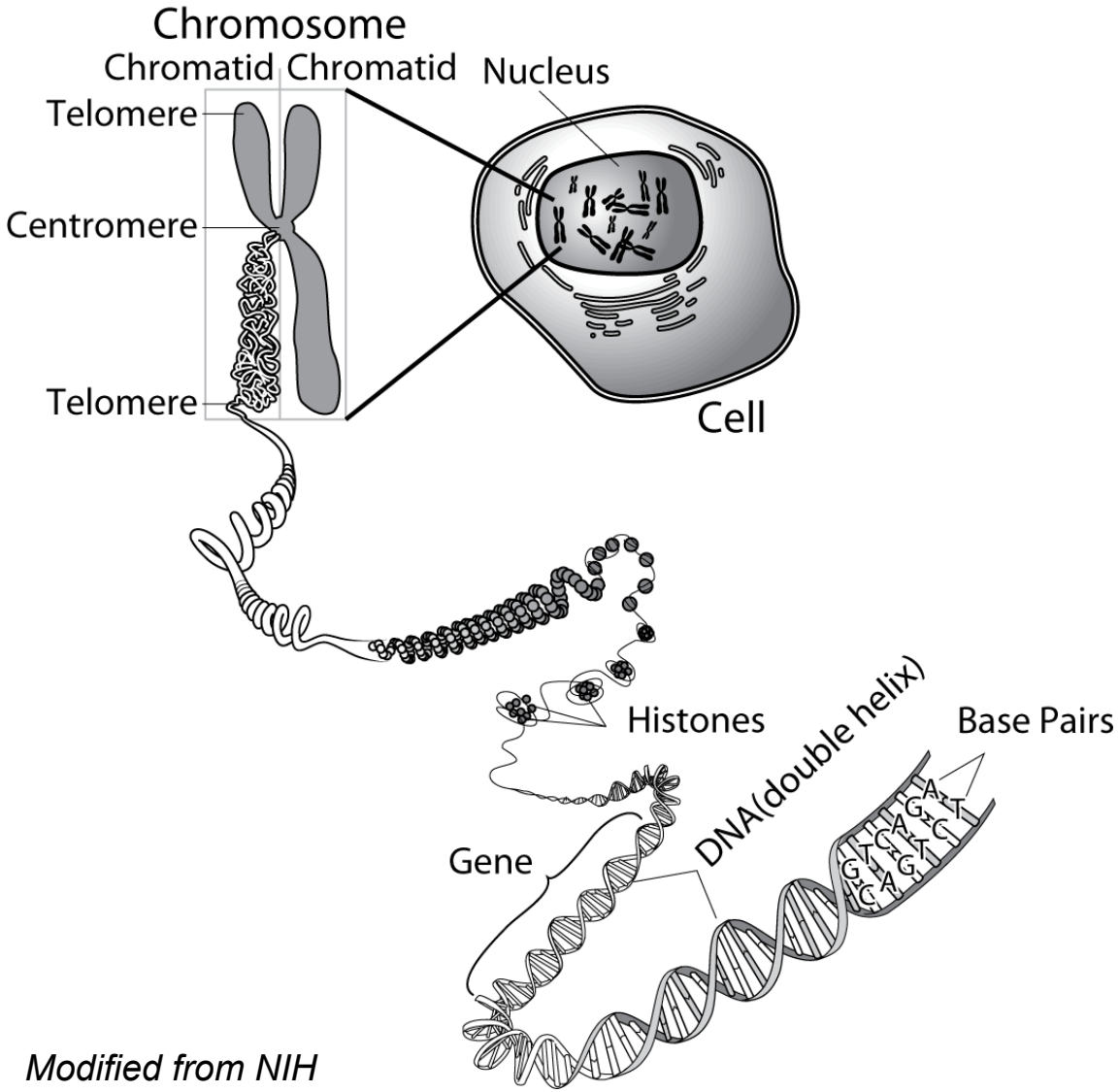
NTNU

Innovation and Creativity

Project – Preprocessor for high throughput sequencing reads

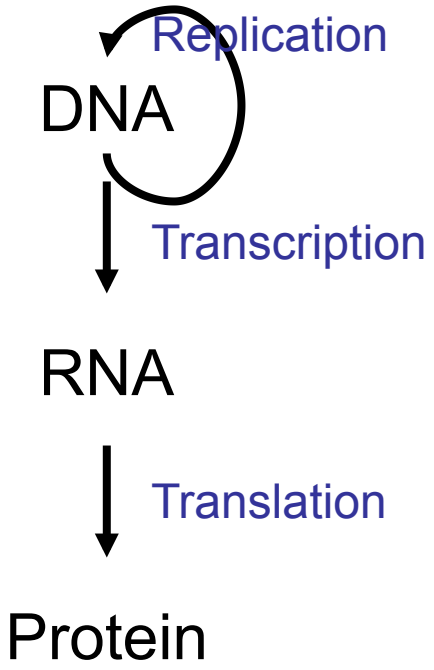
Pål Sætrom

Sequences – basic data structures in cells



Modified from NIH
