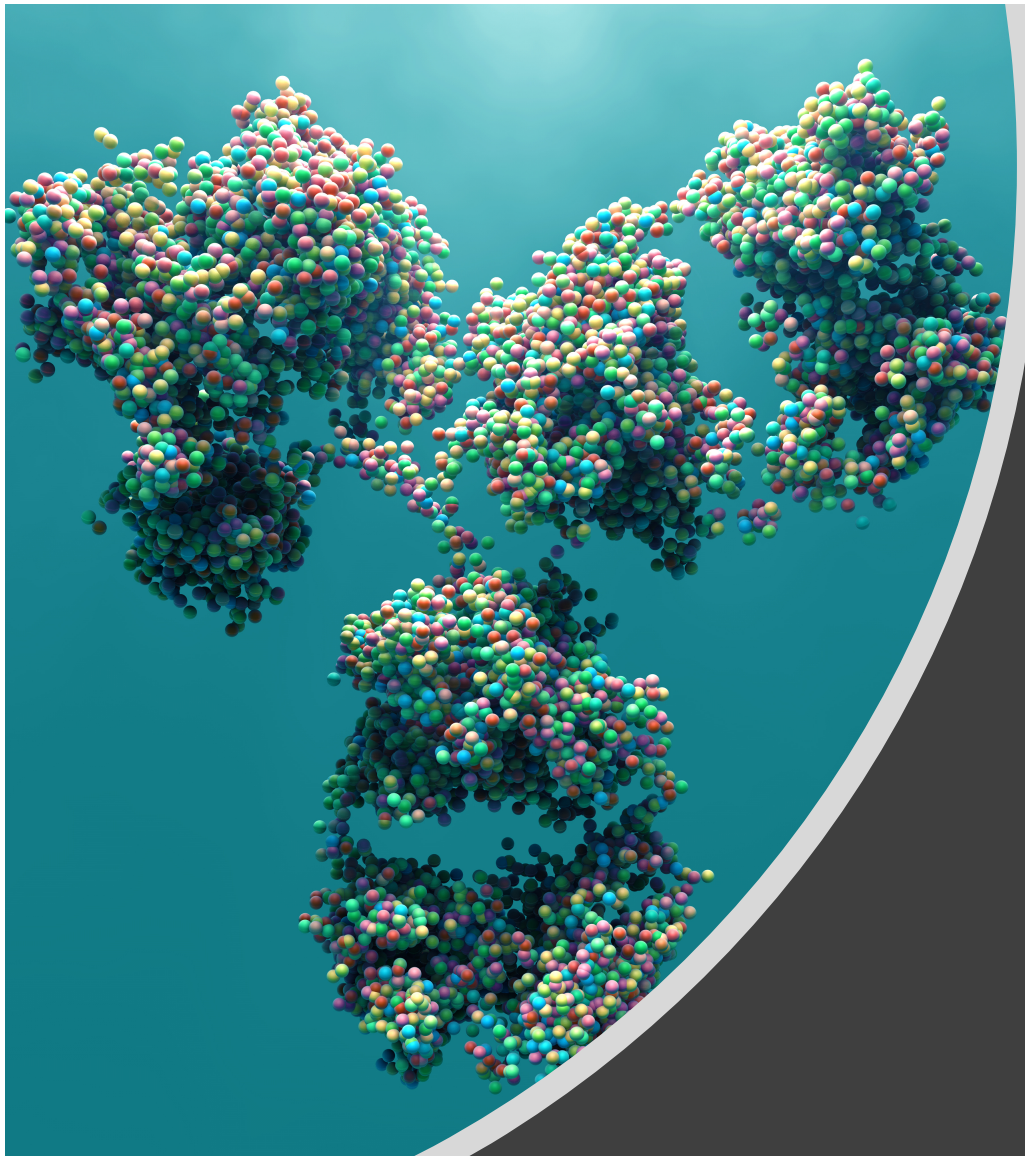
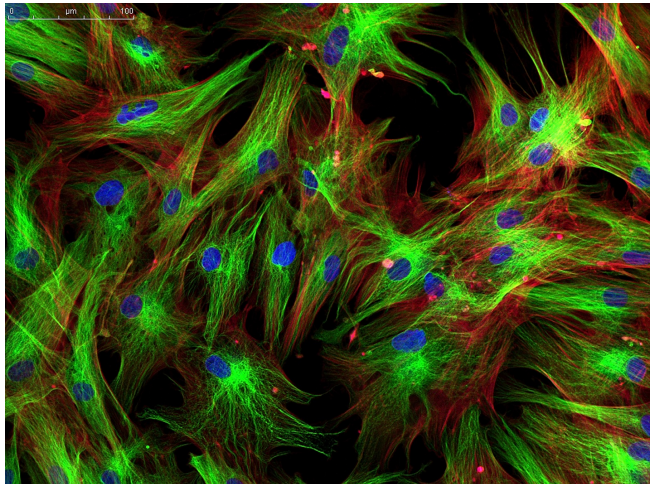


Antibodies as tools for scientific discovery, medicine and diagnostics

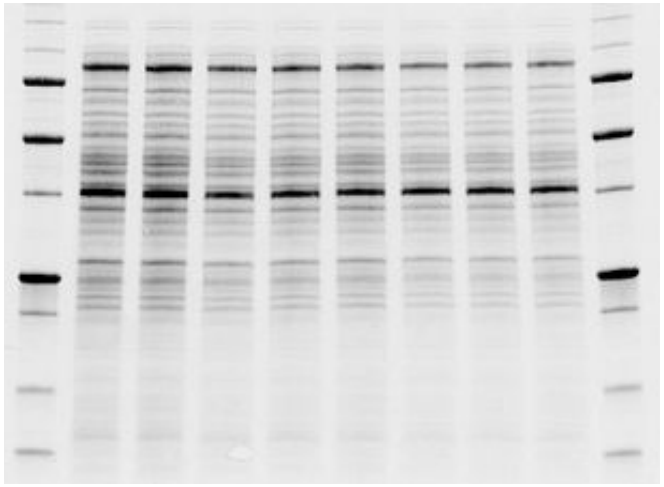
Antibodies are indispensable tools

- Antibodies have high specificity and selectivity for antigens
- On average, months to years of screening and validation must be carried out to have a characterized antibody
- A well characterized antibody can be modified to be used in many assays
  - Depending on antibody the antigen(s) need to be folded or unfolded

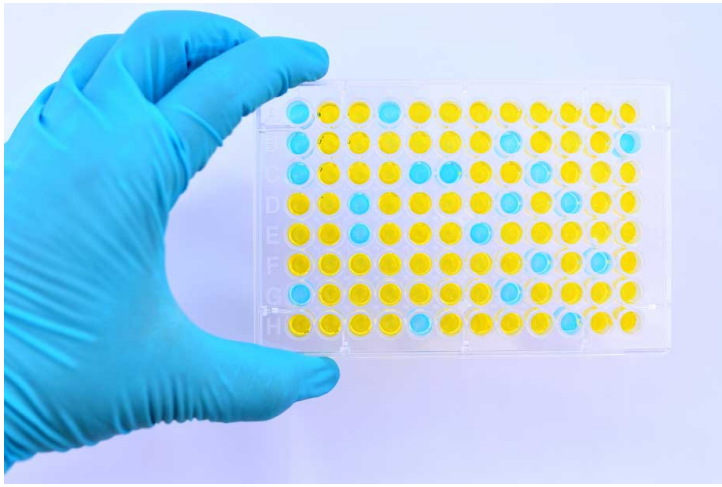
Immunofluorescence
And fluorescence microscopy



