DNA EXTRACTION

Overview of the course

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

Objectives

1. To understand the principles of extracting nucleic acids
2. To perform a DNA extraction.
3. To understand the applications of nucleic acid extraction in cancer research.
4. To understand how to read a research article.

NGS workflow?

1. Sample preparation
2. Sequencing
3. Data analysis

Sample Preparation

1. Extract nuclei acids
2. Fragment nucleic acids
3. Library preparation

Extract DNA

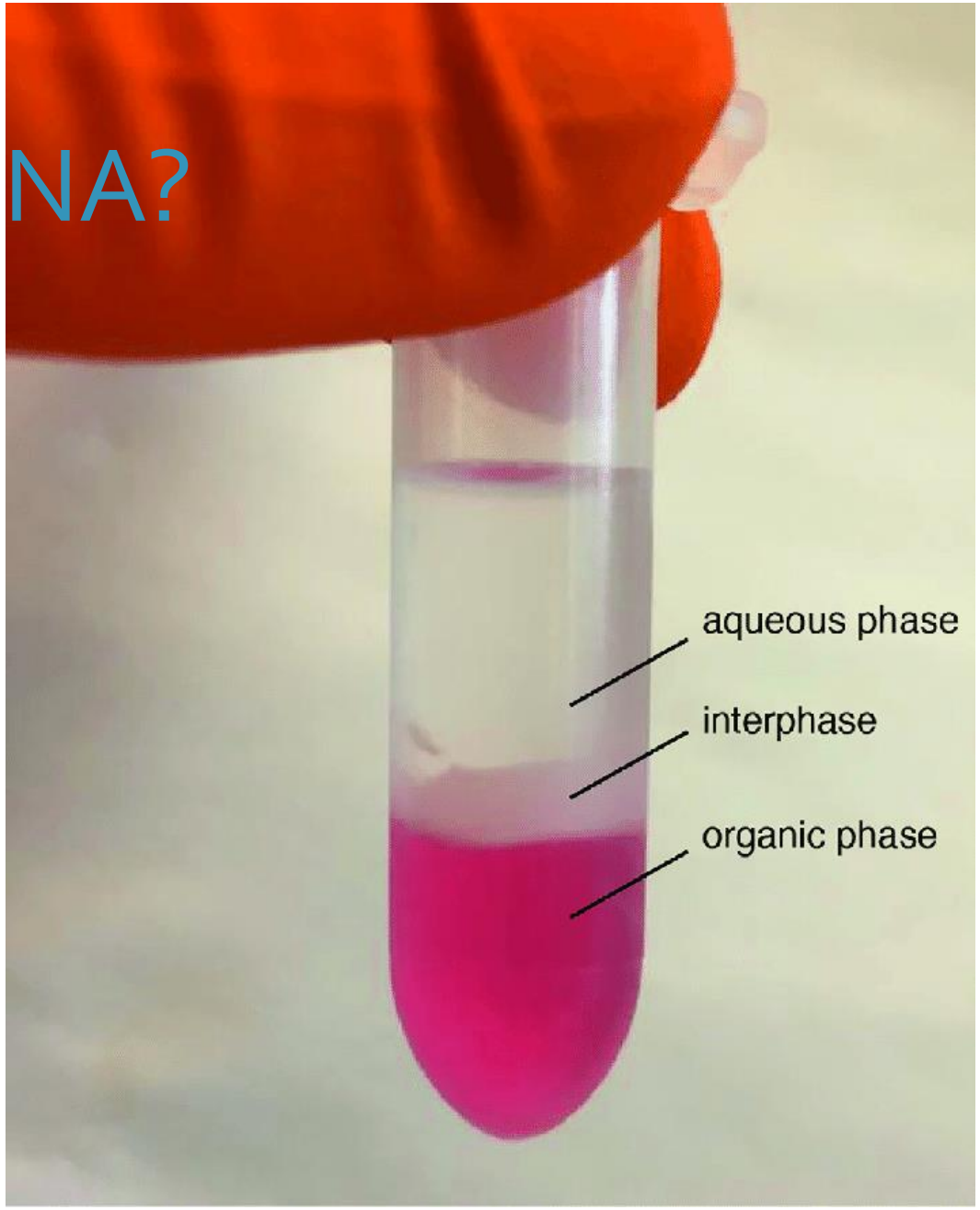
1. Homogenise sample
   - E.g. blender, mortar and pestle
2. Lyse cells
   - E.g. soap, laundry detergent, TRIzol
3. Digest proteins
   - E.g. meat tenderiser, bio laundry detergent, Trizol
4. Precipitate DNA
   - Salt
   - Alcohol

What about extracting RNA?

