNA sequencing workflow. The individual elements of the workflow are detailed in "Planning an RNA-seq study: a quick guide". Protein structure by Richard Wheeler (Zephyris), licensed under Creative Commons 3.0, Wikimedia Commons

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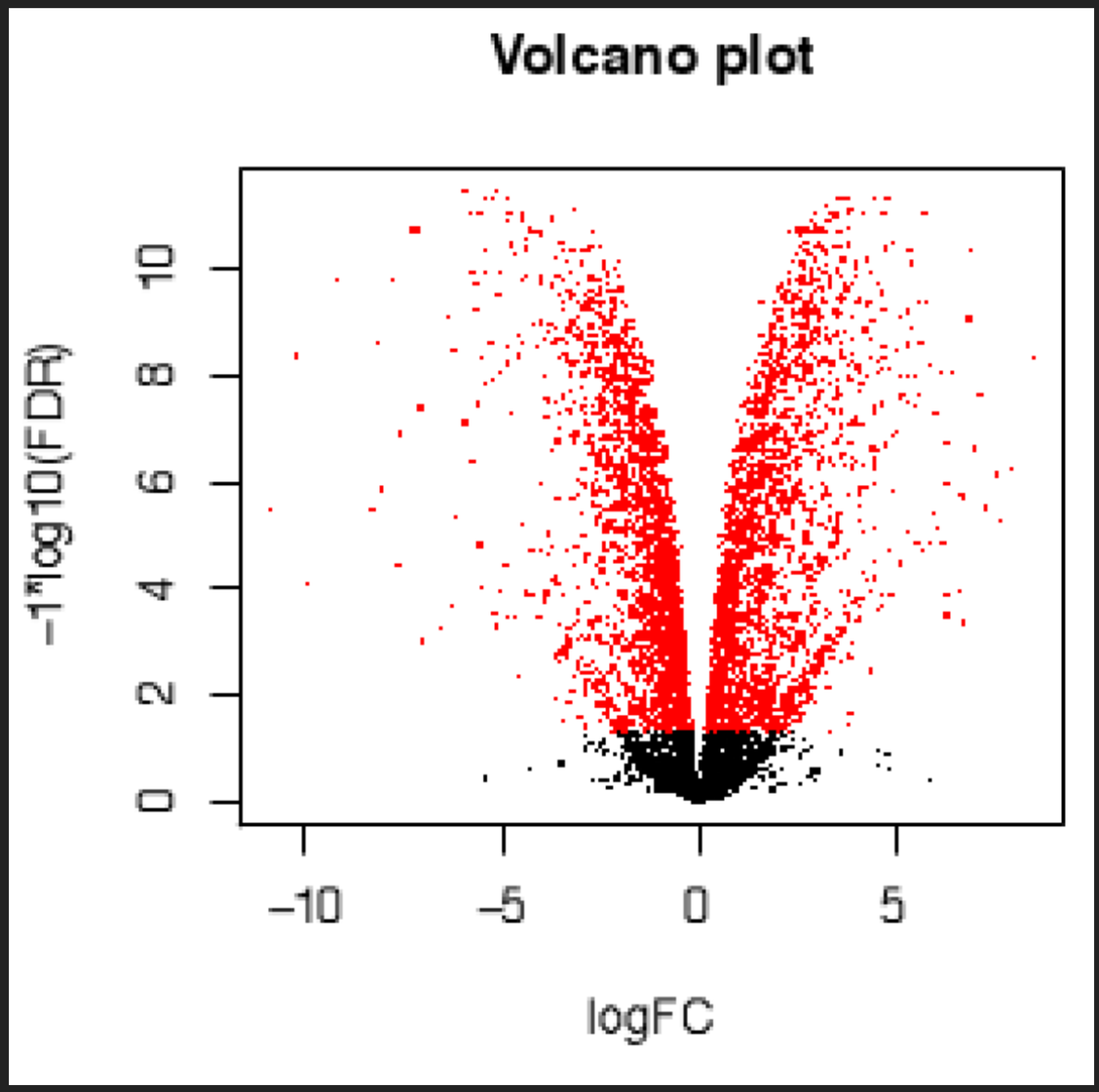

QUALITY CHECK SHORT READ SEQUENCE

- FASTQC "A quality control tool for high throughput sequence data"
  - http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

TRIMMING

- Many different trimmers available
- Trim adapters/low quality reads
- Trim Galore! is one that works well on Hydra

HANDS-ON

- Go to https://github.com/SmithsonianWorkshops/2020-01-28-NMNH-RNAseq/tree/master/Materials
- Click on “2a_Raw Read QA-QC.md”