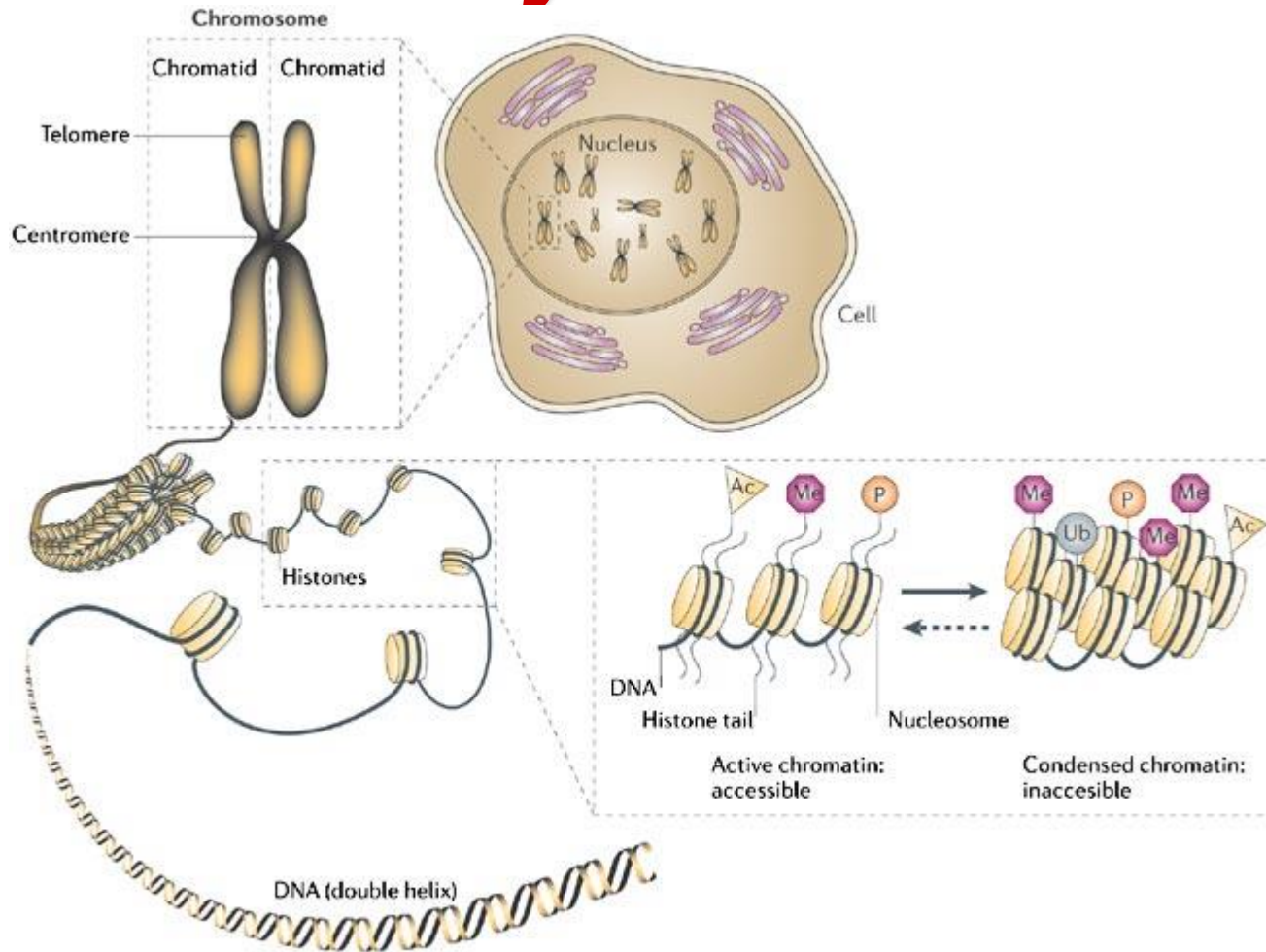


Spirit workshop

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Genetic information in eukaryotic cells



Epigenetics

- Something beyond the gene is contributing to the phenotype – this is the literal meaning of “epi” and “genetics”
- Heritable information
- Nuclear organization
- Influence the gene function
- Development or disease

Epigenetic mechanisms

- DNA methylation,
- chromatin remodeling
 - Covalent histone modifications
 - ATP-dependent chromatin remodeling complexes
- Regulatory ncRNAs

Regulation of gene function

- Transcription initiation (activation)
- Transcription elongation
- Transcription termination
- Splicing
- Nuclear mRNA export
- mRNA stability

Overview of Techniques

Transcription:

- Northern blot

- Steady state RNA - RNA-seq (bulk and scRNA)

- Nascent RNA analysis

 - Nuclear run-on

 - GRO-seq

 - Reporter gene assay

Protein-DNA interactions

- EMSA

- DNA accessibility:

 - FAIRE-seq, DNase-seq, Mnase-seq & ATAC-seq

- Protein localisation:

 - Chromatin Immunoprecipitation (ChIP)

 - DamID

 - Cut & Run

Transcription

What is transcription?