Separate the DNA and RNA!

But how?

- TRIzol or Phenol
- Chloroform



How are high quality nucleic acids isolated?

https://www.labxchange.org/library/items/lb:LabXchange:cea07e6f:lx_simulation:1

Method: DNA extraction stimulation

- Put on PPE.
- Collect tissue: Saliva samples are collected from each individual.
- Lyse the cells: Cells are broken open to release the cell contents.
- Clear the lysate: Separate the DNA from the rest of the cell contents.
- Precipitate the DNA: Bring the DNA out of solution.
- Wash the DNA: Remove any remaining impurities.
- Resuspend the DNA: Dissolve the DNA pellet in water.

QUIZ: DNA vs RNA sequencing workflows

https://create.kahoot.it/share/dna-vs-rna/dc230375-69de-47a5-a02f-690cdac5c35a

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Now, let's extract DNA

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Bulk nucleic acid extraction

- The nucleic acid extraction we have seen so far
- The most common type of nucleic acid extraction
- Nucleic acids are extracted from a population of cells or tissue
- The cell in which the nucleic acid originally came from cannot be determined
- The greatest identification you can make is which sample the nucleic acids came from

Applications of nucleic acid extraction in cancer research

Once nucleic acids are extracted, this tissue information can be used to study cancer.

Why study nucleic acids in cancer research:

- To study the genetic mutations or expression profiles that may lead to cancer
- To develop targeted therapies and personalised medicine.

How are nucleic acids used in cancer research

- PCR and qPCR
- NGS
- Microarrays

NGS in cancer research

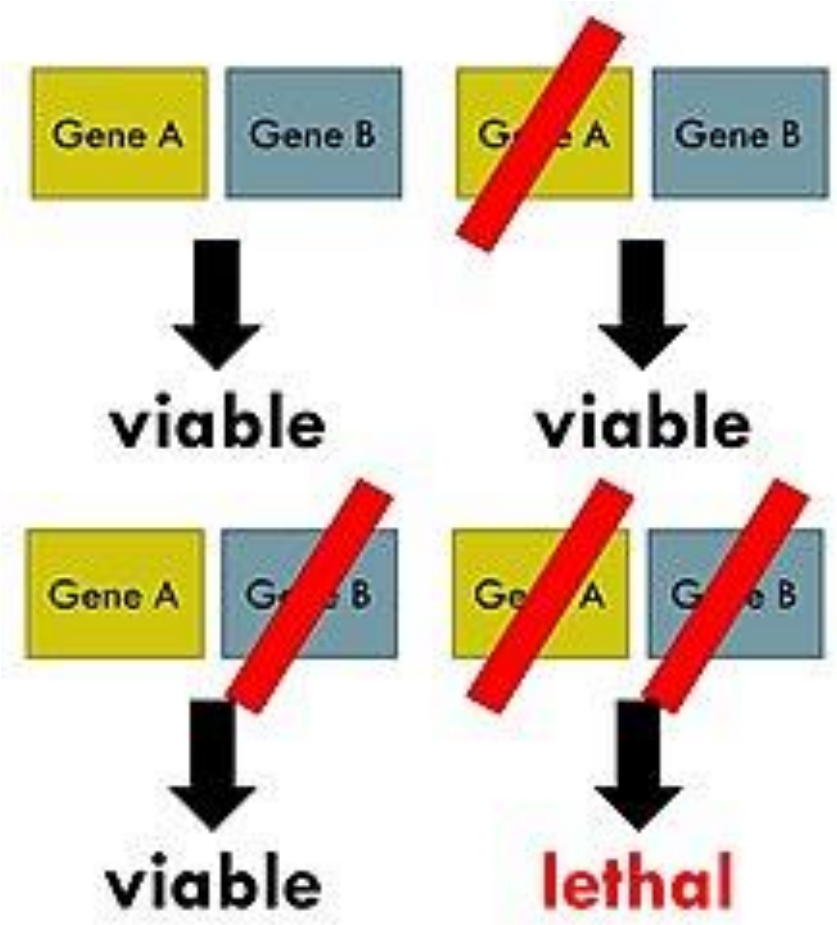
- Whole genome sequencing (NGS)
- Whole exome sequencing (WES)
- RNA sequencing (RNA-seq)

Case Study: Synthetic lethality

What is synthetic lethality?

