

PROTEOMICS, SPATIAL TECHNOLOGIES AND EPIGENETICS

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Overview of the course

| Date | 1 st Lecture | 2 nd Lecture |
|-----------------------|--|---|
| 16 th July | Icebreakers, ground rules and maybe and Intro to Molecular Biology and NGS | |
| 17 th July | Intro to Molecular Biology and NGS | Data analysis 1 |
| 18 th July | Experiment: Nucleic acid extraction | Data analysis 2 |
| 19 th July | Single-cell DNA, RNA and protein technologies | Proteomics, spatial technologies and epigenomics Data analysis 3 |
| 20 th July | Data analysis 4 | Experiment: Staining our own cells |
| 21 st July | Experiment: Gel electrophoresis | Data analysis 5 Data analysis 6 |
| 22 nd July | Preparation for the final presentation | Final presentation and closing ceremony |

Objectives

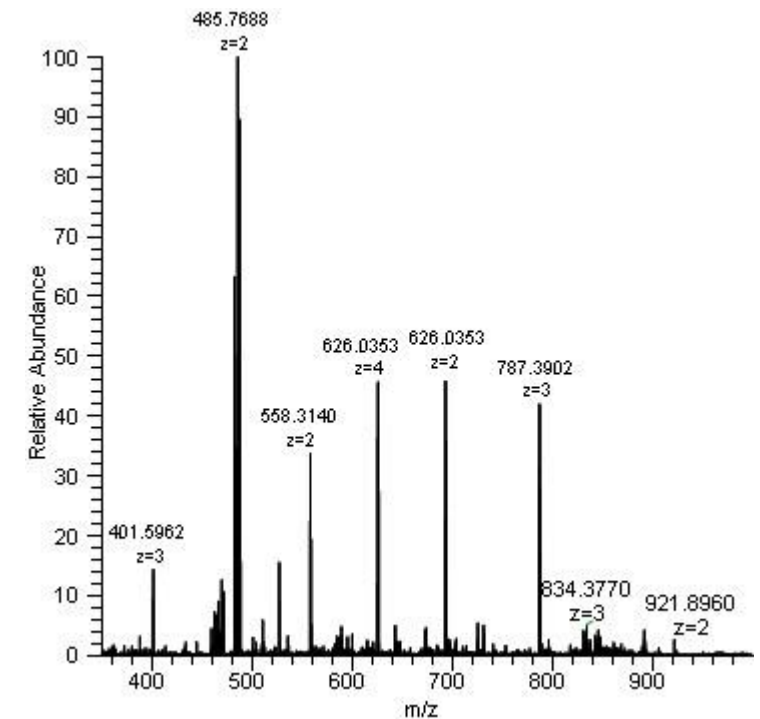
1. To know and understand several proteomics methods used to study cancer.
2. To understand how spatial DNA, RNA and protein technologies are used in cancer research.
3. To understand how epigenomic studies have contributed to cancer research.