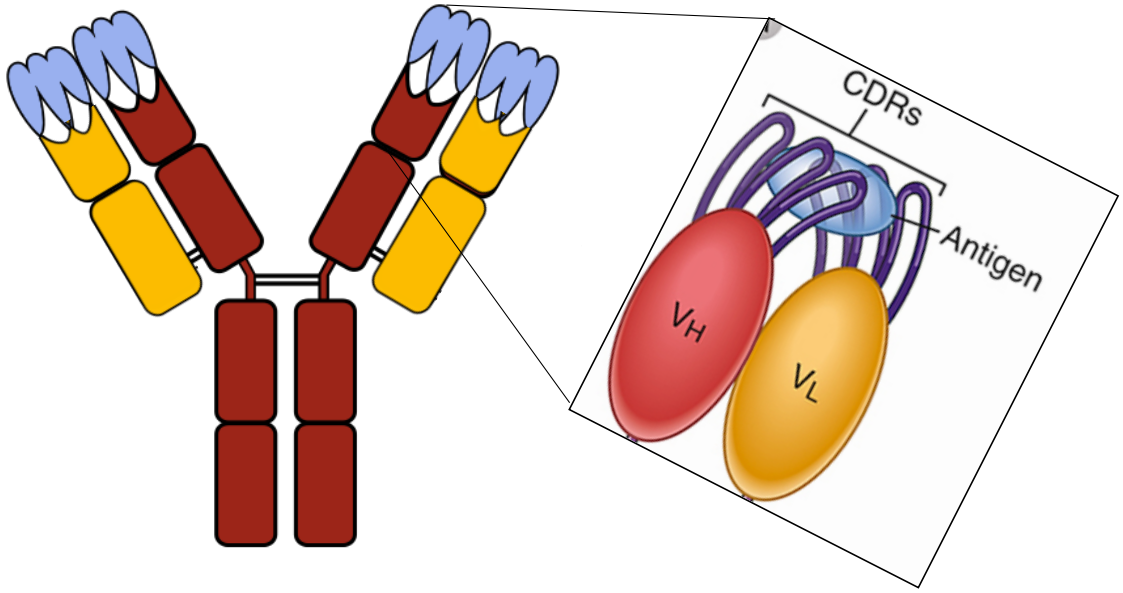
Western Blot



ELISA: enzyme-linked
immunosorbent assay

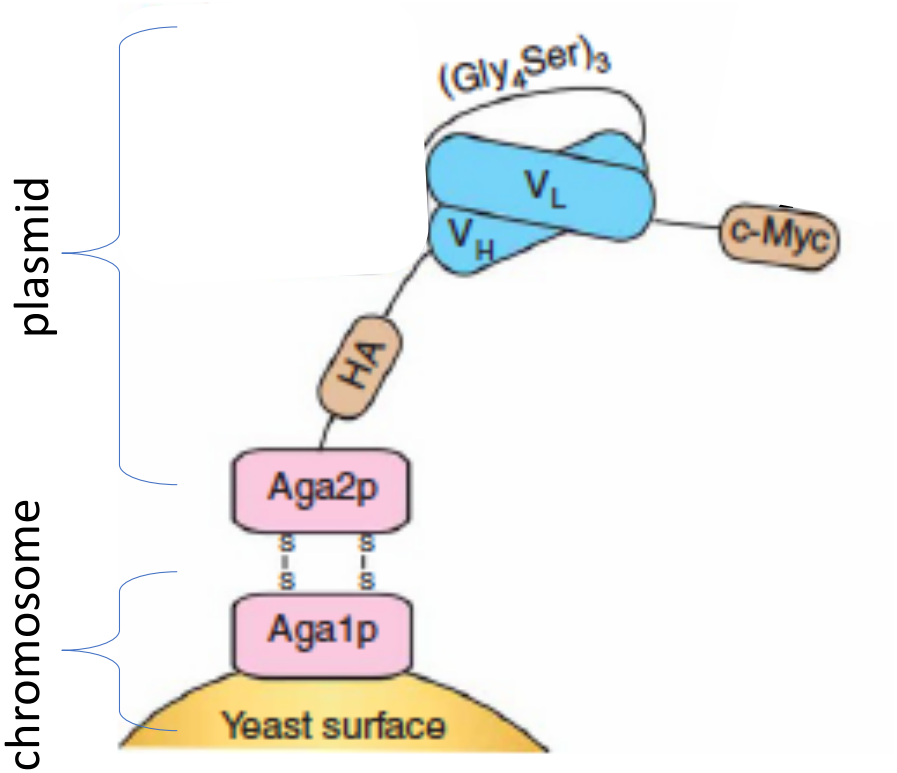


Mod3: Characterization of clones of antibody fragments (scFvs) that bind lysozyme



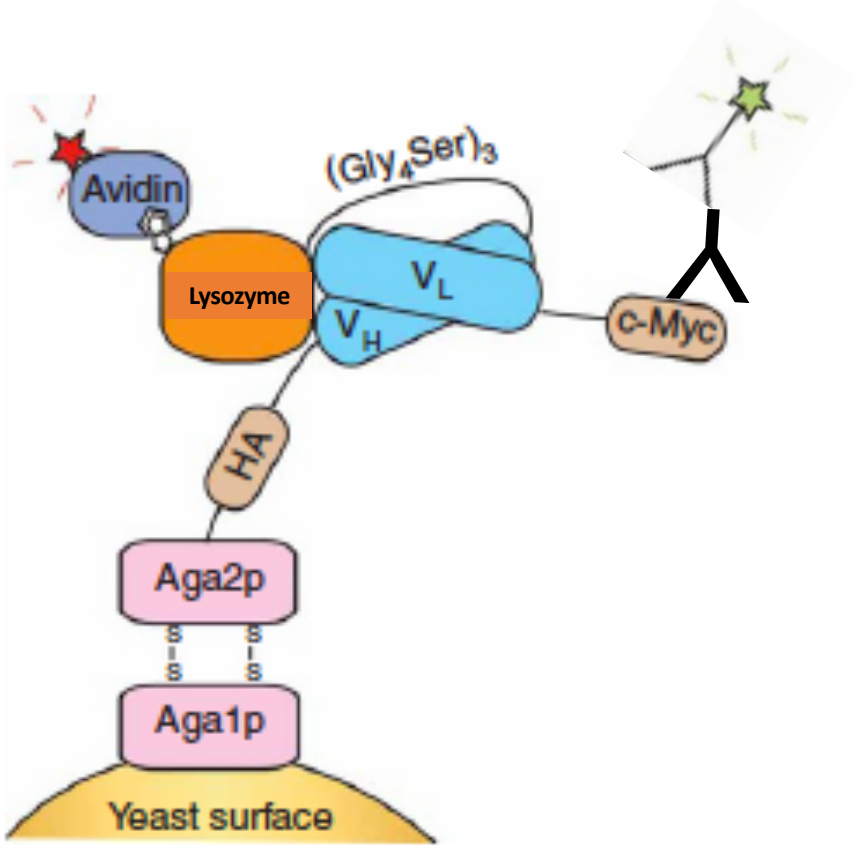
- The goal of this screen is to find a clone with a higher K_d to lysozyme
- Antibody with a lower K_d for its antigen means a more stable interaction and a higher affinity
- On day 1 we (would have) sorted a library of scFv yeast that bind to lysozyme
- We will determine the dissociation constant of a single clone scFv with lysozyme