- It is a type of genetic interaction where the inhibition of two genes is lethal while the inhibition of each single gene is not.
- This can also appear as one gene becoming essential when it's partner is dysfunctional.
- Genes that are synthetically lethal are referred to as synthetic lethal partners
- Such a phenomenon opens new opportunities for cancer treatment.

Case Study: Synthetic lethality



Case Study: Synthetic lethality

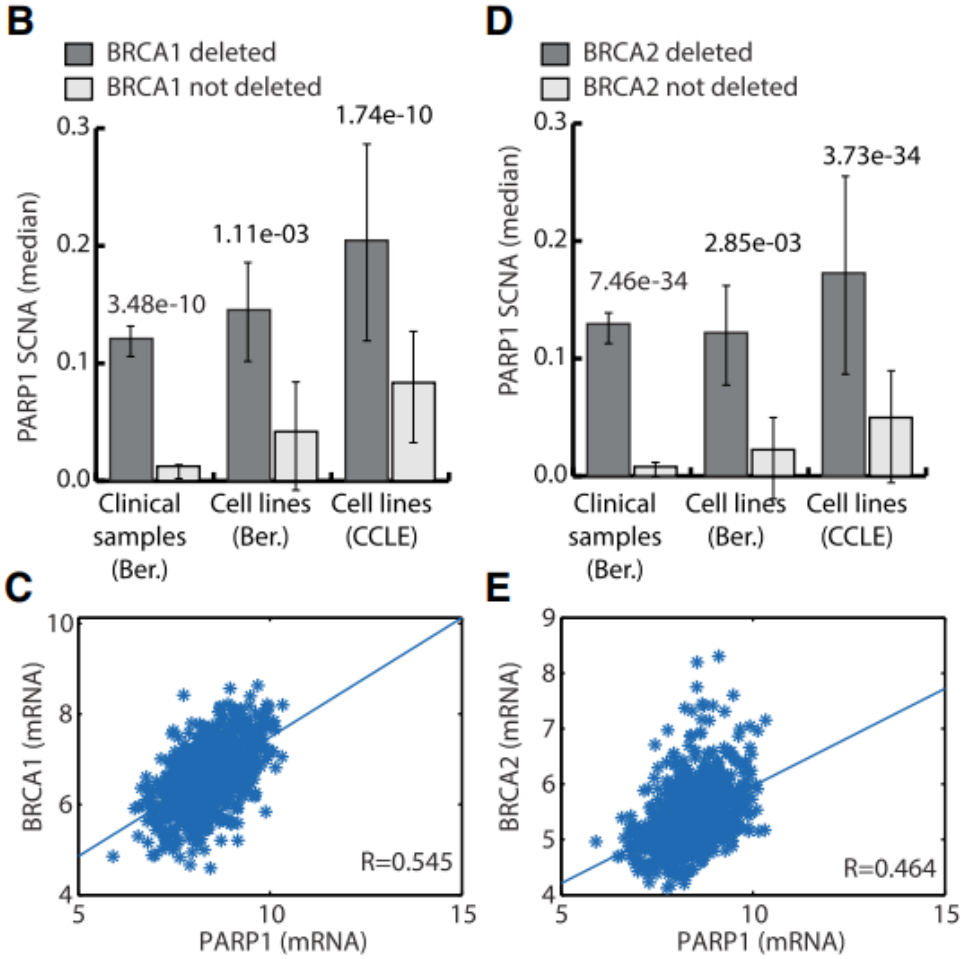
- BRCA1/2 and PARP1 are synthetic lethal partners
- Disruption of these genes are mutually exclusive in cancer cells
- BRCA and PARP are involved in the DNA Damage Response (DDR)
- In cells with a BRCA1/2 mutation, inhibition of PARP causes cell death due to accumulation of irreparable DNA damage

Case Study: Synthetic lethality

- Genetic and transcriptional data can be analysed to predict new synthetic lethal partners
- E.g. DAISY is a genomic and transcriptomic data-driven detection process that can detect synthetically lethal partners
  - Statistical inference strategies were applied to cancer genomic data
  - It is also able to show known synthetic lethal partners, such as BRCA1/2 and PARP1