Proteomic techniques

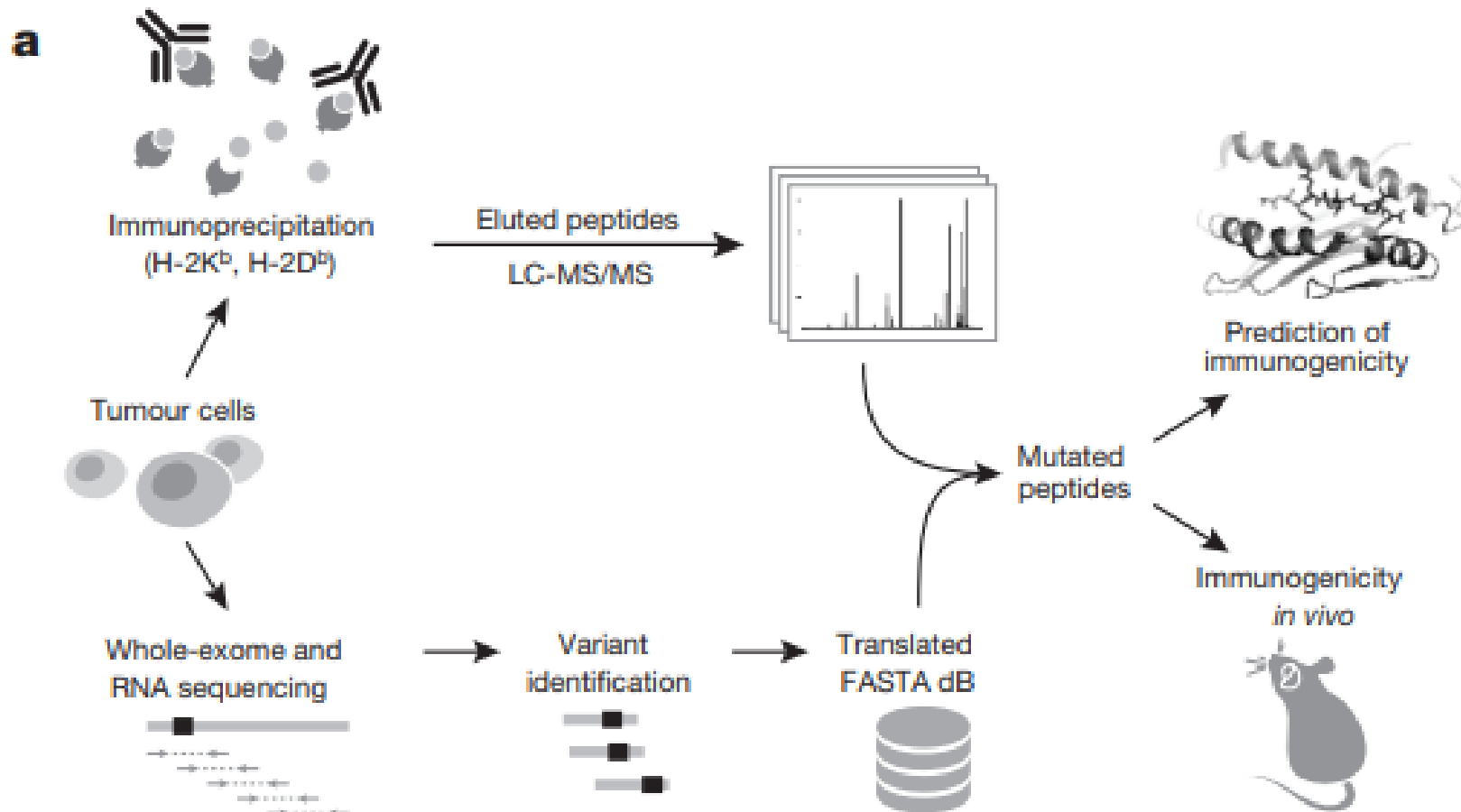
- Proteomics is the large-scale study of proteins
- Proteins are vital to understanding cellular functions and disease mechanisms, especially cancer.
- Examples of proteomic techniques:
 - Mass spectrometry
 - Protein microarrays
 - Gel Electrophoresis

What is mass spectrometry?

- Measures mass-to-charge ratio of charged particles to identify and quantify molecules in a sample.
- Process:
 1. Sample is ionised .
 2. Ionized molecules are then separated in a by their mass-to-charge ratio.
 3. Separated ions are detected.