Yeast display single chain variable antibody fragments (scFv)



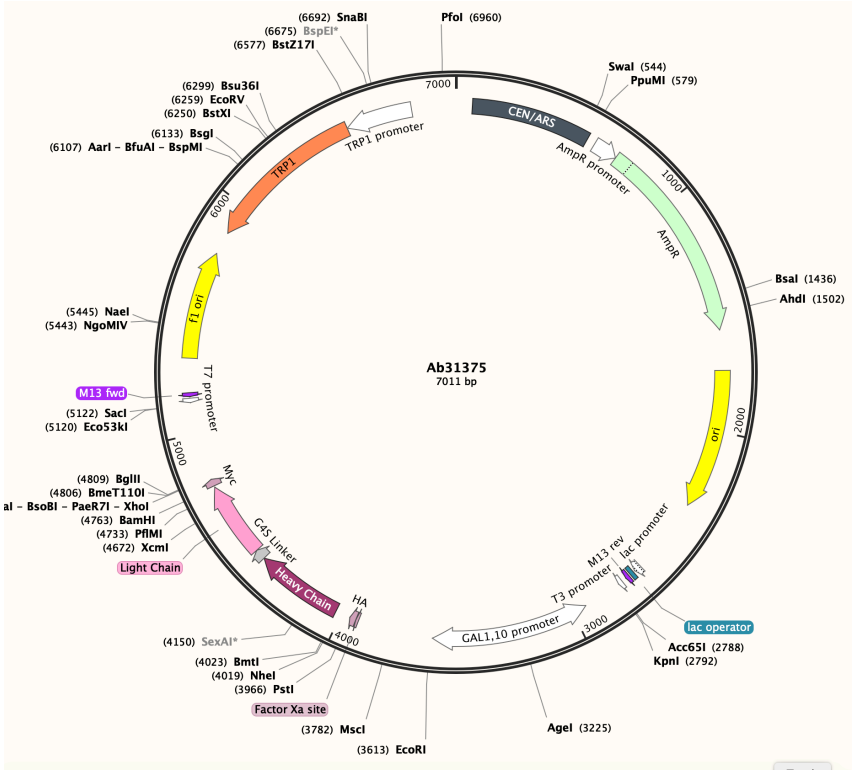
- scFv, single fusion peptide of variable region of light and heavy chain, connected by linker
- scFv fused to Aga2p which attaches to yeast cell wall via a disulfide bond with Aga1p
- The scFv is folded in the endoplasmic reticulum taking advantage of the chaperones and quality-control 'machinery'

Fluorescently labeled antibodies and streptavidin identify scFv expression and antigen binding respectively



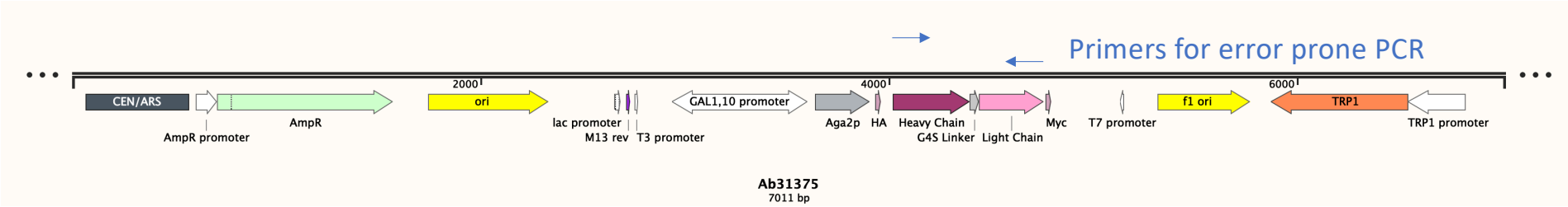
- To measure scFv expression we use a primary antibody to c-MYC and fluorescently labeled secondary antibody against primary antibody constant region
- To measure lysozyme binding we use fluorescently labeled streptavidin

The yeast display plasmid is maintained episomally with nutritional selection, TRP1