http://www.accessexcellence.org/RC/VL/GG/chromosome.php

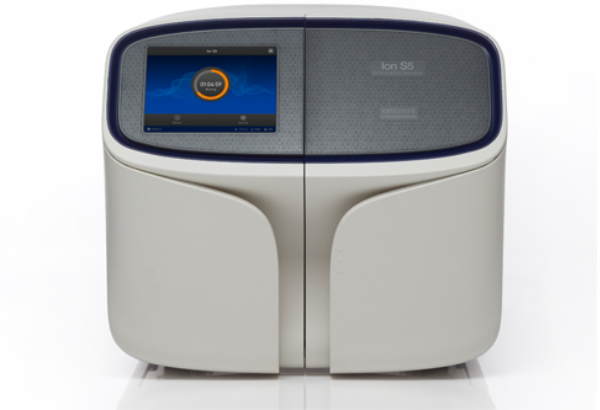
Sequence data



High throughput sequencing – reading the cell's RNA/DNA

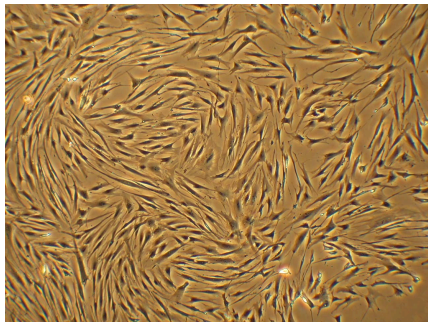
Procedure

1. Isolate RNA/DNA
2. Prepare sequencing library
3. Sequence