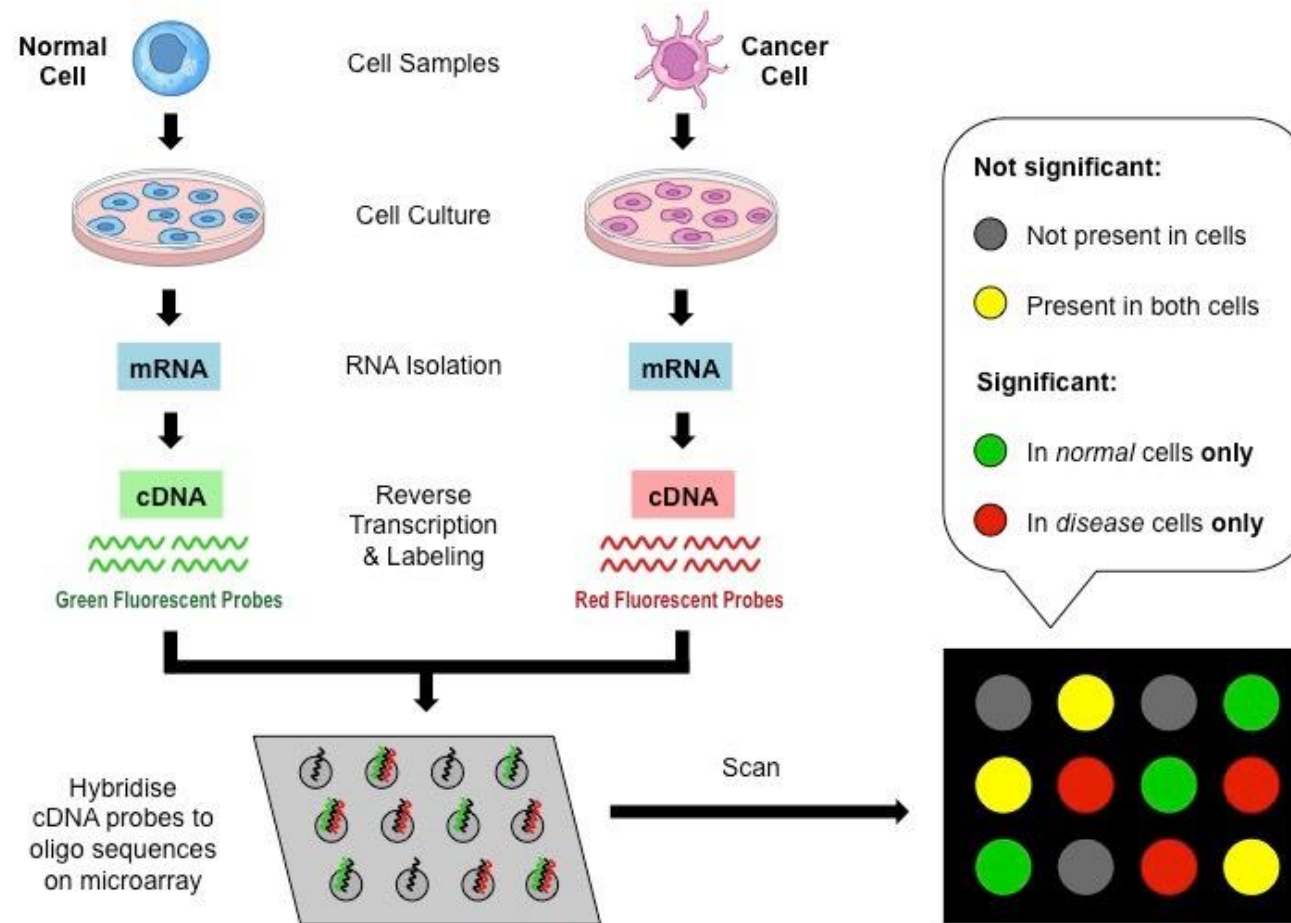
Case study: Mass spectrometry



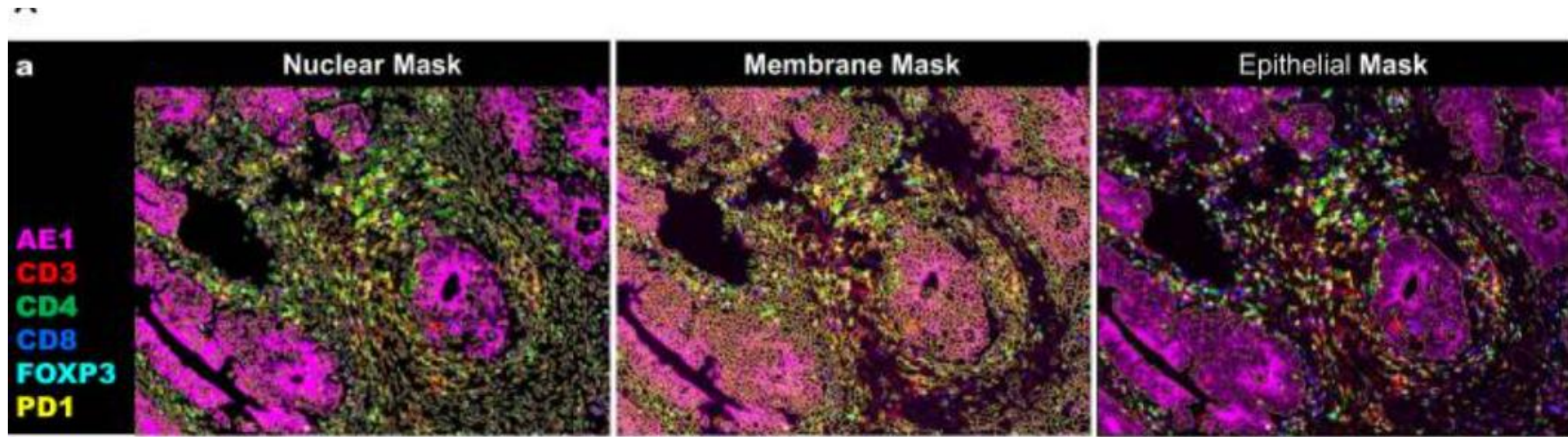
What are microarrays?

- A microarray is a collection of microscopic DNA, RNA, or protein spots attached to a solid surface, used to analyse the expression levels of large numbers of genes
- Process
 1. DNA, RNA, or protein samples are extracted and labelled with fluorescent dyes.
 2. Arrays/microarray chips are prepared. They have DNA, RNA or protein probes attached to their surface
 3. Labelled samples are washed over the array, where they bind or hybridize to their complementary probe.
 4. Microarray is scanned with a laser to detect fluorescent signals; the intensity of these signals indicates the abundance of the nucleic acid or protein

What are microarrays?



