Tutorial 4

Development, cell cycle and stem cells

Immune system

You are now in a position to understand some of the key data on Covid-19 vaccines

You want to figure out whether spike protein is the "best" antigen for vaccination

- You take three SARS CoV2 proteins found on the surface: spike protein (S), nucleocapsid protein (N) and membrane protein (M)

- You make large quantities of these proteins by expressing them in bacteria (*E. coli*). Have a short discussion with your classmates and your TA about how you would do this.

[Concepts to recall from the lectures: DNA can be introduced into bacteria; promoters and operators allow us to transcribe mRNA of a protein in a regulated manner; universal genetic code]

- Next, you take B cells from human donors and add each of the three proteins to the B cells.

You measure the concentration of antibodies produced by B cells in response to each antigen

How will you measure antibody concentration?

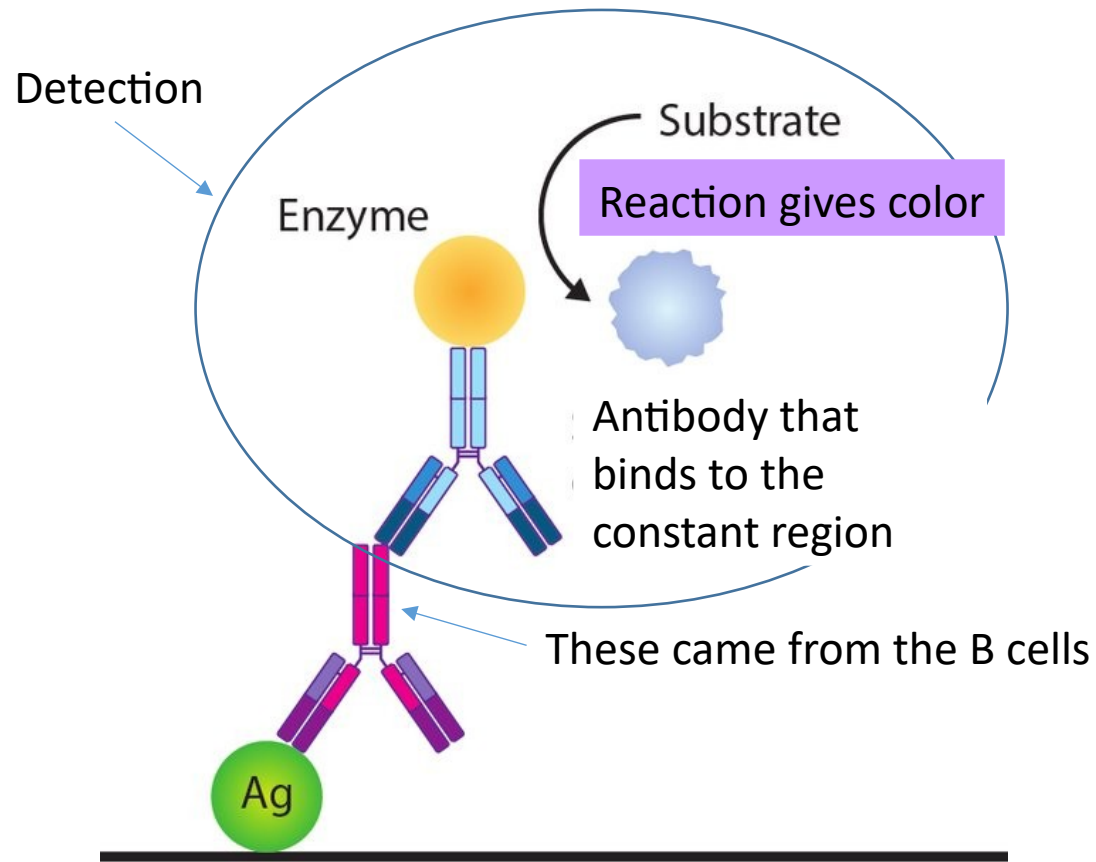Concept to remember: each antibody is highly specific to its antigen

Steps:

We coat different concentrations of antigen on a substrate

Next, we take the extra-cellular medium that the B cells are suspended in (why? because antibodies are released into the extra-cellular medium) and add it to the antigen