Copying the information from DNA as RNA

Why?

Regulate the cell function

Protect the genetic code

Types of transcription systems

Prokaryotic transcription – Cytoplasm

Eukaryotic transcription – Nucleus

Types of RNA

Coding RNAs – translated into proteins

Messenger RNA (mRNA)

Non-coding RNAs

Ribosomal RNA (rRNA)

Transfer RNA (tRNA)

Regulatory RNAs

Antisense RNA

Long non-coding RNA (lncRNA)

Short interfering RNA (siRNA)

Micro RNA (miRNA)

Piwi-interacting RNA (piRNA)

Prokaryotic vs Eukaryotic Transcription

Prokaryotic transcription

- ✓ Transcription and translation occur simultaneously
- ✓ Cytoplasm
- ✓ RNAs released and processed in cytoplasm
- ✓ RNA polymerase – 5 subunits

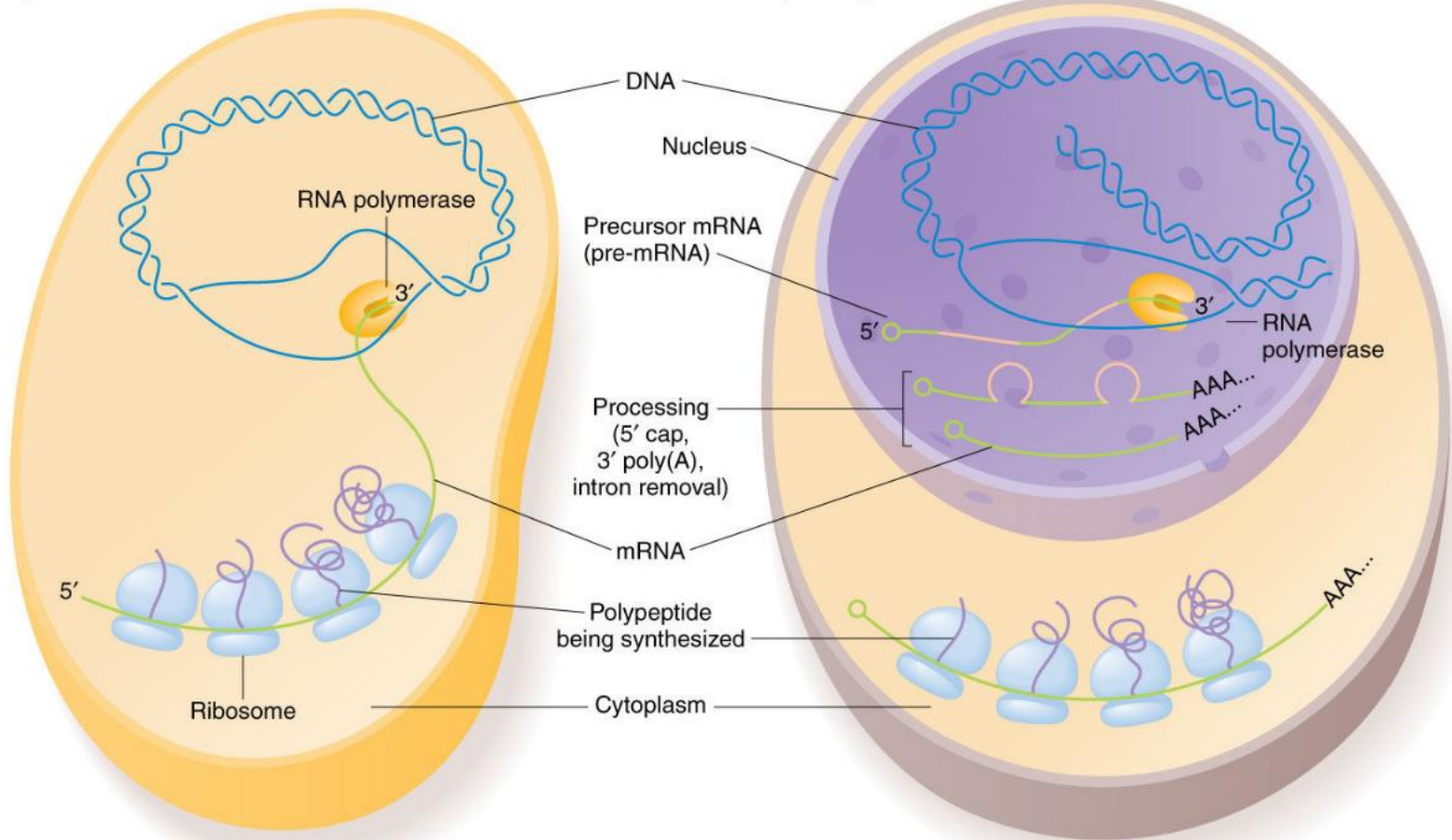