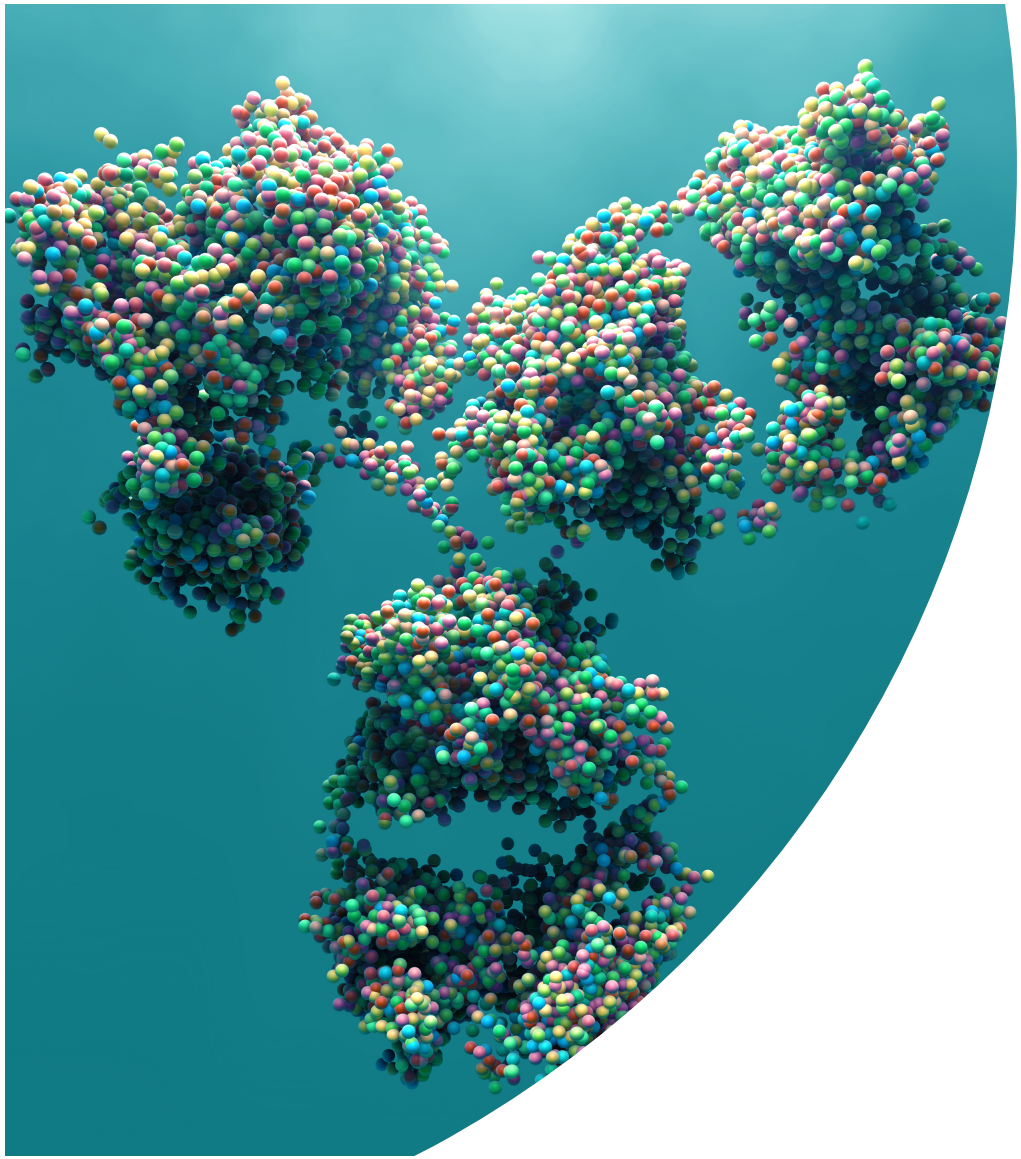


- The strain of yeast we use are incapable of making tryptophan and the media is carefully made to exclude this amino acid
  - The TRP1 gene synthesizes this AA and allows yeast that maintain the plasmid to survive
- This system allows flexibility for directed evolution or rational design
- Our library was made using error prone PCR specific to the scFv sequence
- scFv is inducible via the galactose

Design of Error prone PCR of scFv clone Ab31375



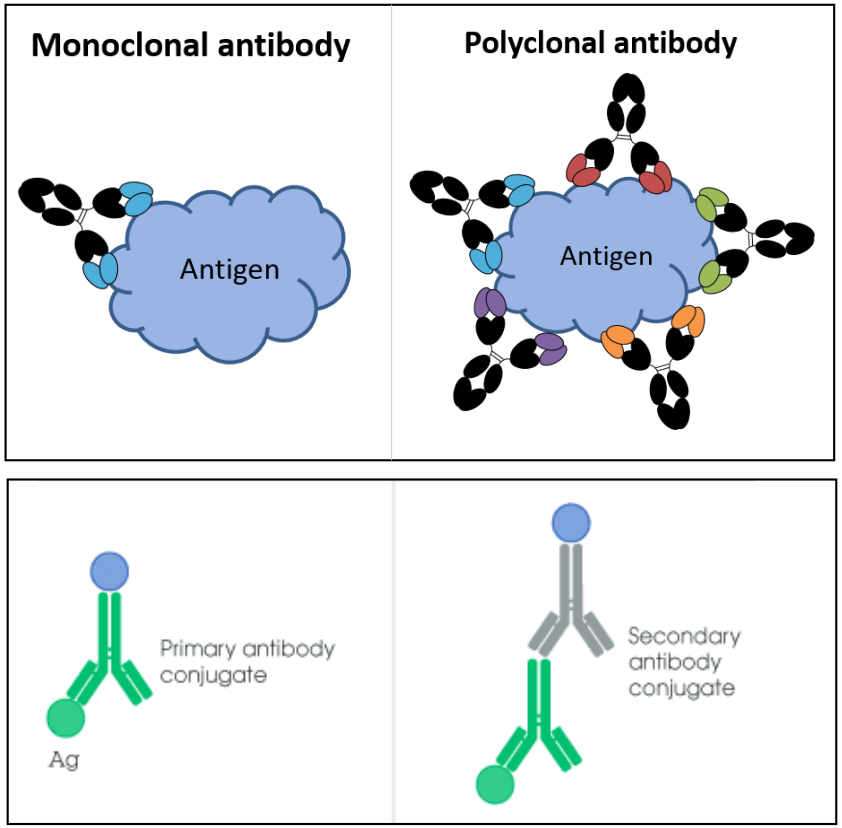
- Clone Ab31375 bind lysozyme with high affinity (measured K_d 6nM)
- Specific PCR buffer conditions and modified dNTPs introduce mutations
- 10 cycles of PCR result in 1-9 amino acid changes in most PCR products
- This product (~900bps) was gel purified and ligated into the backbone plasmid
- The plasmid was transformed into yeast that can not make Trp
- Only yeast that have taken up a plasmid will grow on our Trp- selective media



How would you engineer an antibody to your favorite molecule?

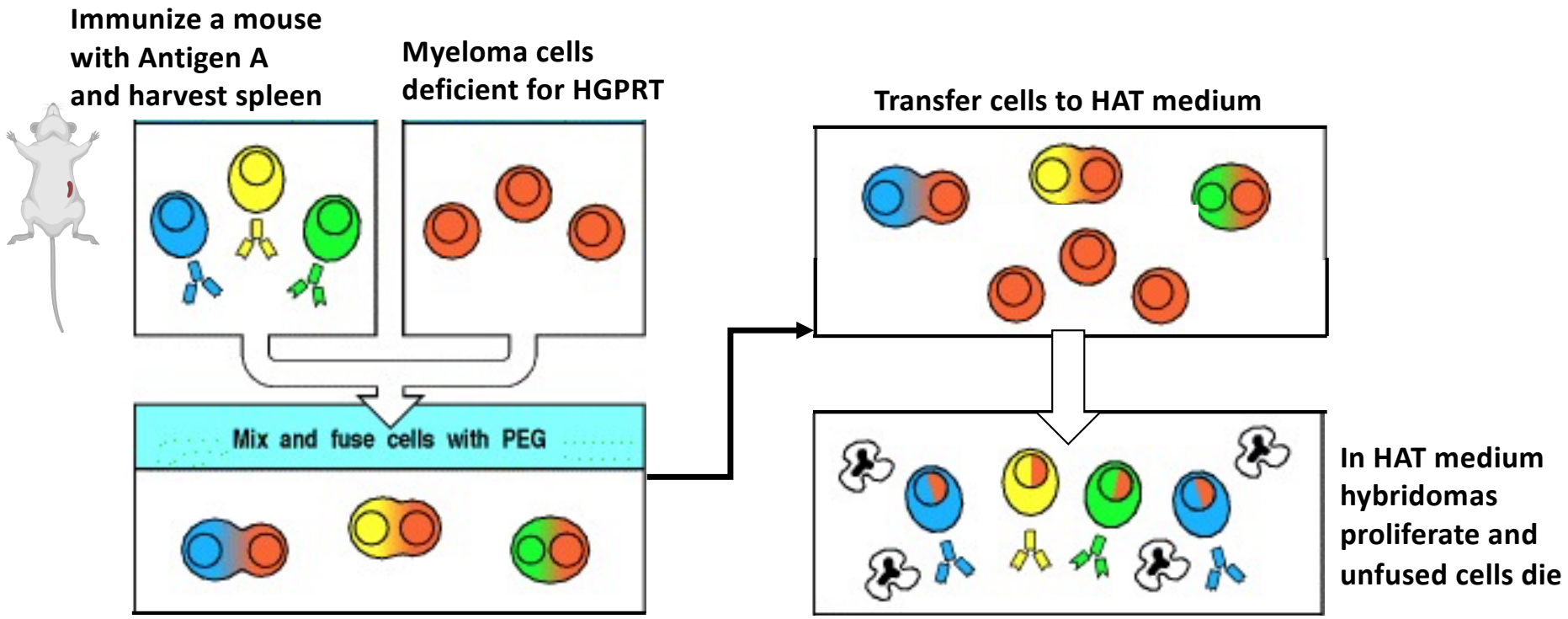
What are considerations for your approach?