Antibodies bind to the antigen and we detect the antibodies
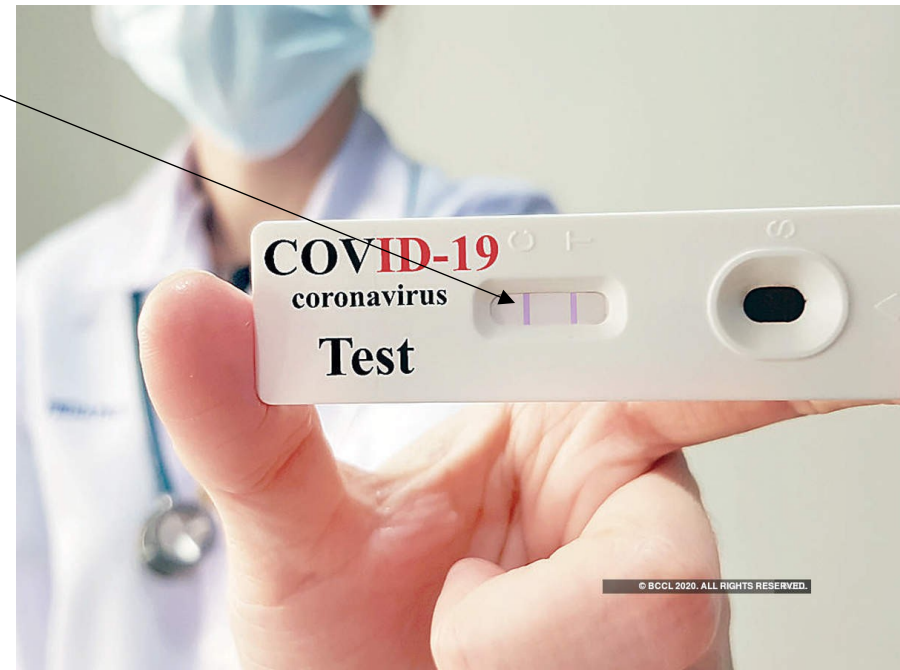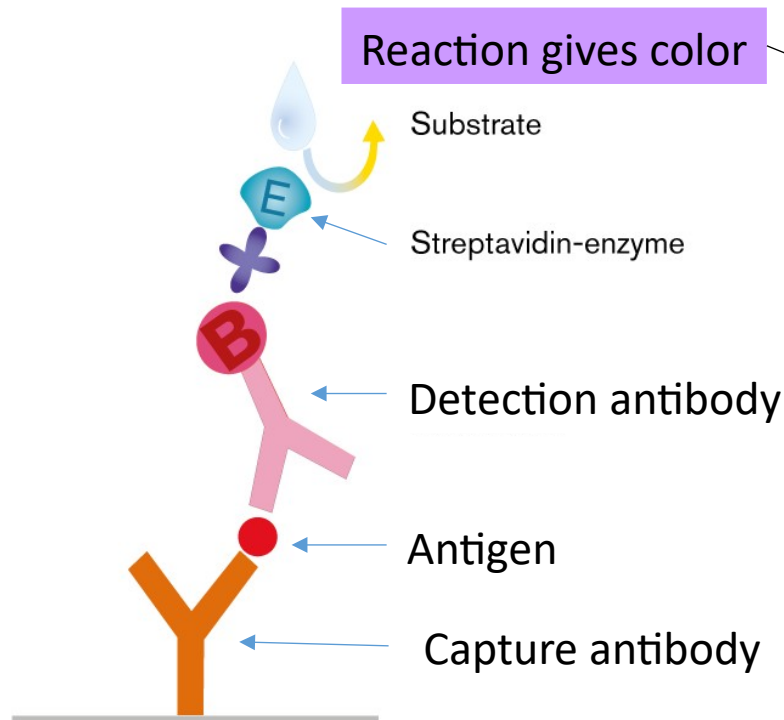
Detecting antibodies

Detection

Substrate

Reaction gives color

Enzyme

Antibody that binds to the constant region

These came from the B cells

Ag

Indirect ELISA

Results

Antigen	Concentration of antibodies (arbitrary units)
S	10,000
N	100
M	10

Which antigen is able to activate memory B cells best?