Cancer case study: Protein imaging and microarrays



Stachtea et al. (2021)

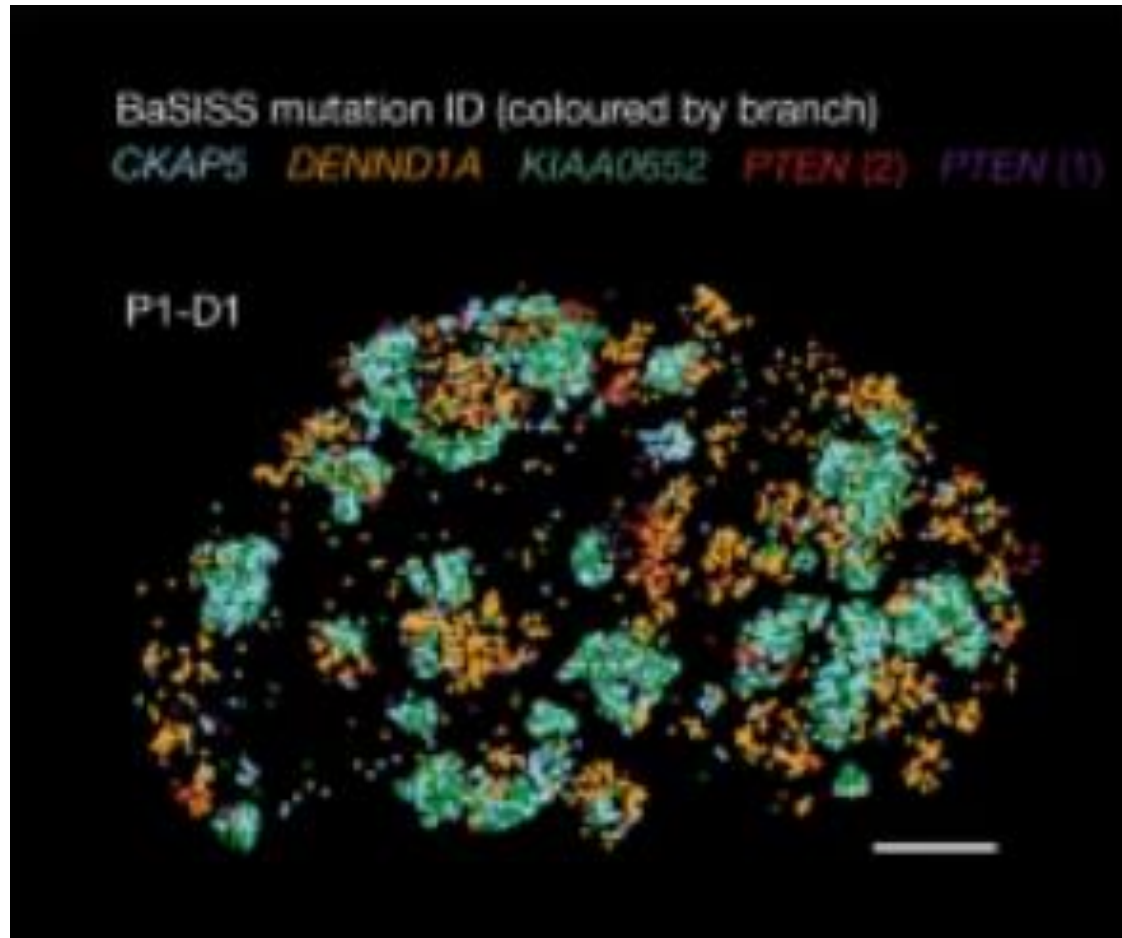
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What is spatial technology?