Antibodies can detect picogram/mL of a molecule

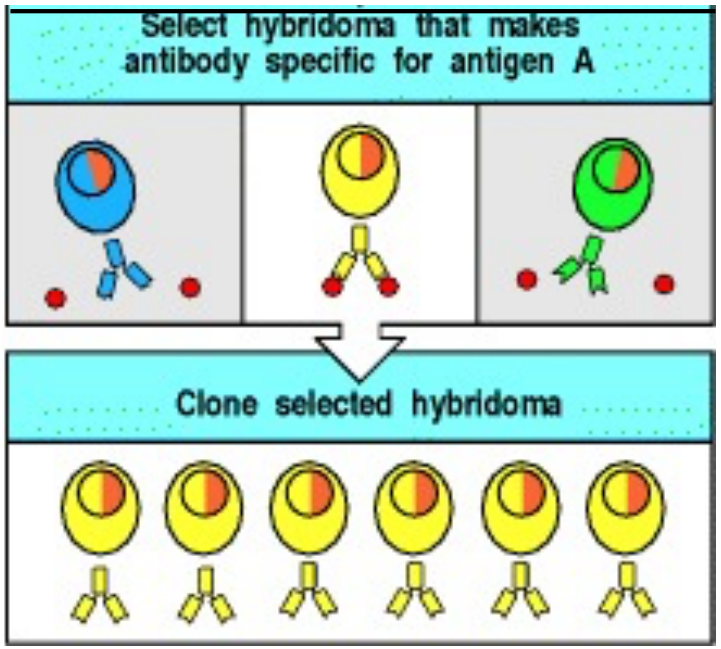


- Antigen: any molecule that can bind specifically to an antibody
  - Antigens can have many epitopes
- Epitope is the specific region/peptide of the antigen recognized by an antibody
- Monoclonal antibodies bind one epitope
- Polyclonal antibodies can bind many epitopes of the same antigen
- Conjugating signaling molecules (dyes, fluorophores) to Abs allow for detection

Monoclonal hybridoma technology results in a renewable source of identical antibodies



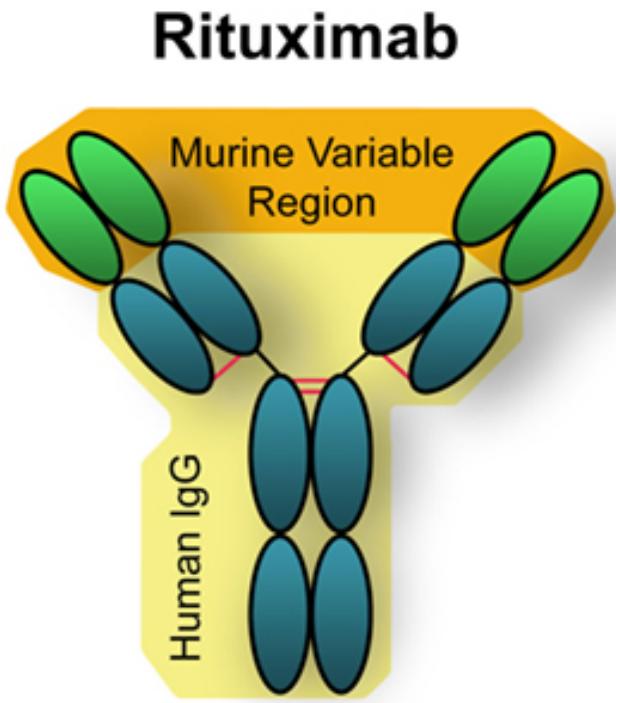
Monoclonal hybridomas can be screened and maintained in culture



Hybridomas can be maintained in cell culture or injected into mice

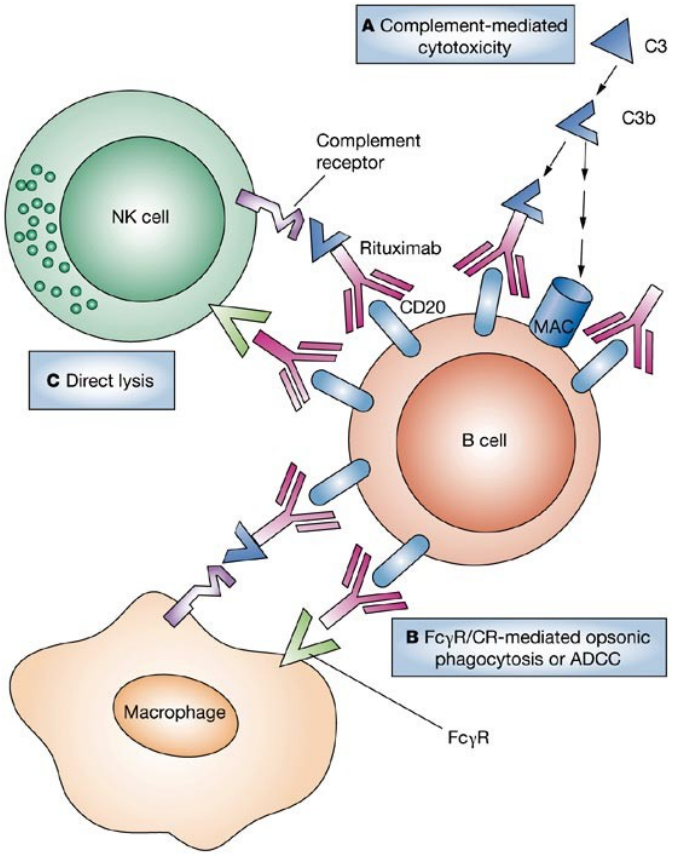
- Discovered in 1975 and won the Nobel prize in 1984
- Humanized mice can produce monoclonal antibodies for medicine
- This process is technically difficult but still the gold standard for development of antibodies that can be used long term