Eukaryotic transcription

- ✓ Transcription and translation do not occur simultaneously
- ✓ Nucleus
- ✓ RNAs released and processed in Nucleus
- ✓ RNA polymerase – 10 -15 subunits

Prokaryotic vs Eukaryotic Transcription

a) Bacterium

b) Eukaryote

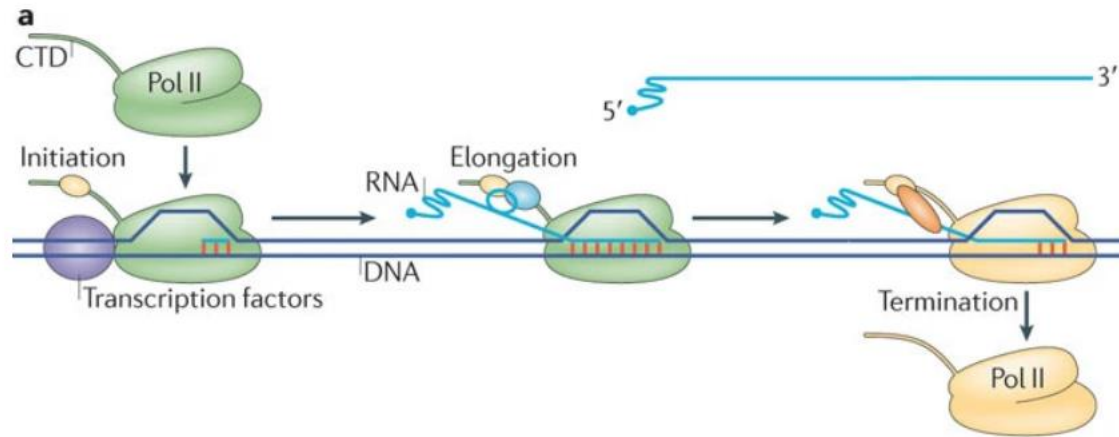


Stages of transcription

Initiation: Binding of RNA polymerase complex to promoter

Elongation: RNA polymerase move through the DNA strand and producing RNA

Termination: Process of end of transcription when RNA polymerase reaches the termination signal



Regulation of transcription

Eukaryotic RNA polymerase II large subunit (RPB1) CTD contain tandemly repeated heptapeptides “YSPTSPS”

Number of repeats in different eukaryotes:

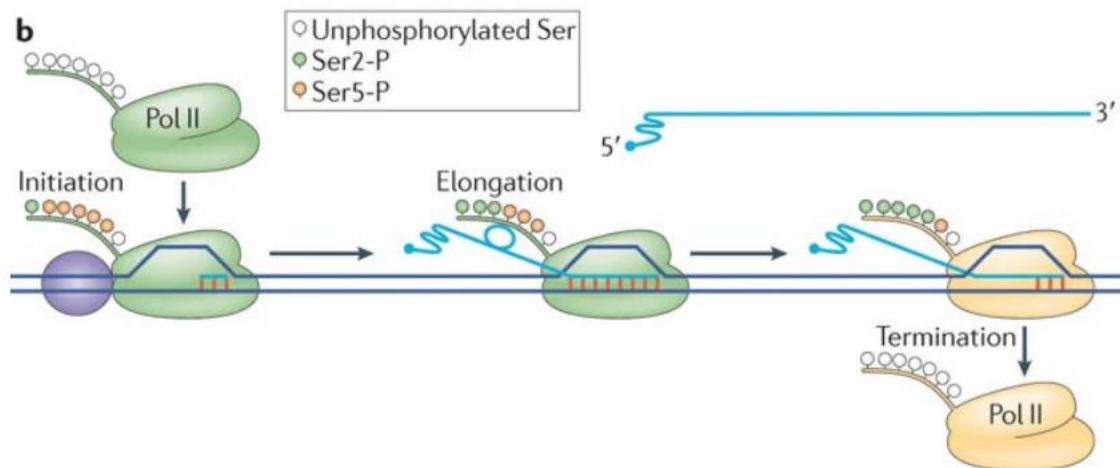
Mammalian – 52

Yeast – 26

Plasmodium – 17

Drosophila – 44

Lower eukaryotes – trypanosomes – none / atypical CTD



Eukaryotic RNA polymerases

Types of RNA polymerases

Type I – ribosomal RNA (rRNA) transcription

Type II – messenger RNA (mRNA) transcription

Type III – transfer RNA (tRNA) transcription

Inhibition of RNA polymerases

Actinomycin D	α -amanitin	DRB	Flavopiridol	Triptolide
Pol I, Pol II & III	Pol II and Pol III	Pol II	Pol II	Pol I and Pol II
I >> II >> III	II >> III			
II: CTD hyperphosphorylation	II: Degradation	II: CTD serine 2 dephosphorylation	II: CTD serine 2 dephosphorylation	II: Degradation
Reversible	Not reversible	Reversible	??	Not reversible