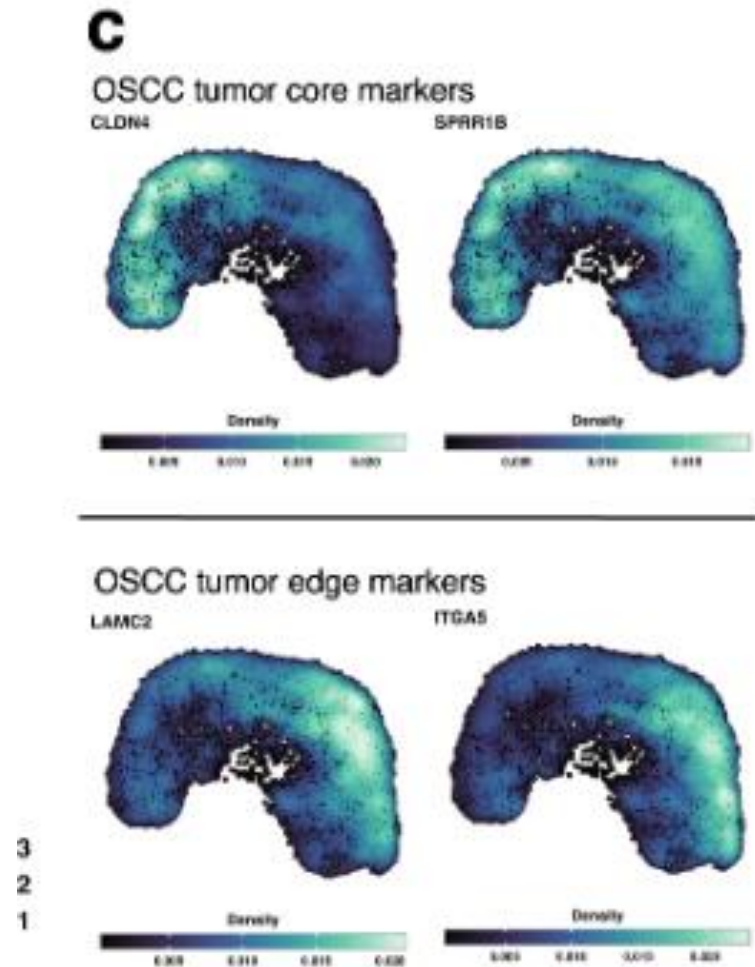
- Spatial technologies allow us to visualise and quantify biological molecules in their native spatial context within cells and tissues.
- This is useful for validating results from other molecular biology methods.
- Examples of spatial technology methods:
 - Spatial transcriptomics
 - Immunohistochemistry (IHC)
 - Fluorescence In Situ Hybridization (FISH)
- Applications in cancer research:
 - Tumour heterogeneity: to see where genetic variants, genes and proteins are expressed in a tumour.
 - Targeted therapy development: to develop treatment that is directed towards the correct location in the tumour

Case study: Spatial genomics

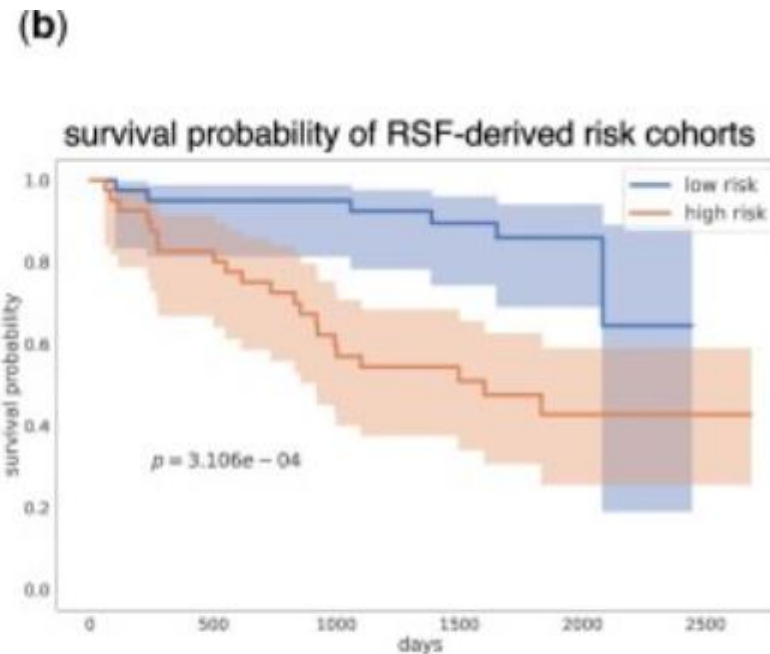
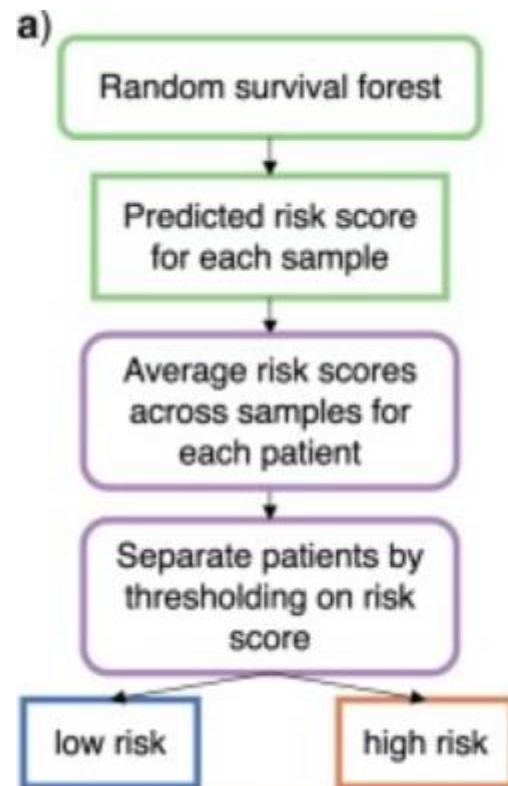