Rituximab is treatment for cancer and autoimmune diseases



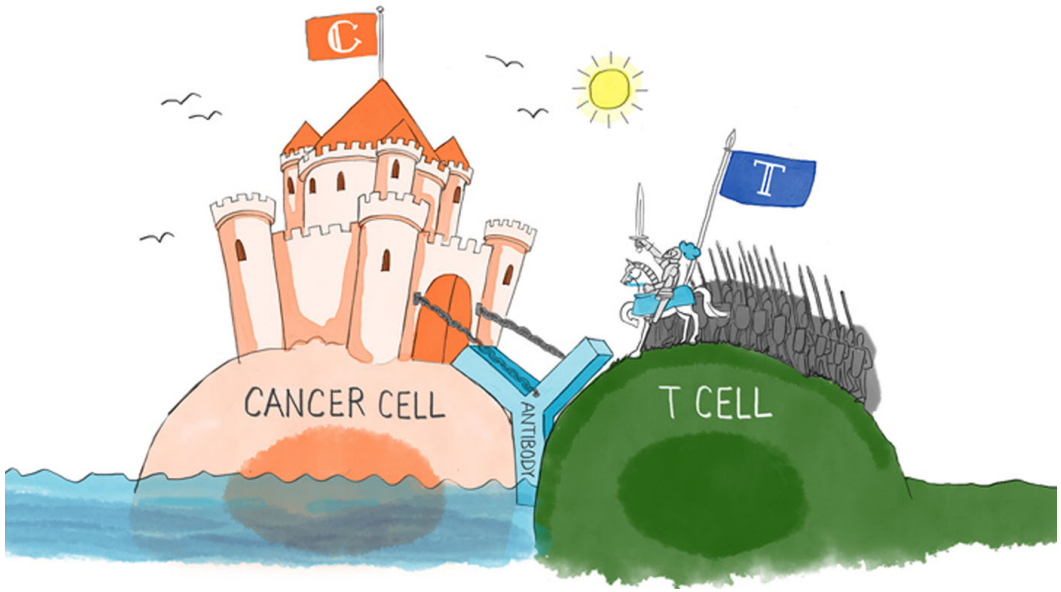
- First antibody FDA approved for cancer treatment in 1997
- Antigen is CD20
- Produced in CHO cells
- Approved for treatment of:
  - non-Hodgkin's lymphoma
  - chronic lymphocytic leukemia
  - rheumatoid arthritis
  - multiple sclerosis

Rituximab targets and kills CD20+ B cells



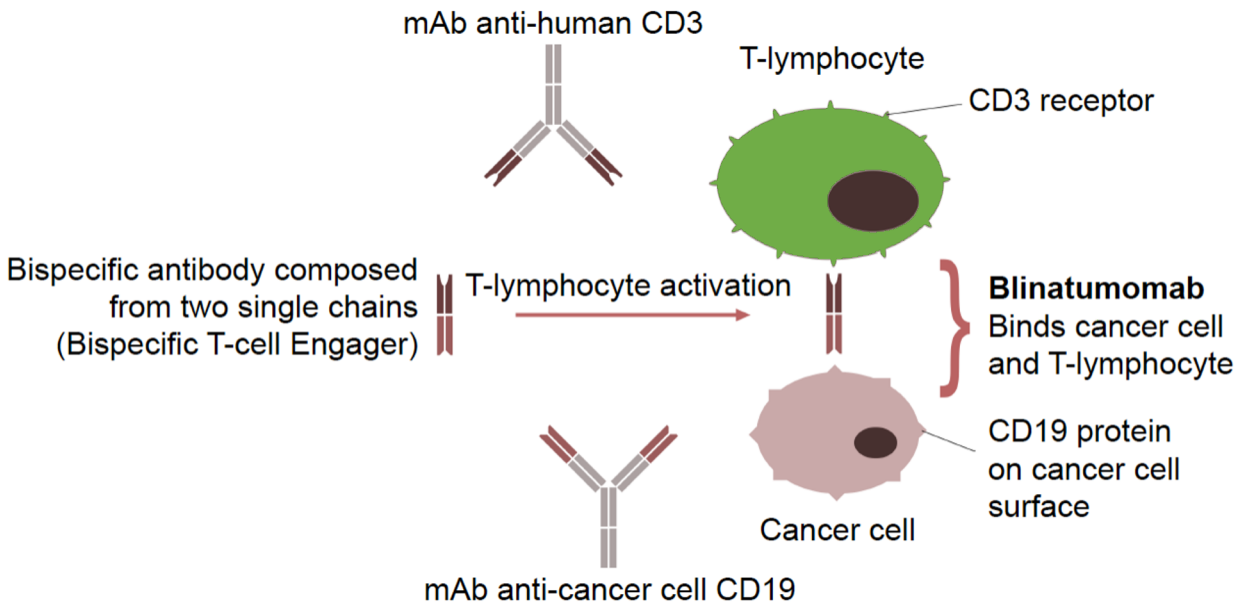
- Binding of Rituximab to CD20+ cells can result in:
  - Complement-dependent cytotoxicity (direct lysis)
  - antibody-dependent cell-mediated cytotoxicity (NK cell)
  - antibody-dependent phagocytosis (macrophage)
- Need better mouse models to study effects of immunotherapy to reduce resistance and side effects
  - Anti-human CD20, mouse models don't mimic the human immune system well enough

Recombinant antibody production led to development of bispecific antibodies



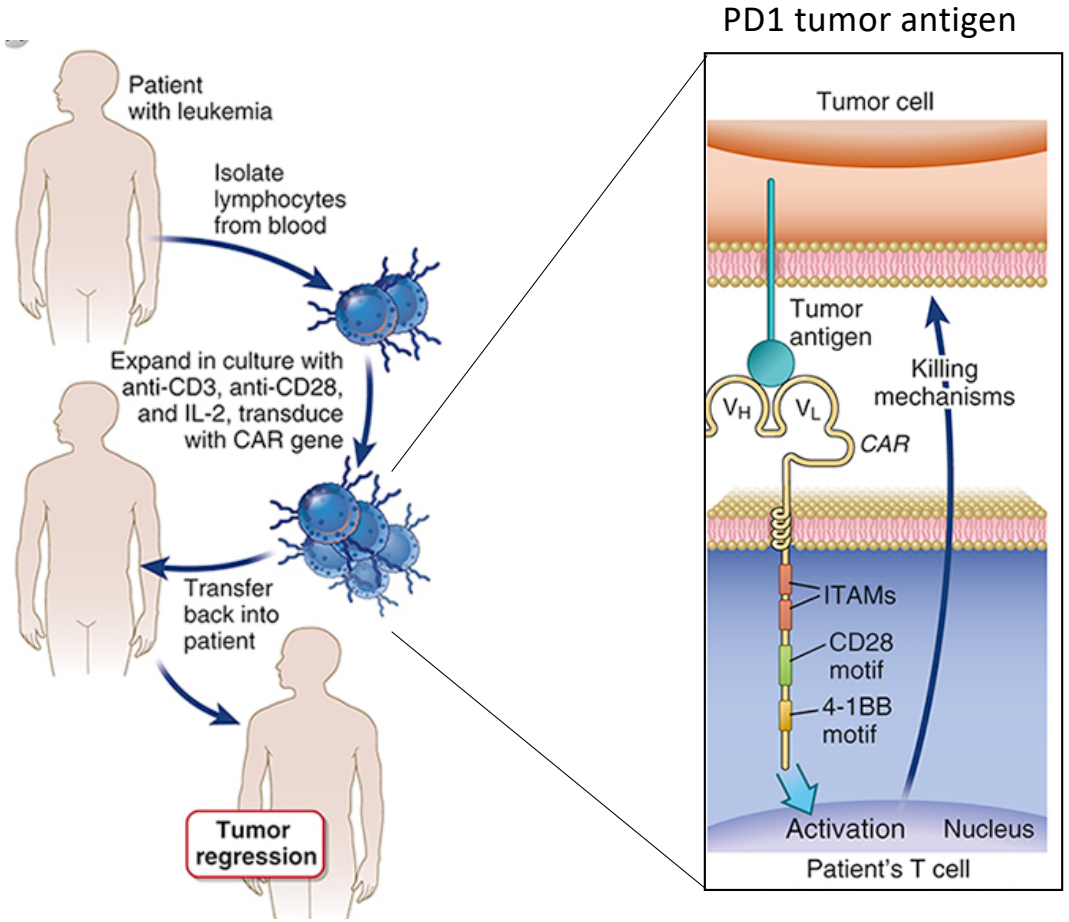
- A bispecific antibody contain two different antigen binding sites in one molecule
- First generated in 1980s but not approved for use as drugs until 2009
- Blinatumomab: a bispecific T cell engager (BiTE) antibody against CD19/CD3 for refractory acute lymphoid leukemia