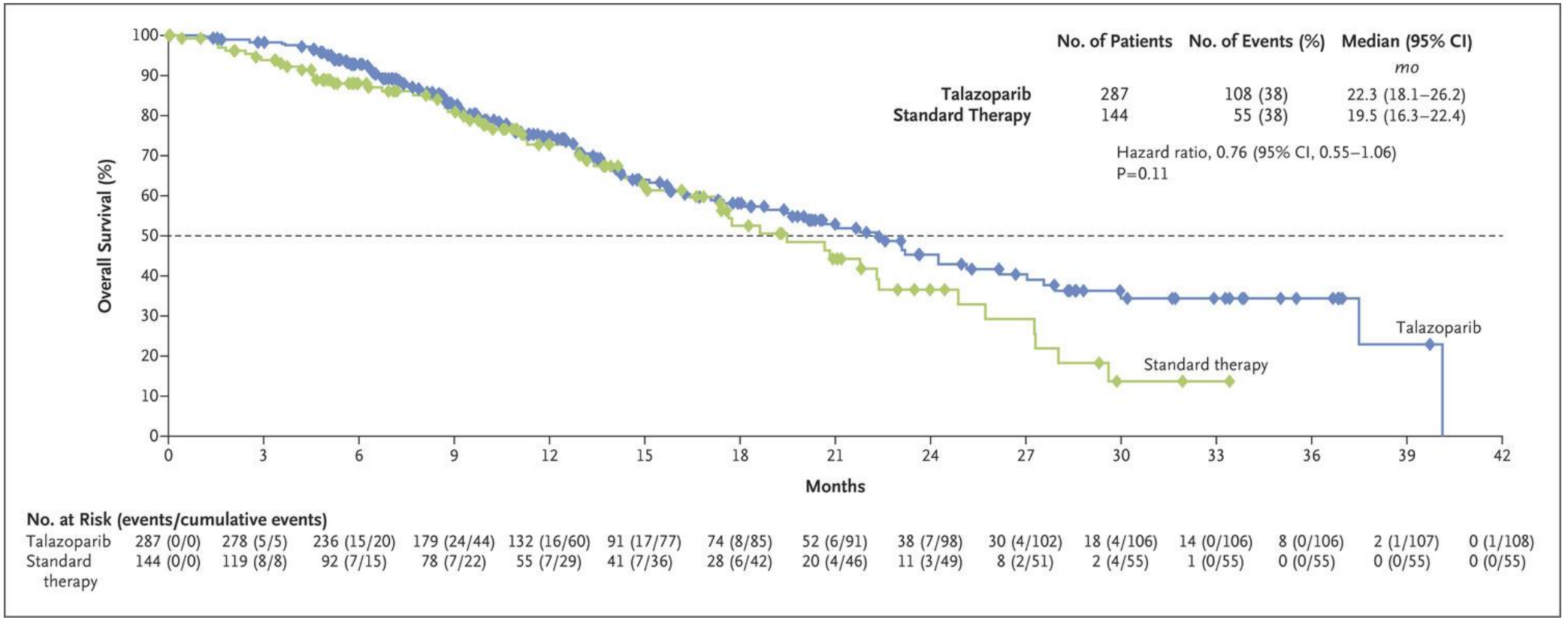
Jerby-Arnon et al. (2014)

Case Study: Synthetic lethality



Jerby-Arnon et al. (2014)

Case Study: Synthetic lethality



Litton et al. (2018)

Case study: Neoantigen Discovery

What are neoantigens?

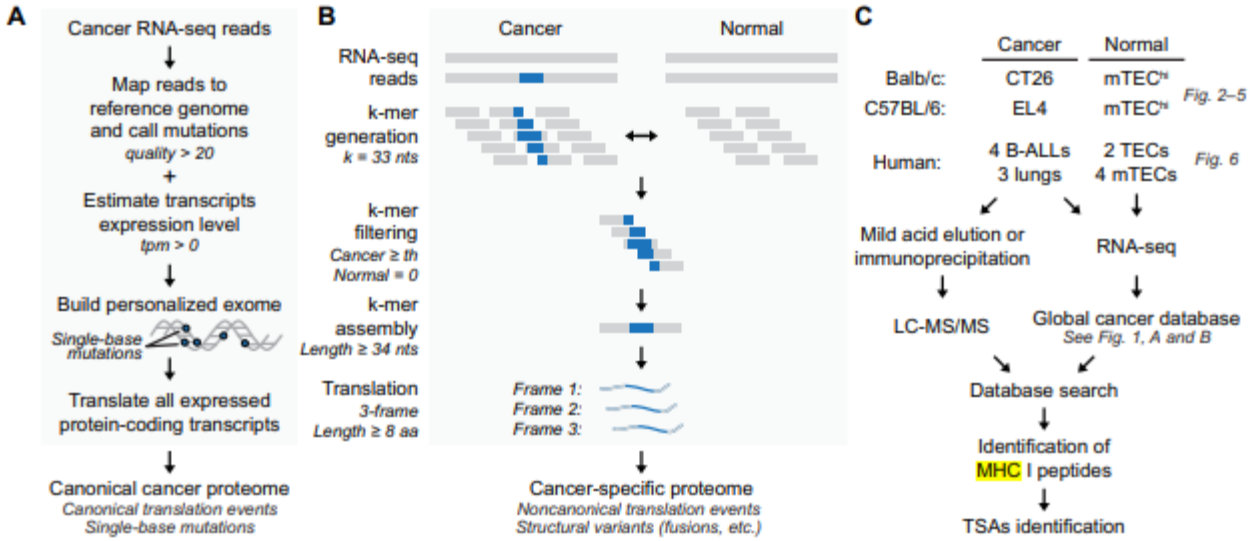
- Neoantigens are antigens that are aberrantly expressed or mutated in cancer cells.
- Should have immune tolerance
- Prime T cell immunity towards cancer-specific antigens
- Typically patient-specific
- To predict neoantigen candidates for vaccination, typically robust pipelines that begin with whole exome sequencing of the tumour are used.