Lomakin et al. (2020)

Case study: Spatial transcriptomics



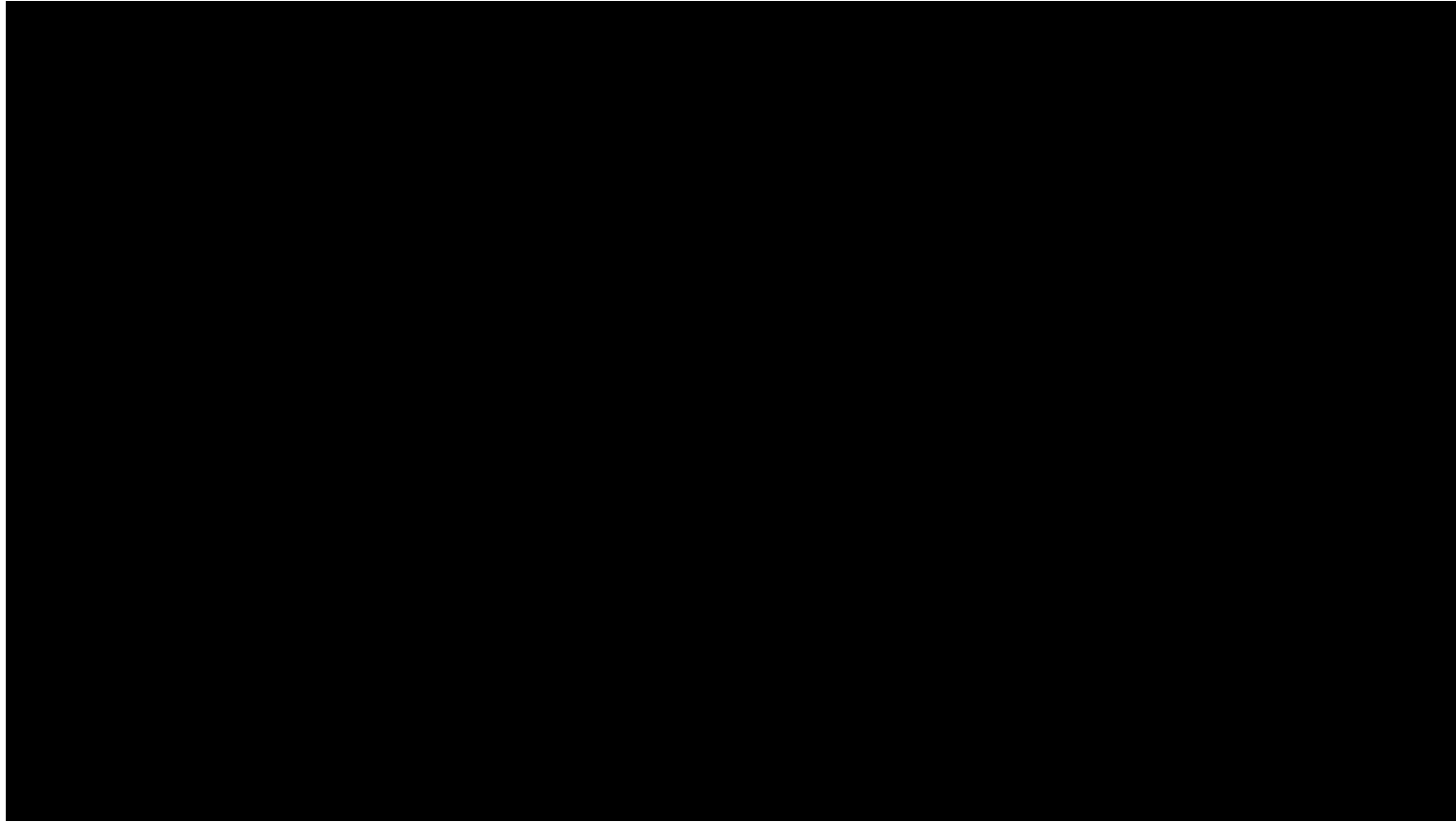
Case study: Spatial proteomics



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What is epigenetics?



What is epigenetics?