The specificity of antibodies is used in Rapid Antigen Tests

- In the previous slide, you detected antibodies, using the antigen
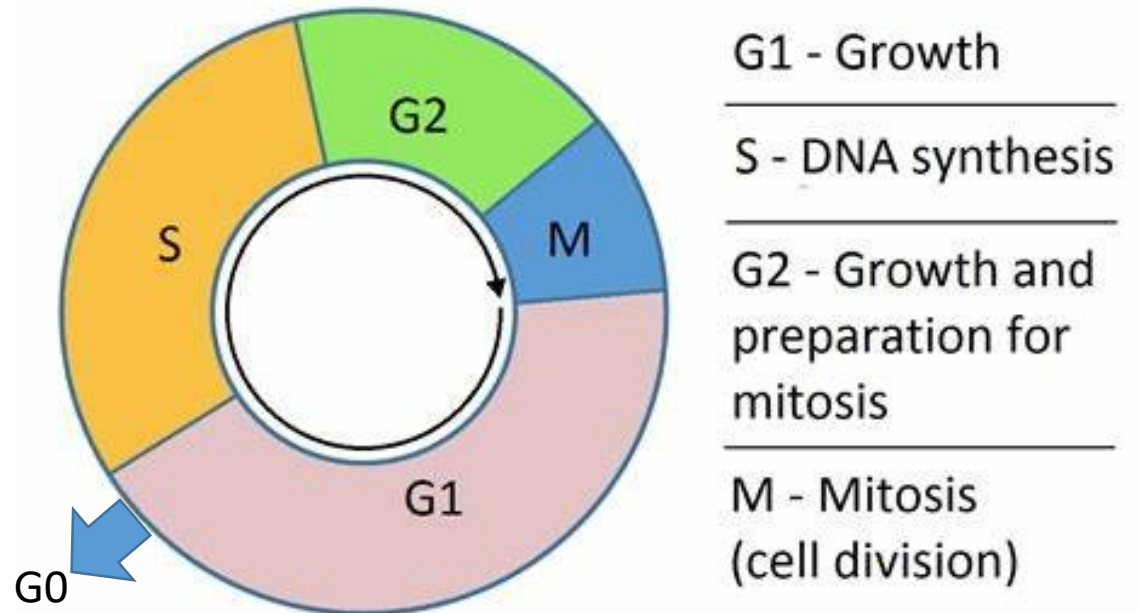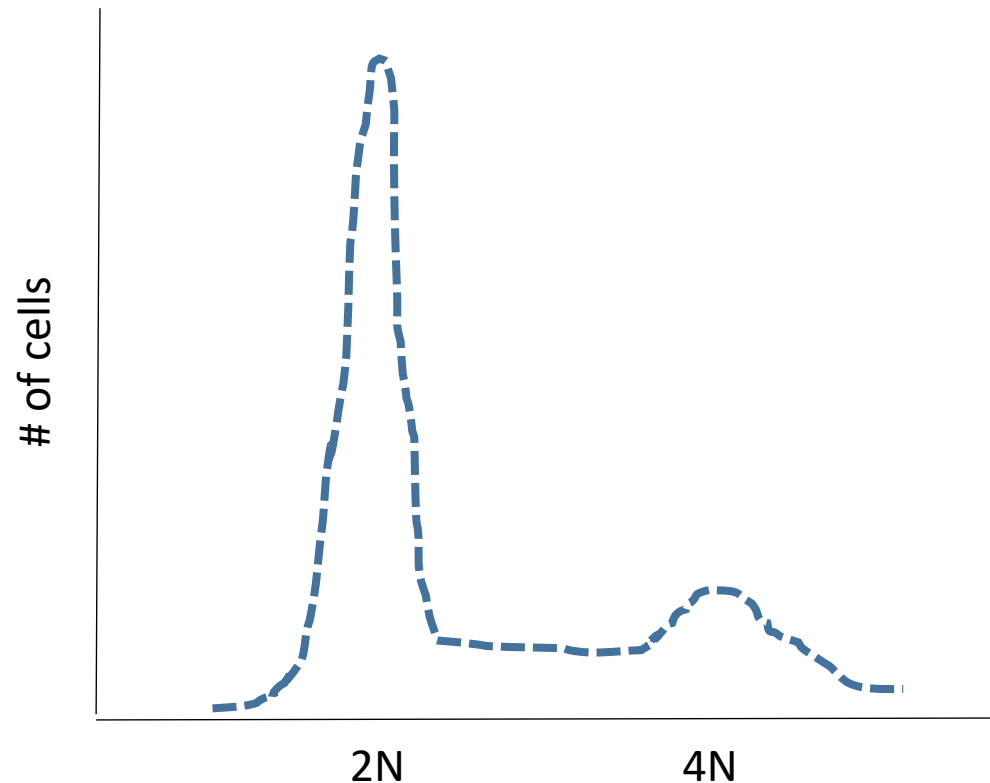- You can also detect antigen, using antibodies

Reaction gives color

Substrate

E Streptavidin-enzyme

Detection antibody

Antigen

Capture antibody

COVID-19
coronavirus
Test

You next isolate T cells and want to perform a FACS assay for proliferation (details not important)