Blinatumomab complexes a T cell with a CD19+ cancer cell resulting in lysis



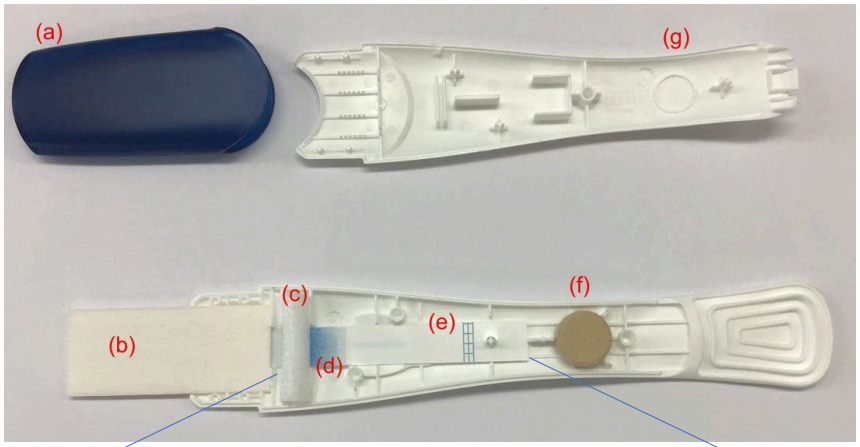
- Issues with dosing and side effects
  - continuous intravenous administration of the drug is required
- Low cytotoxicity because only involves CD3+ T cells

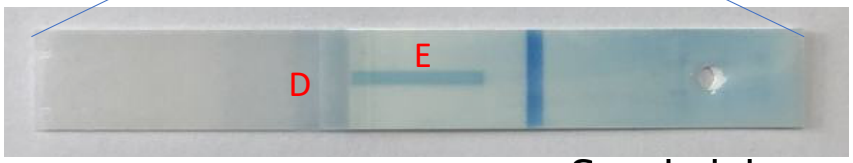
CarT (Chimeric antigen receptor T) cell immunotherapy is not antibody based



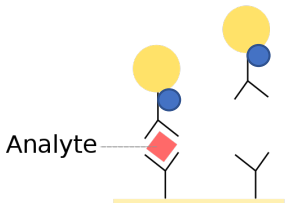
- T cells are isolated from patient's blood
- Genetically modified to express CARs (inset)
- Modified cells are returned to patient
- Intense systemic inflammation eradicates cancer cells
  - Immediate side effects life threatening in some cases
  - Cost is approximately $400,00-500,00 per treatment

A pregnancy test is a Lateral flow immunochromatographic assay device



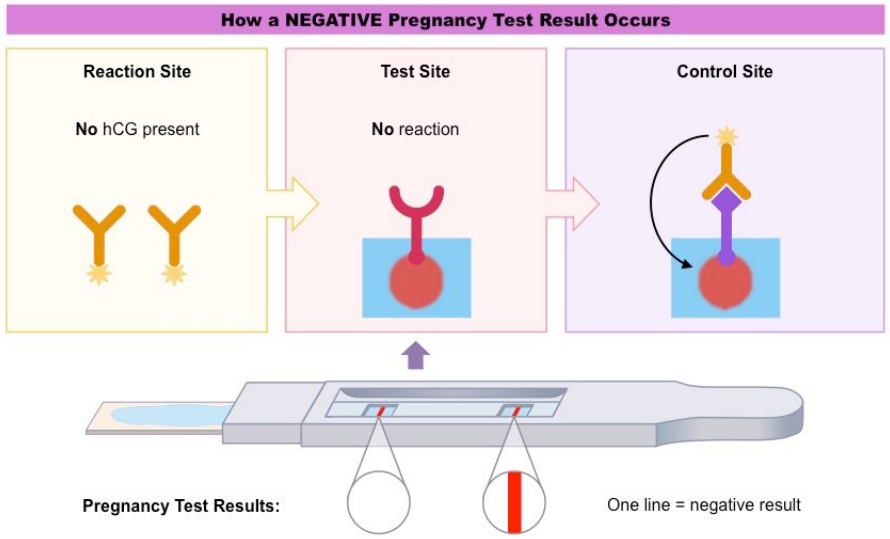


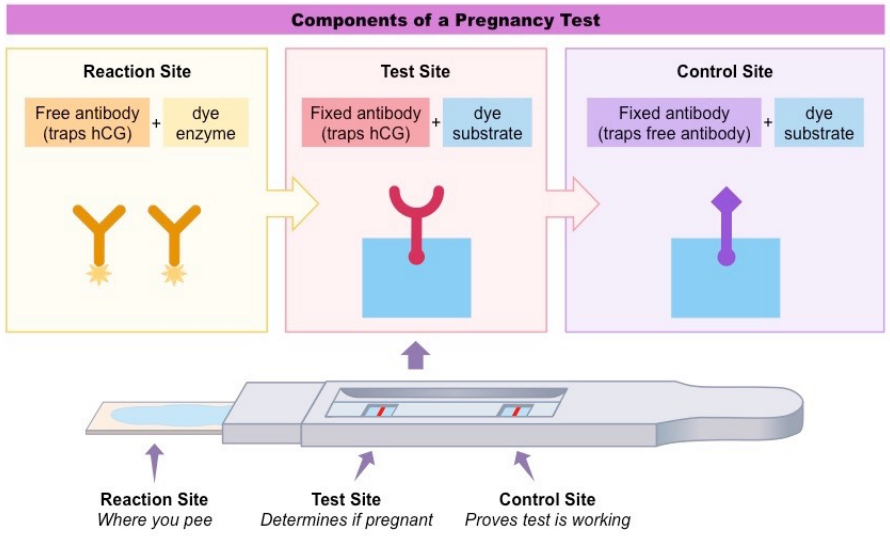
Sandwich



- A pregnancy test is a widely used antibody based diagnostic device
  - anti-hCG (human chorionic gonadotropin, hormone)
- (B)Absorbent pad, a filter helping to remove any proteins or bacteria in the urine that may affect the assay's performance, leaving mostly water and the hCG protein.
- (D) Conjugate pad with Latex microbeads coated in an antibody specific to hCG that is conjugated to blue dye
- (E) Nitrocellulose membrane with antibody test line
  - Halfway along this test strip is a stripe of a second antibody.
  - This antibody also binds to hCG, but to a different region from the antibody attached to the latex beads (sandwich assay)
  - As the beads flow through the test strip
    - Negative beads with Ab flow through strip and end at control site
    - Positive: hCG binds antibodies at (D) on the latex beads also bind to the antibodies in the test strip, stopping them from flowing through the test strip.