- Then peptides based on mutations identified in the patient are designed and their ability to bind to MHC molecules and induce T cell responses need to be predicted and, in some cases, validated.

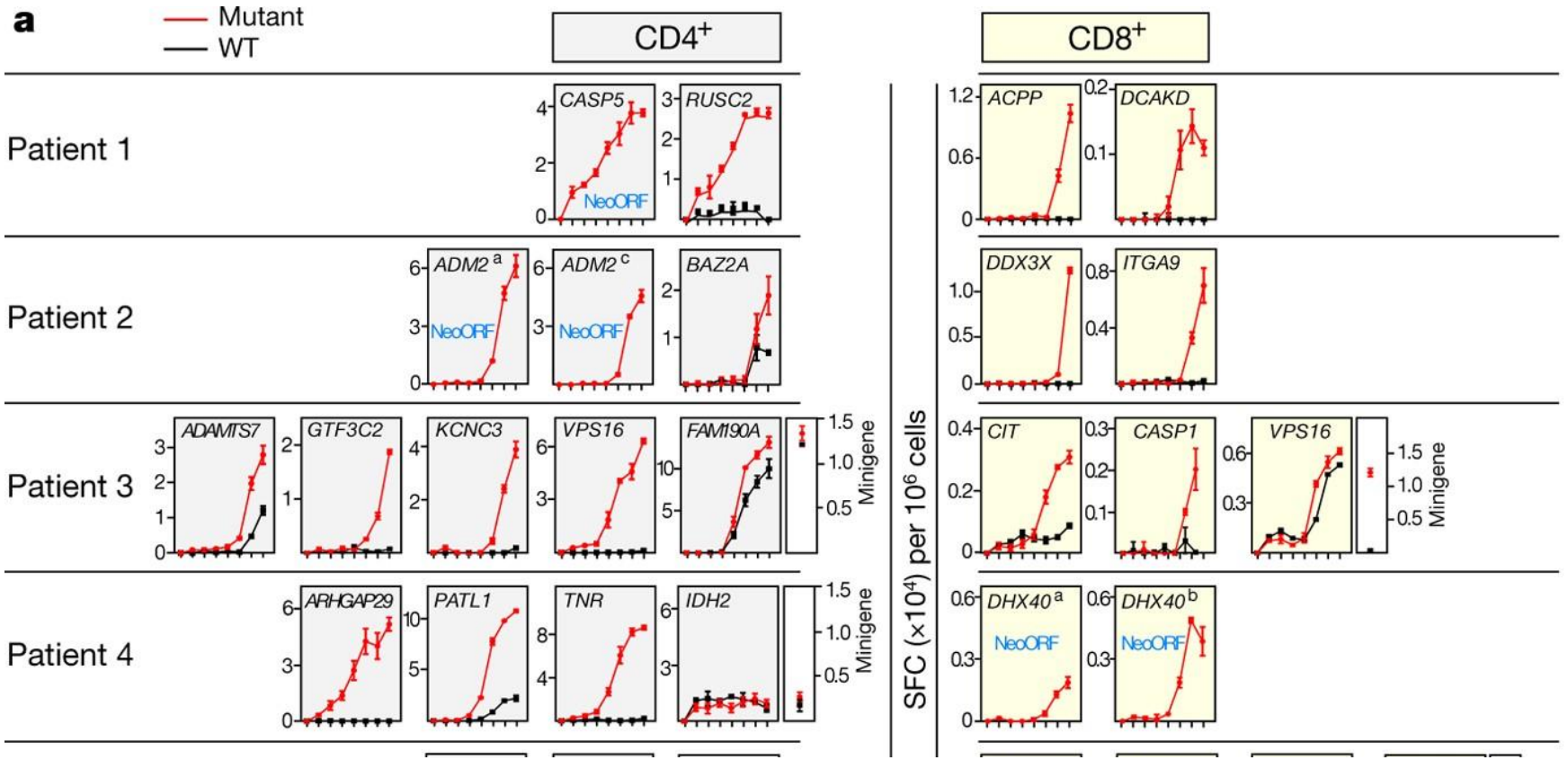
Case study: Neoantigen Discovery

Laumont et al. (2018)

- Discovered 40 tumour-specific antigens using proteogenomics



Case study: Neoantigen Discovery



Ott et al. (2017)

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4. To understand how to read a research article.

Types of Biomedical research articles

- Original Research: Reports new data from experiments or clinical trials.
- Review Articles: Summarize existing knowledge on a topic.
- Case Studies: Detailed analysis of a single patient

Structure of original research articles

1. Abstract
2. Introduction
3. Methods
4. Results
5. Discussion
6. Conclusion

1. Abstract

An overview of the article. It contains:

- Background and aim of the study
- Methods
- Results
- Conclusion

2. Introduction

- Background information
- Provides context for where the research sits in the field and why it's needed
- Outlines research aims

3. Methods

- Provides details on how the experiments were conducted
- Should have enough detail to carry out the same experiment

4. Results

Contains all the objective data:

- Text description
- Graphs
- Tables
- Pictures

5. Discussion

- Author's interpretation of the results
- Author's opinions
- Implications and significance of results
- Limitations
- Directions for future research

6. Conclusion

- Summary of key findings
- Highlights the practical or theoretical implications.

How to read a research article

1. Skim
2. Detailed read-through
3. Critical evaluation

1. Skim

1. **Title and Abstract**: Understand the focus and scope of the study.
2. **Figures and Tables:** Get an overview of the data and key results.

2. Detailed read-through

1. **Introduction**: Gain background knowledge and understand the research question.