Methods to detect RNA

Northern blot – steady state RNA

RNA sequencing – steady state RNA

Bulk RNA-seq

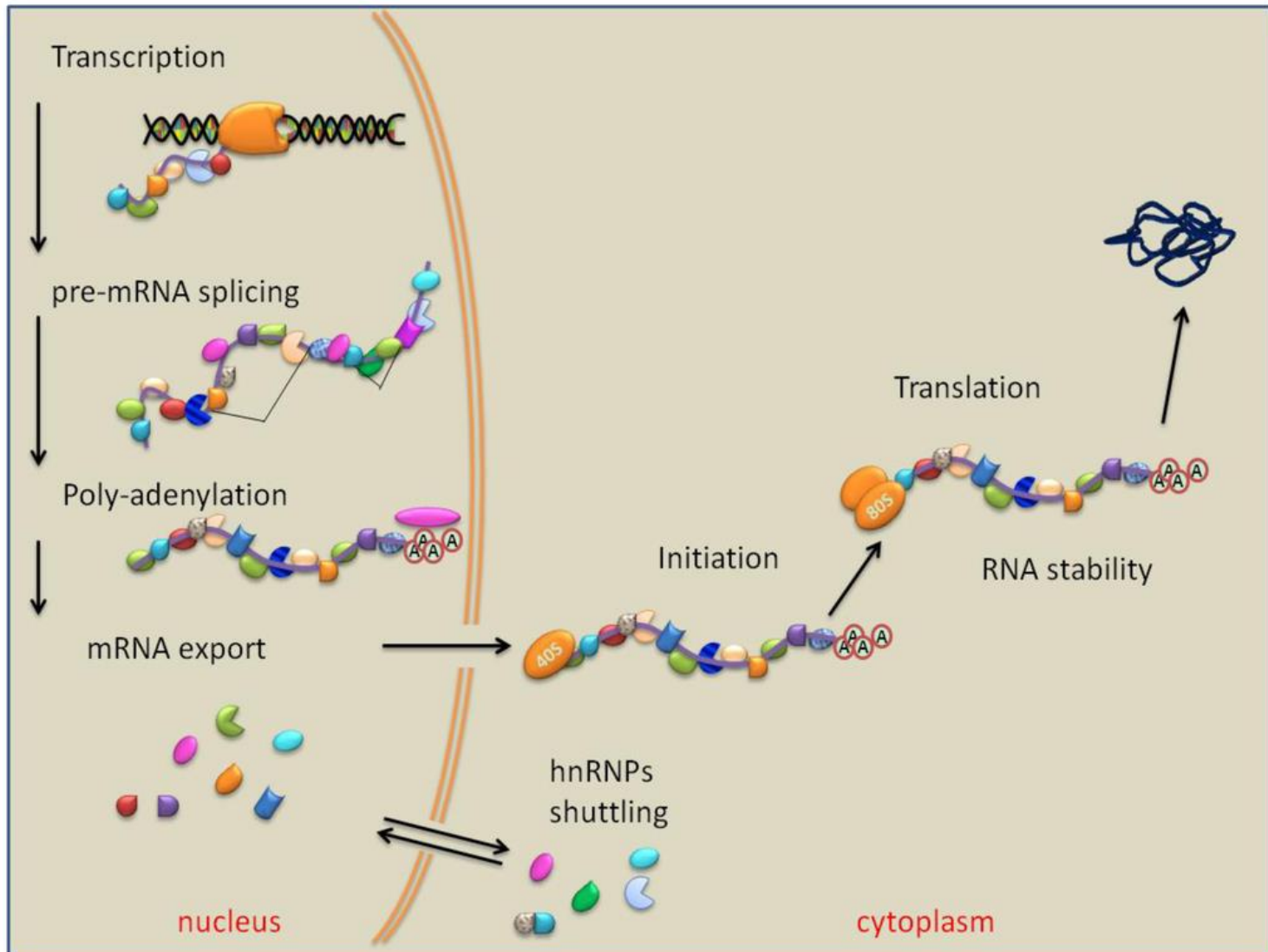
Single cell RNA-seq

RNA microarray – not very much used due to limitation

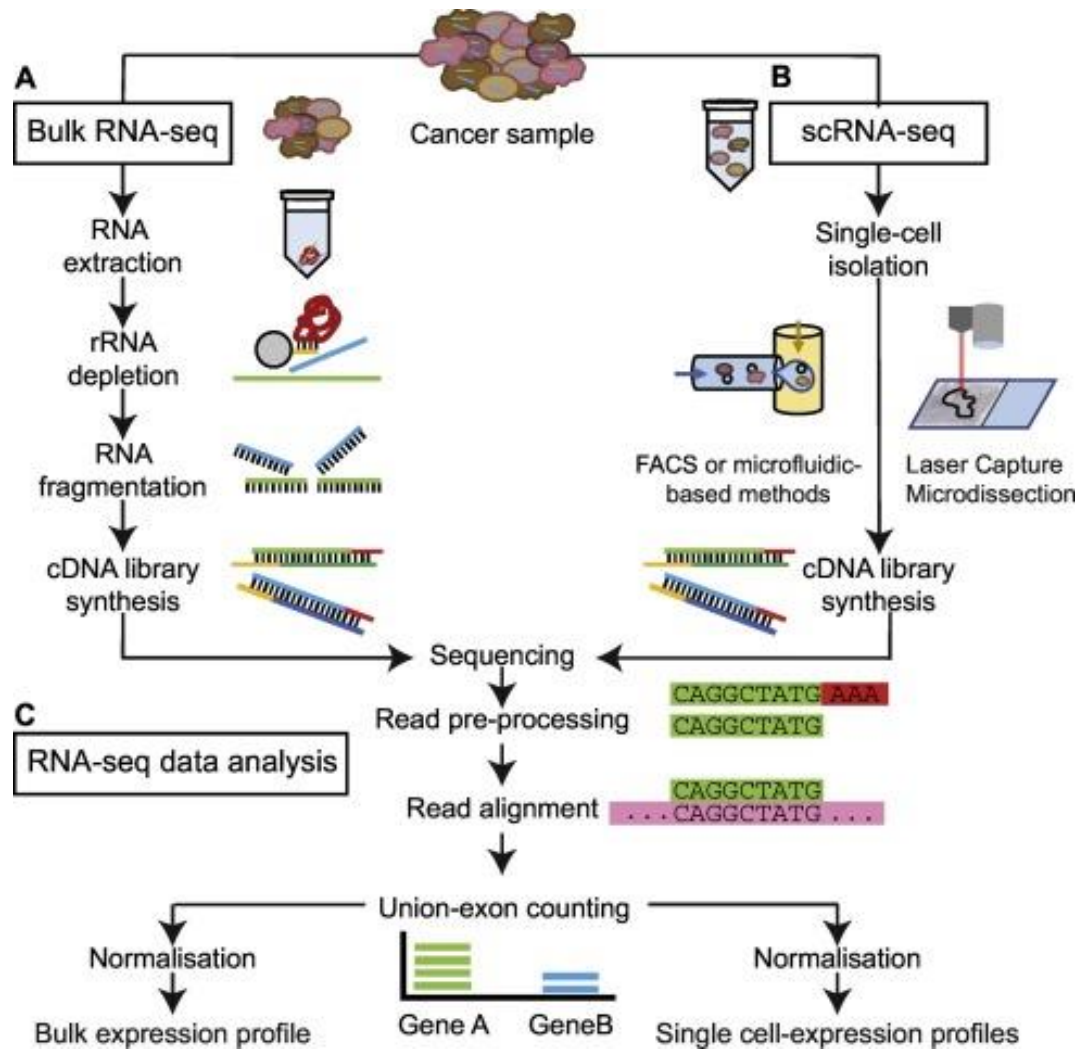
Nuclear run-on – nascent RNA