- The study of heritable changes in gene expression that do not involve changes to the underlying DNA sequence.
- Epigenetic changes can change gene expression, and this can contribute to tumour development and progression.
- Examples of epigenetic techniques:
 - ChIP-sequencing
 - ATAC-sequencing

Applications of epigenomic studies in cancer research

- Epigenetic changes can activate oncogenes or silence tumour suppressor genes, contributing to cancer progression and resistance to treatment.
- Applications:
 - Biomarker discovery
 - Personalised therapy

What is ChIP-seq

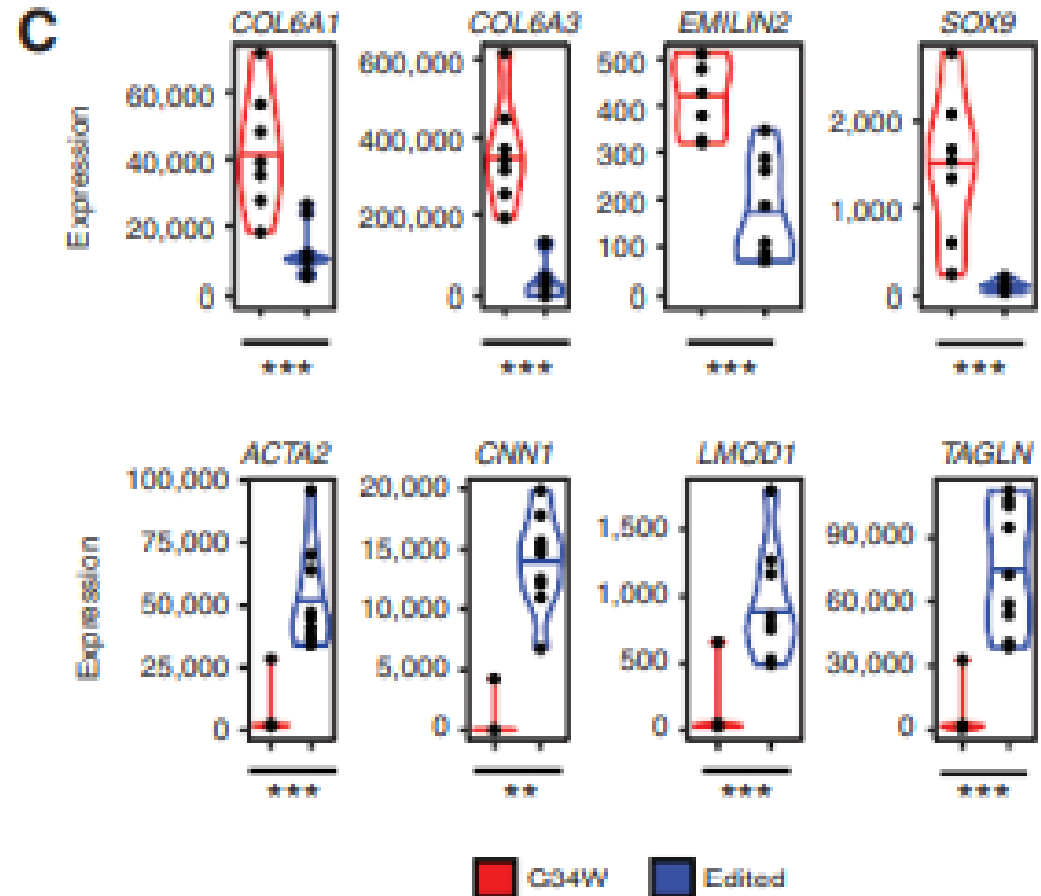
- Chromatin Immunoprecipitation followed by sequencing (ChIP-seq)
- Used to analyse protein interactions with DNA
- Steps:
 1. Cross-linking DNA and proteins
 2. Immunoprecipitating DNA-protein complexes with specific antibodies
 3. Sequencing the DNA
 4. Mapping the sequences back to the genome to identify binding sites.

Case study: ChIP-seq

B

| Upregulated genes in G34W | P |
|---|----------|
| Cell-cell adhesion | 8.87E-19 |
| Extracellular matrix organization | 5.54E-15 |
| Extracellular structure organization | 6.44E-15 |
| Cell-cell adhesion via plasma-membrane adhesion molecules | 1.22E-12 |
| Tube morphogenesis | 1.30E-12 |