2. **Materials and Methods**: Evaluate the study's design and reproducibility.
3. **Results**: Analyse the data and note key findings.
4. **Discussion**: Understand the interpretation of the results and their implications.

3. Critical evaluation

1. **Validity and Reliability**: Assess the accuracy and consistency of the methods and data.
2. **Strengths and Weaknesses**: Identify the strengths and limitations of the study.
3. **Relevance and Impact**: Consider the study's importance and potential impact on the field.

Additional tips

- Read critically
- Read creatively
- Use the search tool

Let's analyse a paper

Pan-cancer analysis of whole genomes

https://doi.org/10.1038/s41586-020-1969-6

The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium

Questions

1. What is the main aim of the research article? Which figure(s), in your opinion, present the key experimental findings of this paper. Justify your selection?
2. Which gene is most frequently mutated across cancers? Do you know any functions of this gene?
3. Why may mutational drivers not have been found in all the genomes studied.
4. What are the clinical applications of this study?
5. Does this study have any limitations?

1. Aim

Answer: To conduct an integrative analysis using multiple cancer WGS databases.

Where to find answer: **Abstract** and **The pan-cancer analysis of whole genome**

Abstract

enabled systematic documentation of this variation at the whole-genome scale[1–3]. Here we report the integrative analysis of 2,658 whole-cancer genomes and their matching normal tissues across 38 tumour types from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA). We describe the generation of the PCAWG resource,

The pan-cancer analysis of whole genome

Given the recent meta-analysis of exome data from the TCGA Pan-Cancer Atlas[23–25], scientific working groups concentrated their efforts on analyses best-informed by whole-genome sequencing data.