4. Analyze data

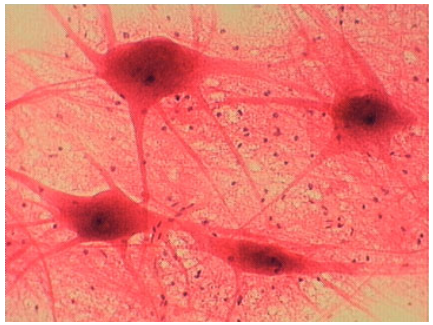


1. Isolate RNA/DNA

Connective tissue



Brain



Muscle

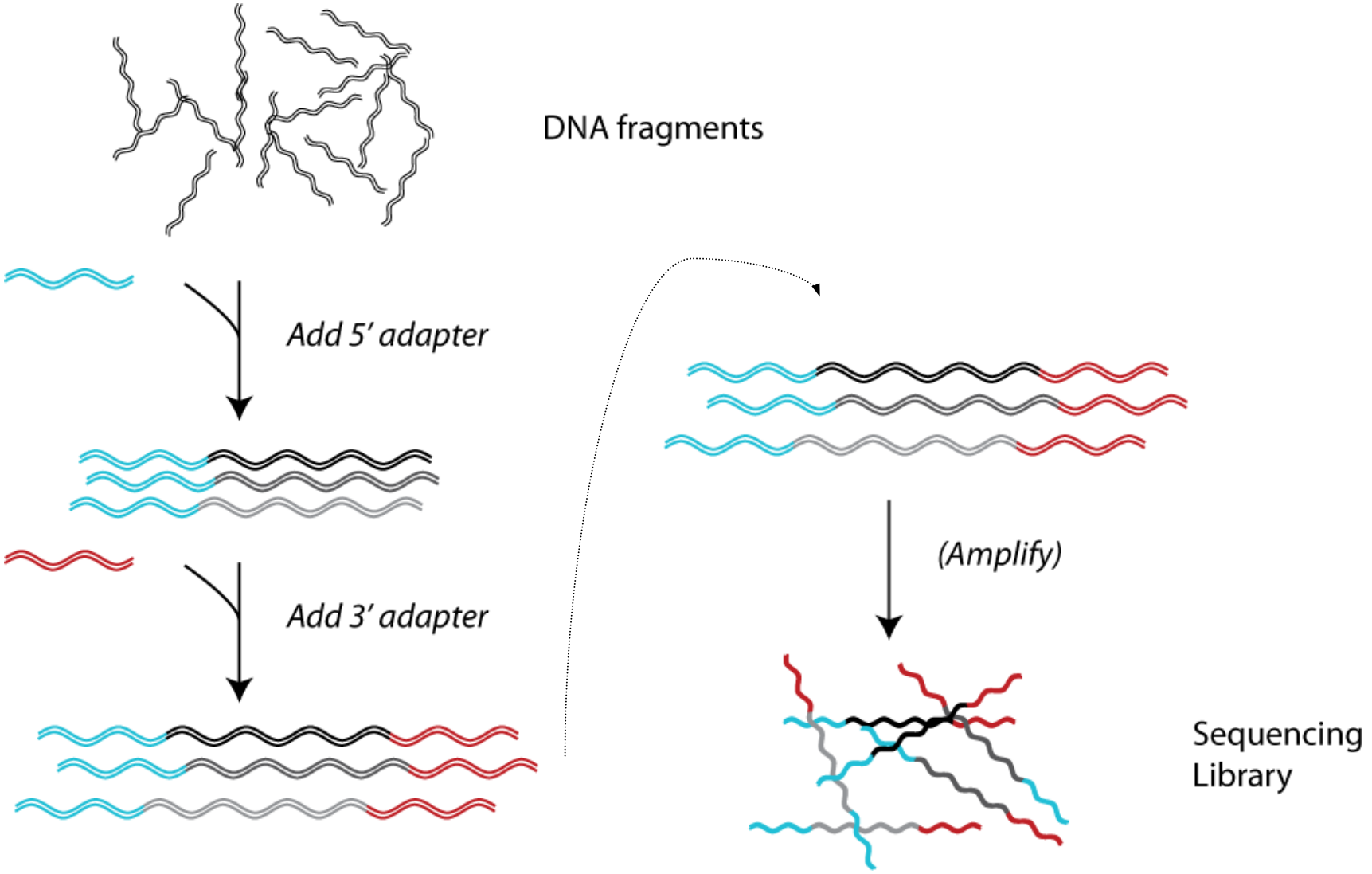
1. Tissue sample
2. “Break” cells (liq. N, blender, chemicals)
3. Chemical reactions to isolate RNA or DNA