Global run-on sequencing (GRO-seq) – nascent RNA

Fate of mRNA

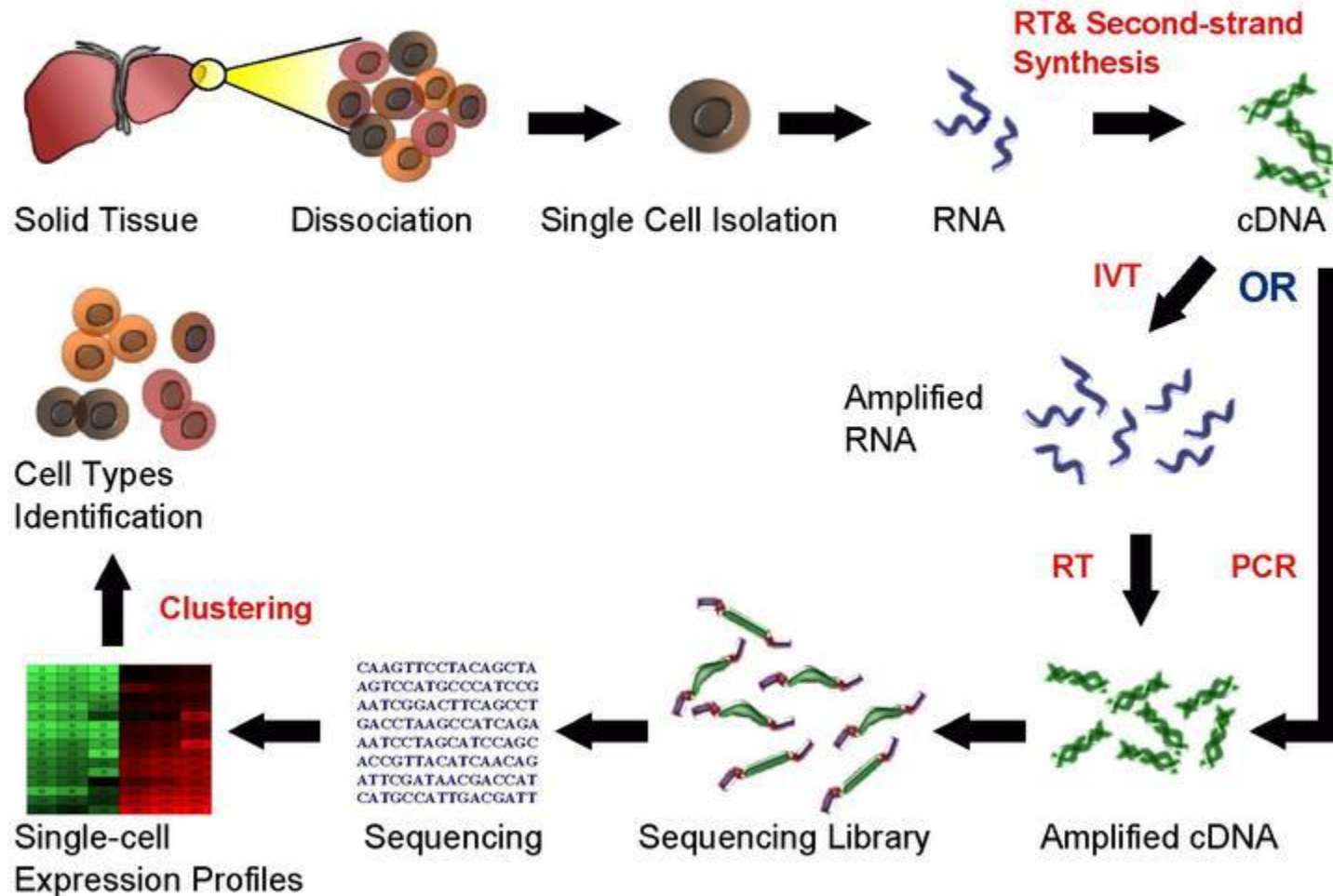


RNA-seq analysis

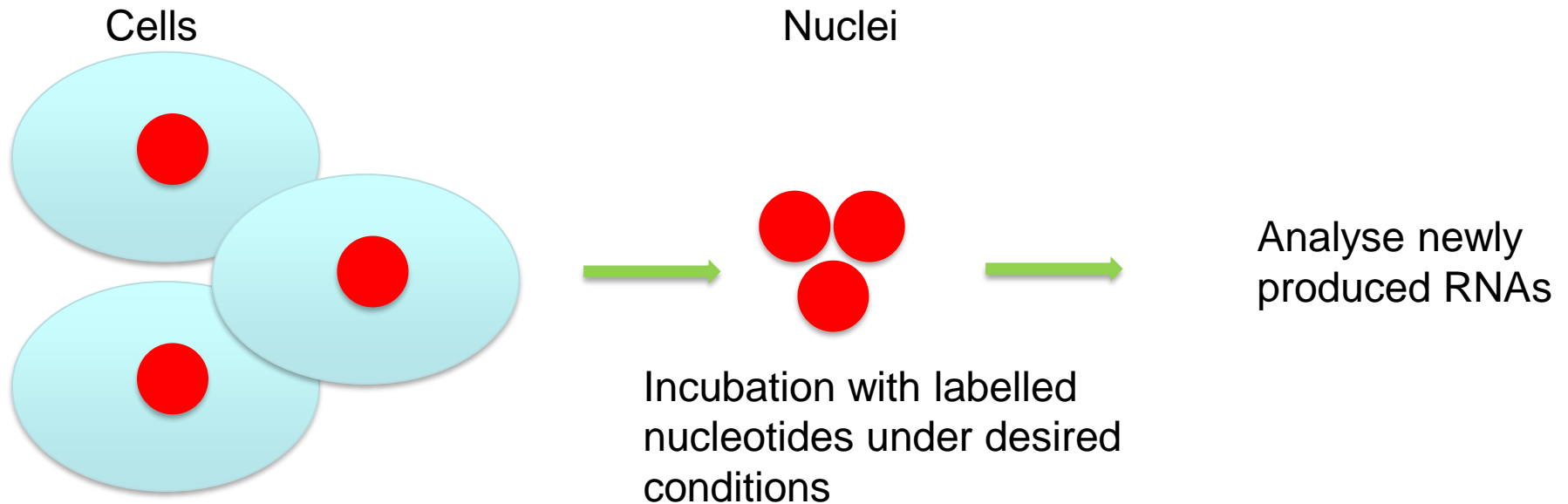


RNA-seq analysis

Single Cell RNA Sequencing Workflow



Nuclear run-on assay



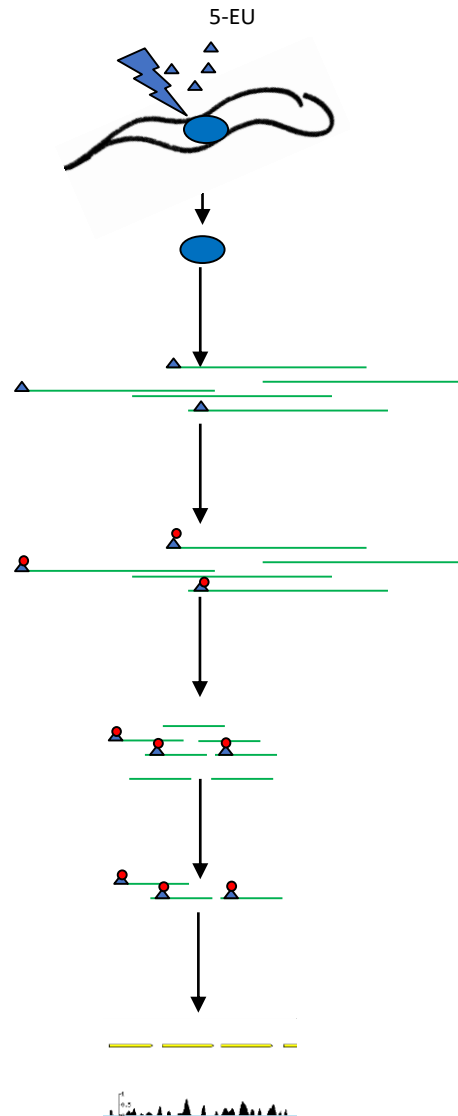
Important for transcriptional control

Helps to eliminate any post transcriptional regulation – Steady state RNA levels

Problems:

- Nuclear integrity ?
- Isolation procedure can alter the physical and functional status of the cells

Labelling of nascent RNAs with 5-Ethynyl Uridine (5-EU)