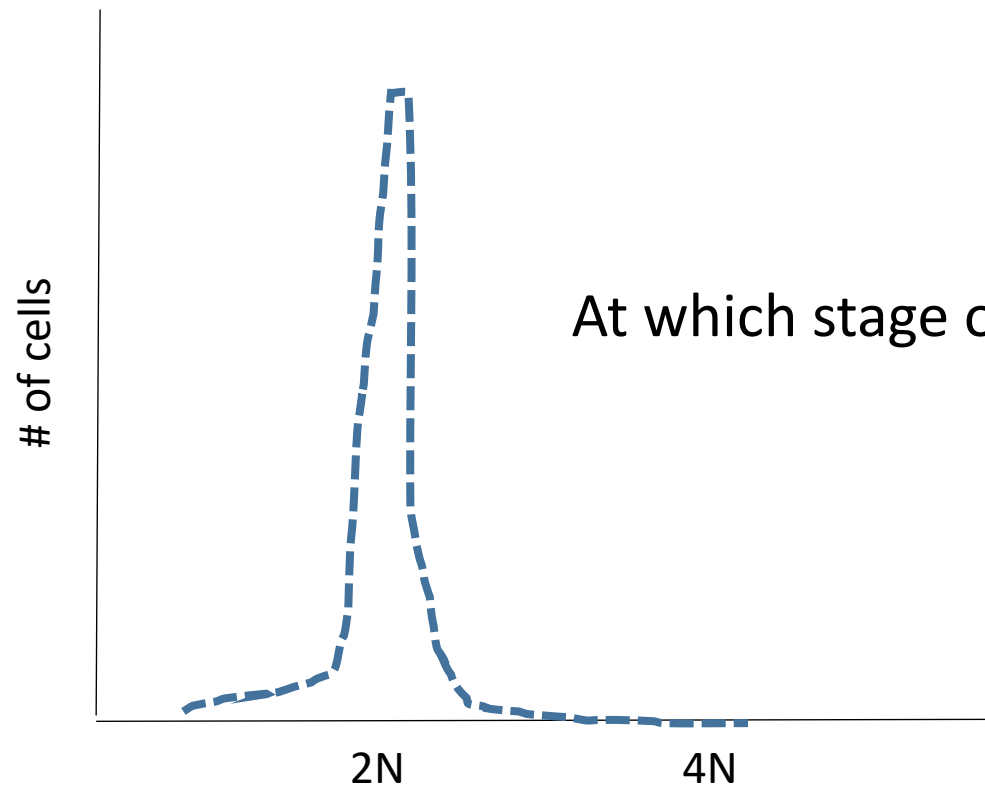In the data, N is one copy of the genome, 2N is diploid.

The output of the assay is a measure of the genome content of the cell population.

Below is shown a graph of a dividing cell population.

Based on the genome content, mark the phase of the cell cycle on the graph.

G1 - Growth

S - DNA synthesis

G2 - Growth and preparation for mitosis

M - Mitosis (cell division)

You take T cells that have not been treated with any antigen and perform FACS

At which stage of the cell cycle are these T cells?