→ RNA/DNA sample

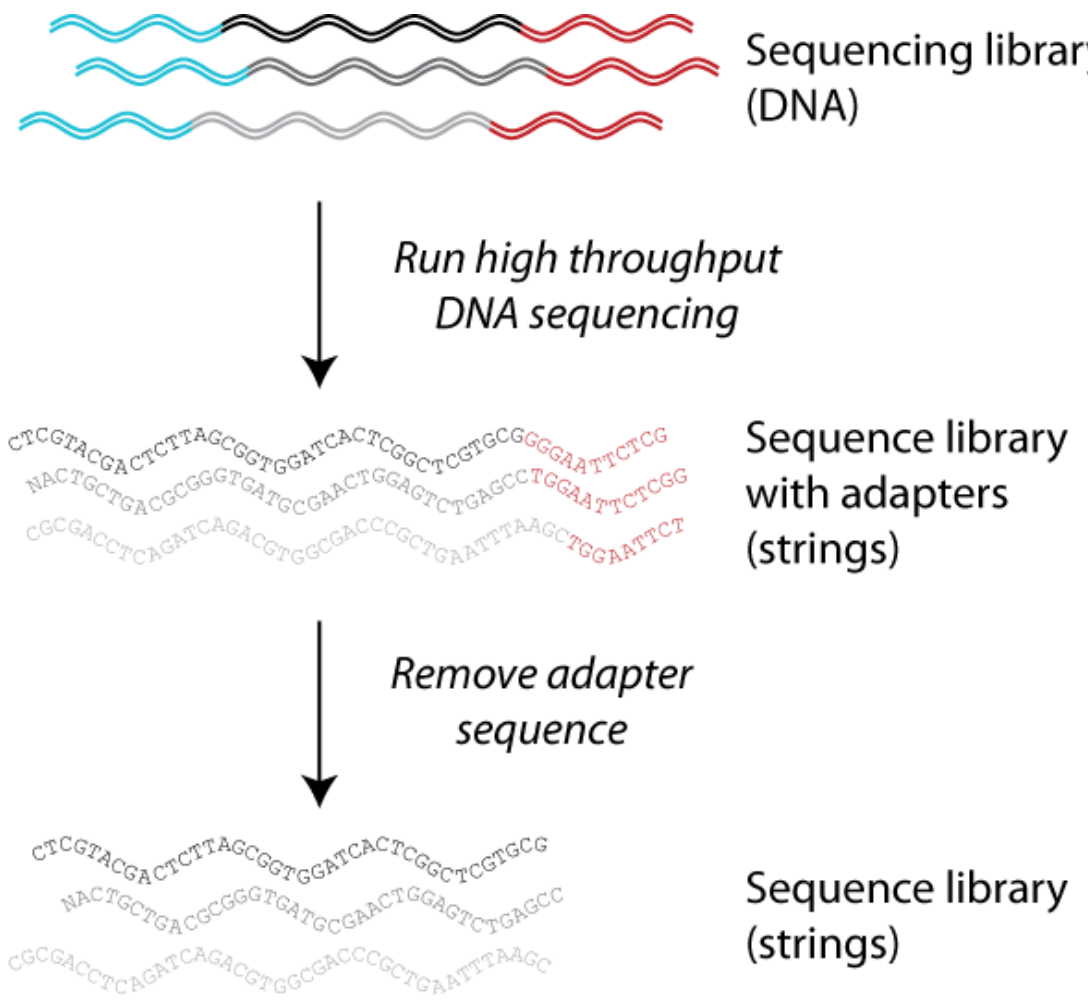
Simple DNA: Salt; soap; alcohol

Pictures:
Photo Researchers, Inc., Iowa State Univ,.
Stephanie Saade, USA Today

2. Prepare sequencing library



3. + 4. Sequence and analyze data

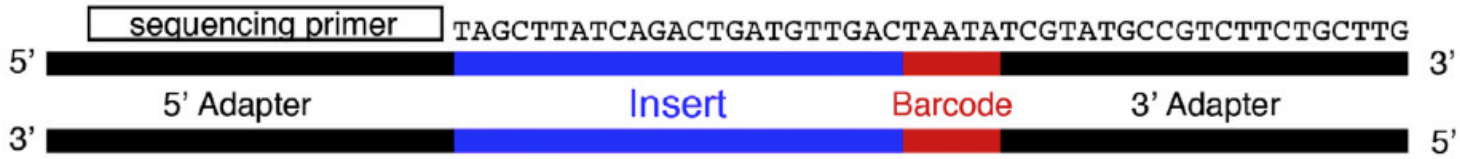


Barcode sequencing - unique adapter per sample

- Sequencing reaction produces lots of data
  - ~ 300 * 10^6 sequences (reads)
  - Cost: ~ NOK 8000-16000
  - Default: single sample per reaction
- Some applications (RNA seq.) requires less data per sample
  - Small RNAs: ~ 10 * 10^6 reads sufficient
- Using unique adapter per sample
  - Allows multiplexing multiple samples
  - “Barcode” read during sequencing

Barcode sequencing – Resulting data

PCR product



fastq converted to fasta

```
@HISEQ_ID
TAGCTTATCAGACTGATGTTGACTAATATCGTATGCCGTCTTCTGCTTGAA
+HISEQ_ID
CCCFFFFFHHHHHJJJJJHIJJIJGJJJJGHGHHJHIGFGGIJJJJIIEGF
```

fasta checked for valid barcode and adapter

redundant read of subsample member tracked

```
>seq1271271 subsample HISEQ_ID pooled.fa
TAGCTTATCAGACTGATGTTGT
```

fasta files split into individual files by subsample (header contains read id and read frequency)

```
>seq76|193312
TAGCTTATCAGACTGATGTTGT
```

Farazi et al., (2012) *Methods*

Project

- Task 1 – Perfectly matching adapter fragments
- Task 2 – Imperfectly matching adapter fragments
- Task 3 – Finding the adapter sequence
- Task 4 – De-multiplex barcoded library

- Individually or in pairs

- Deliverable 1: Project report
- Deliverable 2: Oral presentation

Project report

- Parts: Introduction, Methods, Results and Discussion, References
- Figures and Tables to present results
- Pseudo code to describe algorithms
- Follow standard for scientific reports
  - Clear, consistent, unambiguous presentation
  - Consistent (standard) formatting

Deadline: October 31, 23:59.