1. Aim

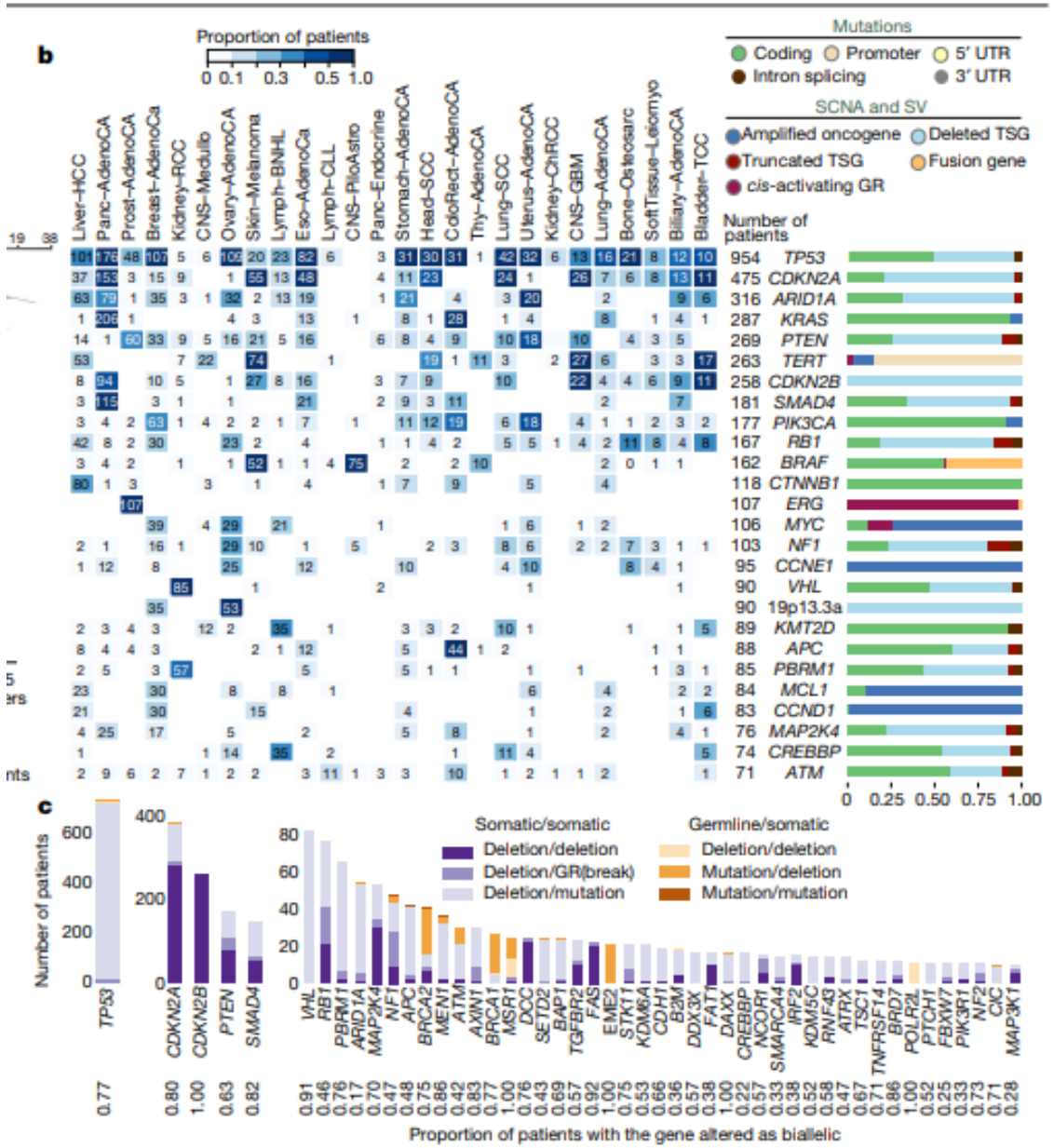


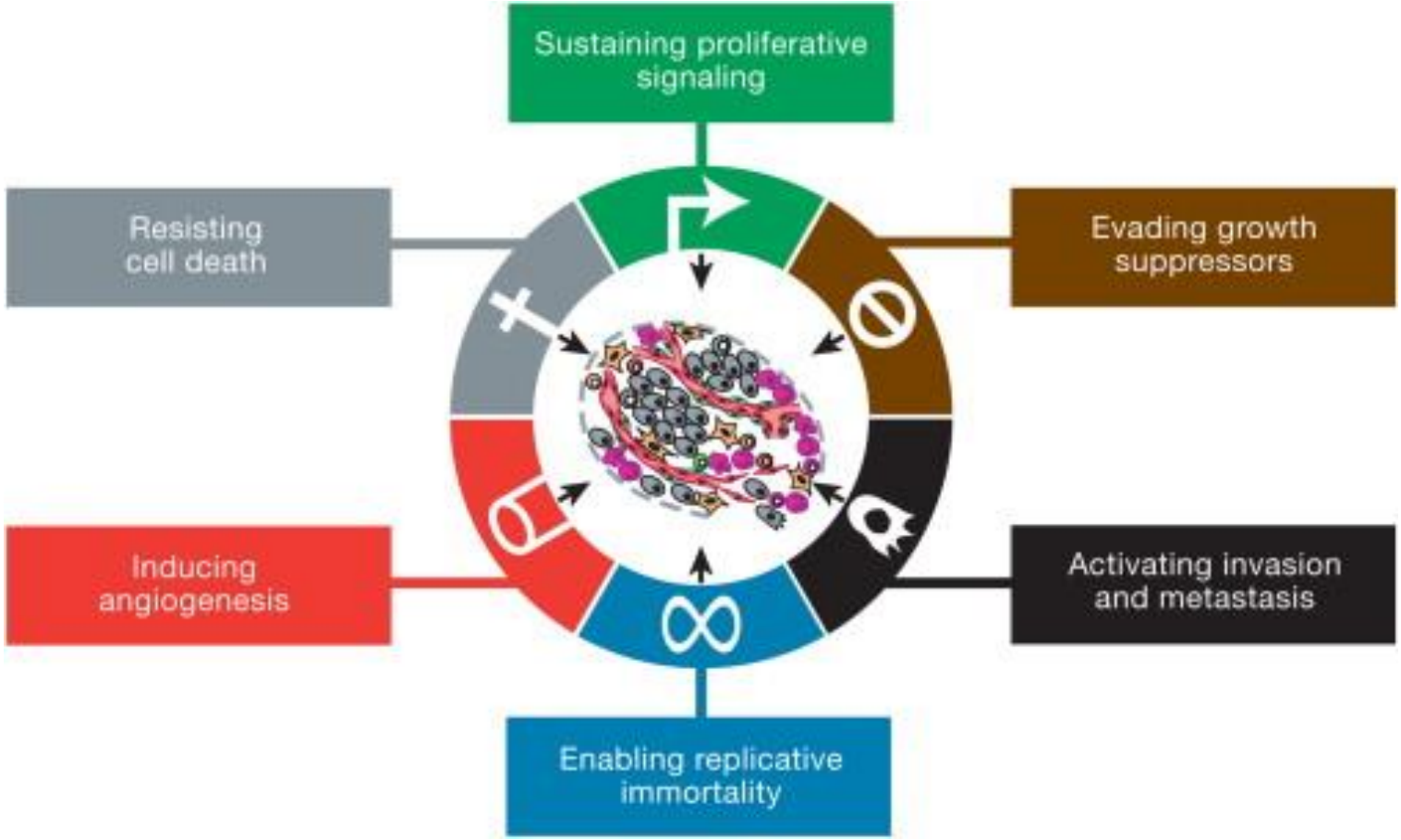
Figure 2b

2. Most frequently mutated gene

Answer:

- TP53
- It encodes p53—one of the most studied tumour suppressor genes
- Frequently found mutated in tumours
- If not directly mutated, then pathways/interactions are likely mutated in tumours.
- Halts the cell cycle before S phase via p21 or before mitosis via 14-3-3σ
- Triggers caspase-dependent apoptosis. I.e., p53 is pro-apoptotic and anti-cell-cycle.
- Where to find answer: **Abstract** and **The pan-cancer analysis of whole genome**

2. Most frequently mutated gene



Hanahan and Weinberg (2011)

3. Why are mutational drivers not always found?

Answer:

- Poor quality samples
- Inadequate sequencing
- Failure of bioinformatic algorithms
- Tumour may be driven by gene that has not yet be described for that tumour type (i.e. novel drivers)

Where to find answer: PCAWG tumours with no apparent drivers

4.Clinical applications

Answer: Drug targets and precision medicine

Where to find answer: **Discussion**, **conclusion** and **creativity**

Conclusion and future perspectives

The promise of precision medicine is to match patients to targeted therapies using genomics. A major barrier to its evidence-based imple-

5. Limitations

- Answer: Lack of ancestral diversity
- Where to find answer: **Methods**

Methods

(Supplementary Table 1). Using population ancestry-differentiated single nucleotide polymorphisms, the ancestry distribution was heavily weighted towards donors of European descent (77% of total) followed by East Asians (16%), as expected for large contributions from European, North American and Australian projects (Supplementary Table 1).

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Summary

- To extract nucleic acids from cells you must homogenise the sample, lyse the cell membrane, digest proteins, precipitate nuclei acids.
- Extracted nucleic acids can be used to study cancer biology. For example:
  - Synthetic lethality
  - Neoantigens
- Original research articles typically consist of an abstract, introduction, methods, results, discussion and conclusion.
- To read a paper you must:
  1. Skim
  2. Read in detail
  3. Evaluate critically

Any questions?