You treat T cells with each of the three antigens and perform FACS

Treatment with N and M antigens

Treatment with S antigen
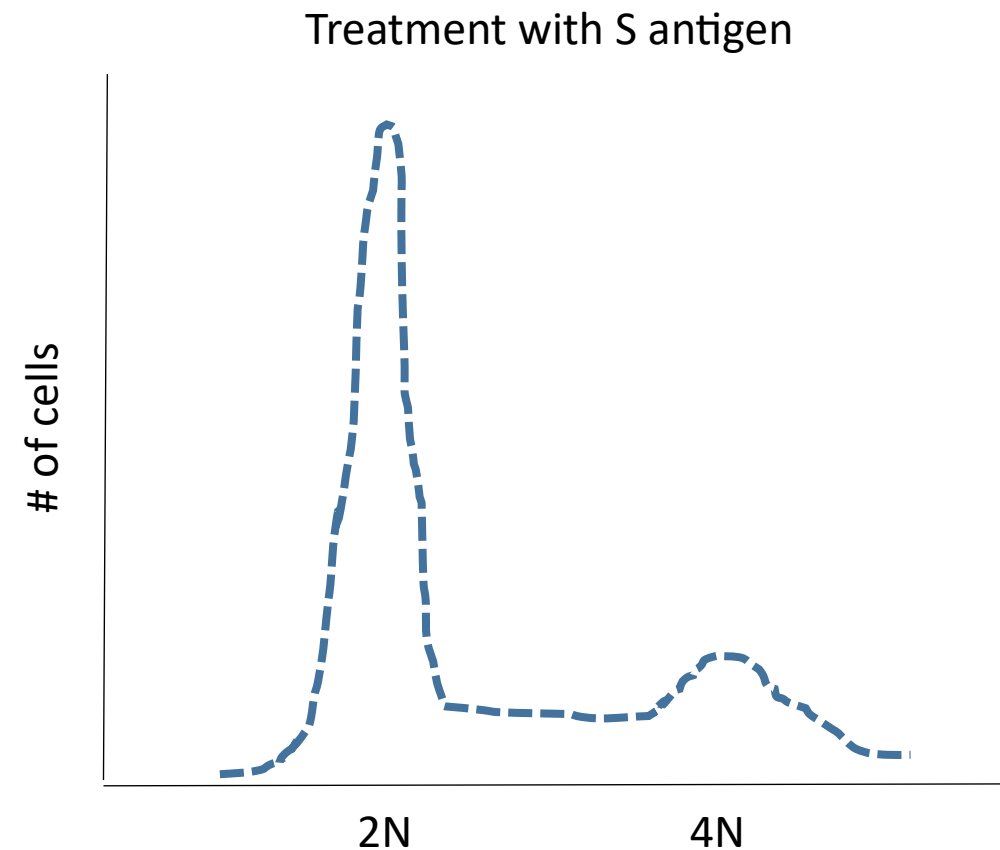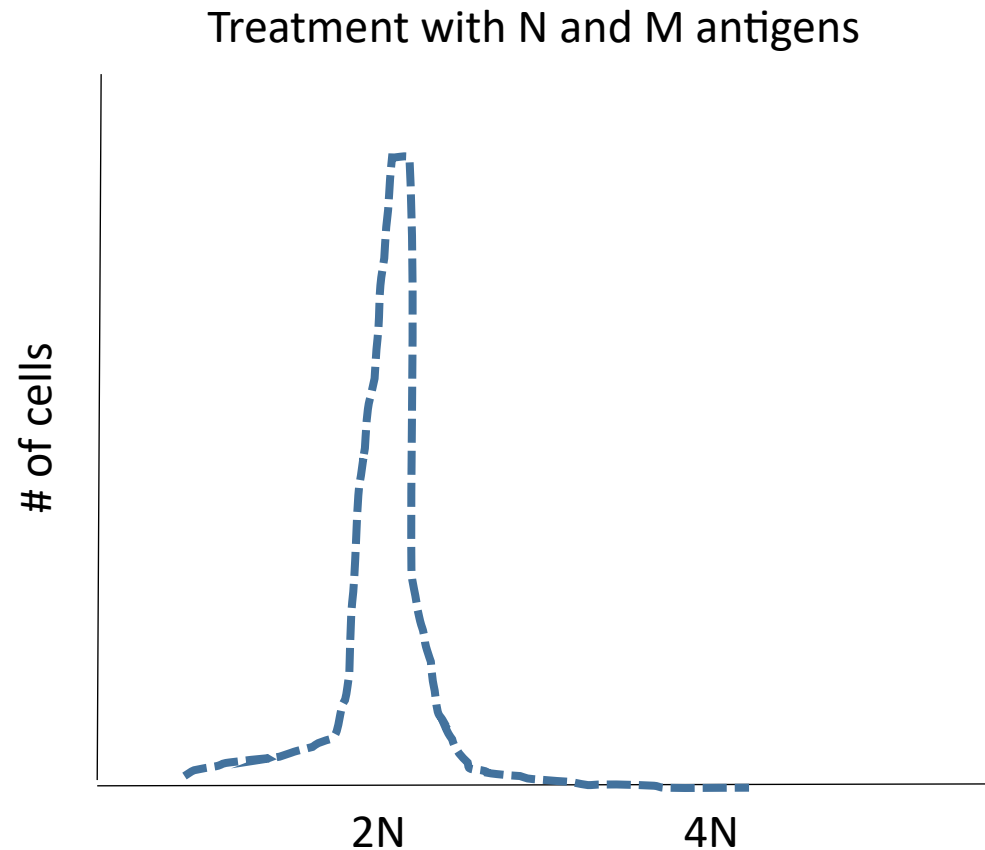
Which antigen is causing T cells to proliferate? Explain briefly, based on the data.

Which SARS CoV2 antigen would you choose for your vaccine?