10 min

Isolation of Nuclei

RNA isolation

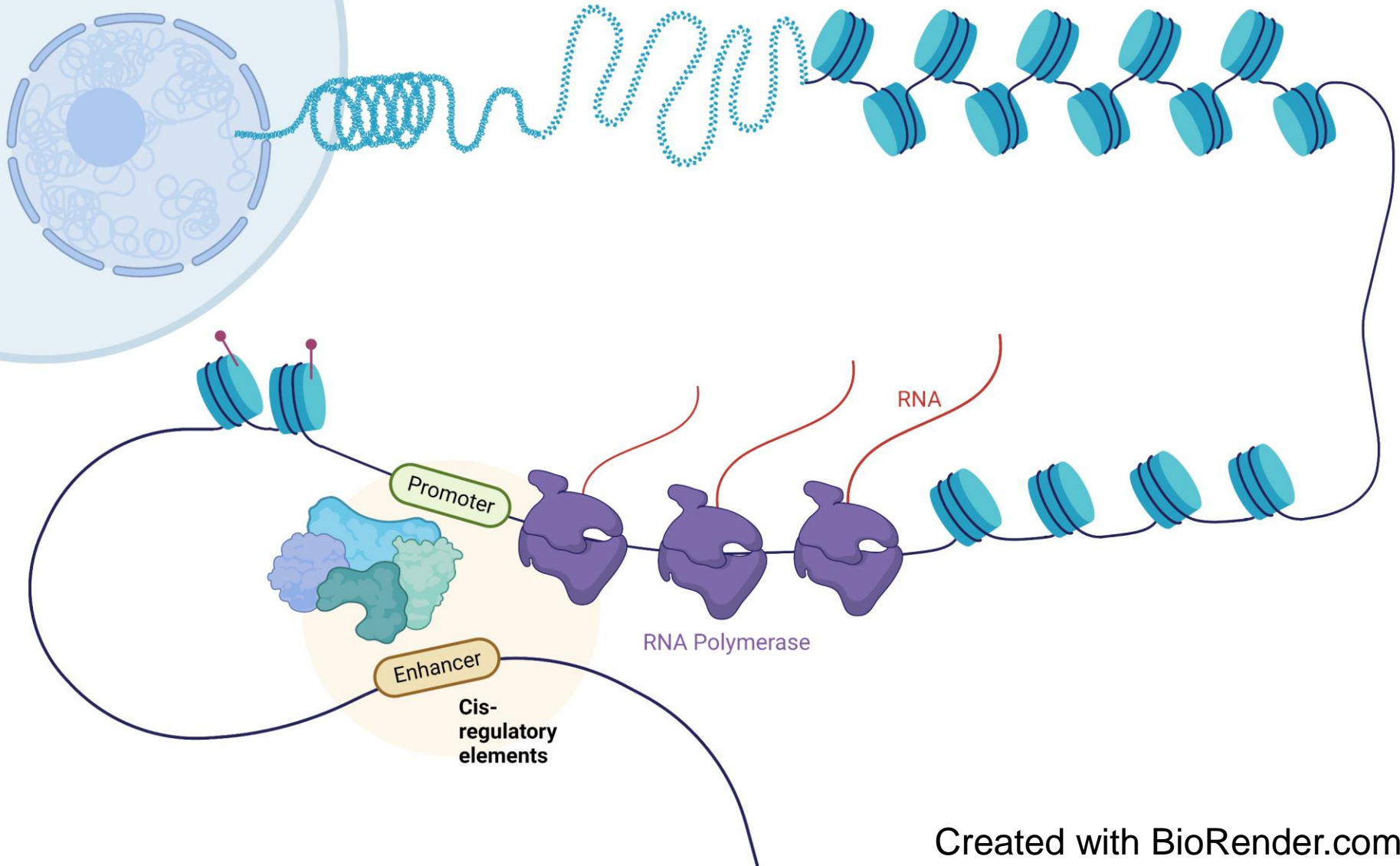
Add azide-biotin to 5-EU using click-chemistry

RNA fragmentation (optional)

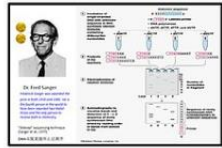
Purification of biotin labelled RNAs & quantification

Nascent RNA-seq / Global Run-On sequencing (GRO-seq)

Regulatory elements in transcription



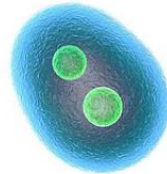
History of sequencing



Sanger method



Human Genome Project



Complete eukaryotic genome



Second generation sequencer: 454 GS20



Research Human Microbiome Project



Nanospace sequencing

1981

1995

2001

2007

2011

2019

1977

1990

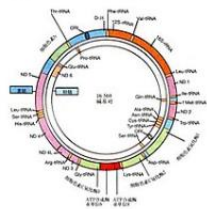
1996

2005

2008

2014

Human mitochondrial genome sequence



Complete cell genome



Complete the Human Genome Project



Second generation sequencer: Genetic Analyzer 2



Third generation sequencer: PacBio RS



The third stage Human microbiome project



Activities

Database mining

NCBI – Blast database

VEuPathDB - Eukaryotic Pathogen, Vector and Host Informatics Resource

Next Generation Sequencing (NGS) analysis

Introduction to Galaxy platform – free for academics

Other options

NGS file format

Fasta

```
>NG_008679.1:5001-38170 Homo sapiens paired box 6 (PAX6)
ACCTCTTTTTCTTATCATTGACATTTAAACTCTGGGGCAGGTCCTCGCGTAGAACGCGGCTGTCAGATCT
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CCTCCGCTCCCAGGTAACCGCCCGGGCTCCGGCCCCGGCCCGGCTCGGGGCCCCGCGGGGCTCTCCGCTG
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```

Fastq

```
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Sequence | TTAATTGGTAAATAAATCTCCTAATAGCTTAGATNTTACCTTNNNNNNNNNTAGTTTCTTGAGA
+ sign & identifier | +HWI-EAS209_0006_FC706VJ:5:58:5894:21141#ATCACG/1
Quality scores | efcffffcfeefffcfffffddf`feed]`_]_Ba_^__[YBBBBBBBBBRTT\]] [] dddd`
```