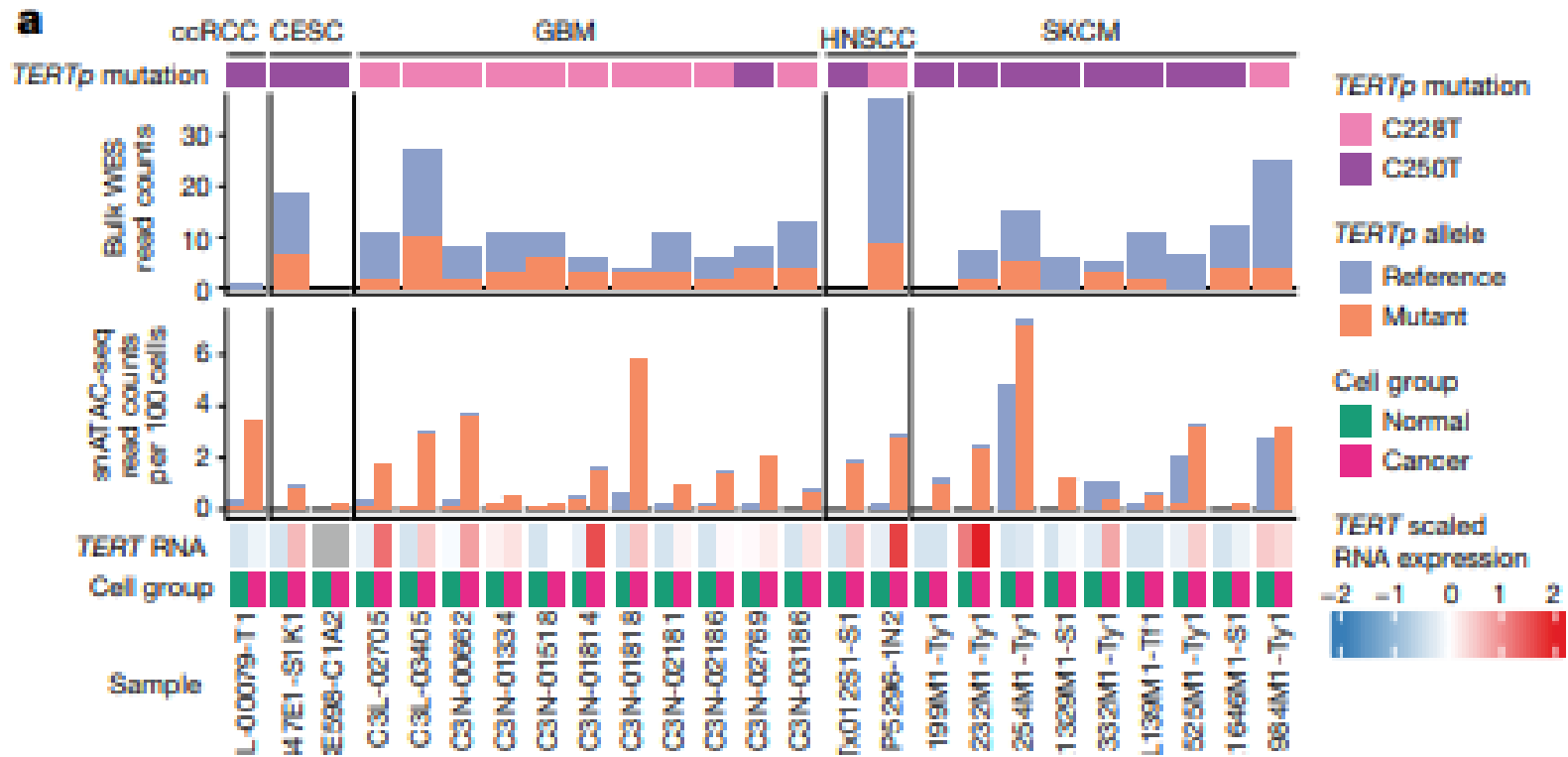
| Downregulated genes in G34W | P |
|---|----------|
| Actin filament-based process | 2.20E-09 |
| Homophilic cell adhesion via plasma membrane adhesion molecules | 4.61E-08 |
| Muscle contraction | 2.56E-07 |
| Actin cytoskeleton organization | 2.76E-07 |
| Regulation of cell morphogenesis | 9.68E-07 |



What is ATAC-seq?

- Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq)
- Used to assess chromatin accessibility across the genome.
- It involves using a transposase enzyme to probe open chromatin regions and insert sequencing adapters directly into the DNA.
- Steps:
 1. The transposase inserts sequencing adapters into open and accessible parts of chromatin.
 2. The fragments are then purified, amplified, and sequenced.

Case study: ATAC-seq



ChIP-seq vs ATAC-seq

| | ChIP-seq | ATAC-seq |
|--------------------|---|---|
| Purpose | Identifies where proteins bind to DNA. | Assesses regions of open chromatin across the genome. |
| Method | Uses antibodies to precipitate DNA-protein complexes. | Uses transposase to probe open chromatin regions. |
| Output | DNA regions bound by specific proteins. | Accessible chromatin regions indicating active transcription. |
| Application | Mapping transcription factor bindings, histone modifications. | Identifying active regulatory regions. |

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Any questions?