This design is used in many diagnostic devices and only necessitates a specific antibody





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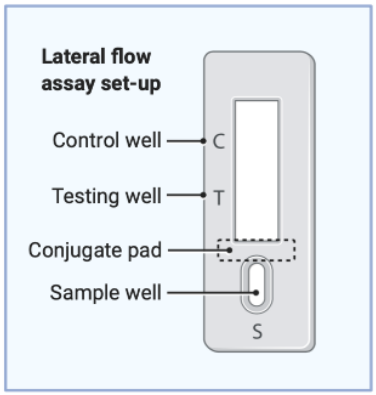
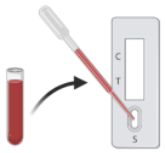
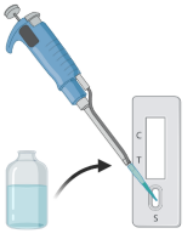
Serologic Diagnostic Test: COVID-19 Detection





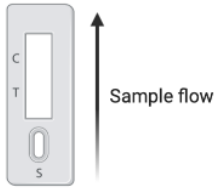
1 Sample loading

Add drop of blood or serum in sample well (S).



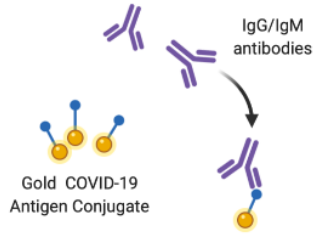
2 Buffer loading

Add dilution phosphate saline buffer to sample well.



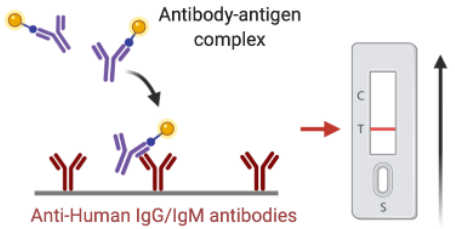
3 Sample incubation

Capillary action moves sample across lateral flow test.



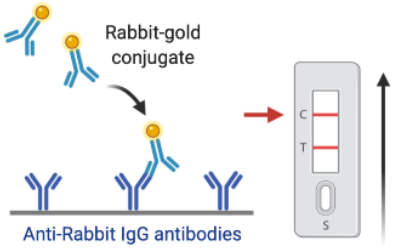
4 Antibody-antigen recognition

Antibodies with specificity for COVID-19 bind to gold COVID-19-antigen conjugates in conjugate pad.



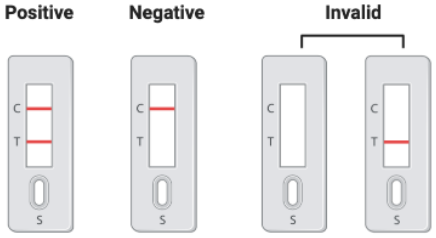
5 COVID-19 antibody detection

Sample enters testing well (T) and COVID-19 antibody-antigen complex binds to immobilized anti-human IgG/IgM antibodies.



6 Control antibody detection

Rabbit antibody-gold conjugate binds to immobilized anti-rabbit IgG antibodies.



7 Interpreting results

Positive: one strip each in C well and T well
Negative: one strip in C well

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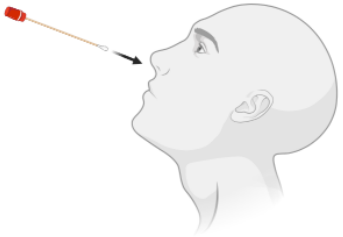
COVID-19 Diagnostic Test through RT-PCR

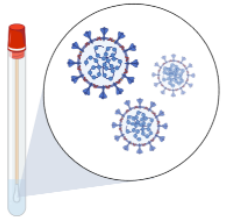
1 Nasopharyngeal swab <15 min

Cotton swab is inserted into nostril to absorb secretions.



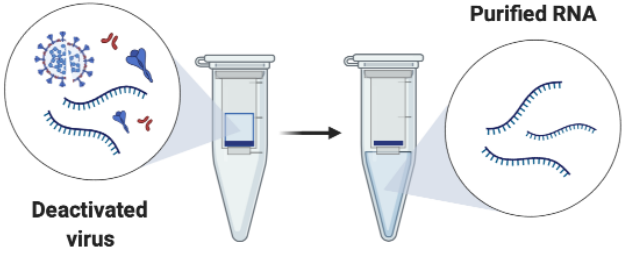
2 Collected specimen 0-72 h

Specimen is stored at 2-8°C for up to 72 hours or proceed to RNA extraction.



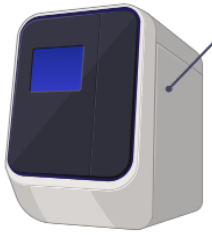
3 RNA extraction ~45 min

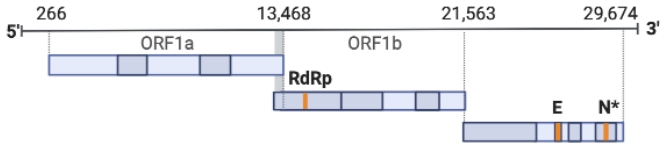
Purified RNA is extracted from deactivated virus.



4 RT-qPCR ~1 h per primer set

Purified RNA is reverse transcribed to cDNA and amplified by qPCR.





Primers and probes for screening

E gene First-line screening tool

E_Forward: ACAGGTACGTTAATAGTTAATAGCGT
E_Probe1: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
E_Reverse: ATATTGCAGCAGTACGCACACA

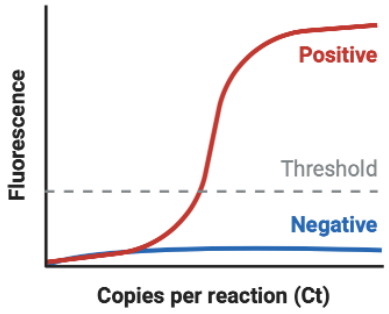
RdRp gene Confirmatory testing

RdRp_Forward: GTGARATGGTCATGTGTGGCGG
RdRp_Probe1: FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
RdRp_Probe2: FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
RdRp_Reverse: CARATGTTAAASACACTATTAGCATA

* N gene testing is not further used because it is slightly less sensitive.

5 Test results real-time

Positive SARS-CoV2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).



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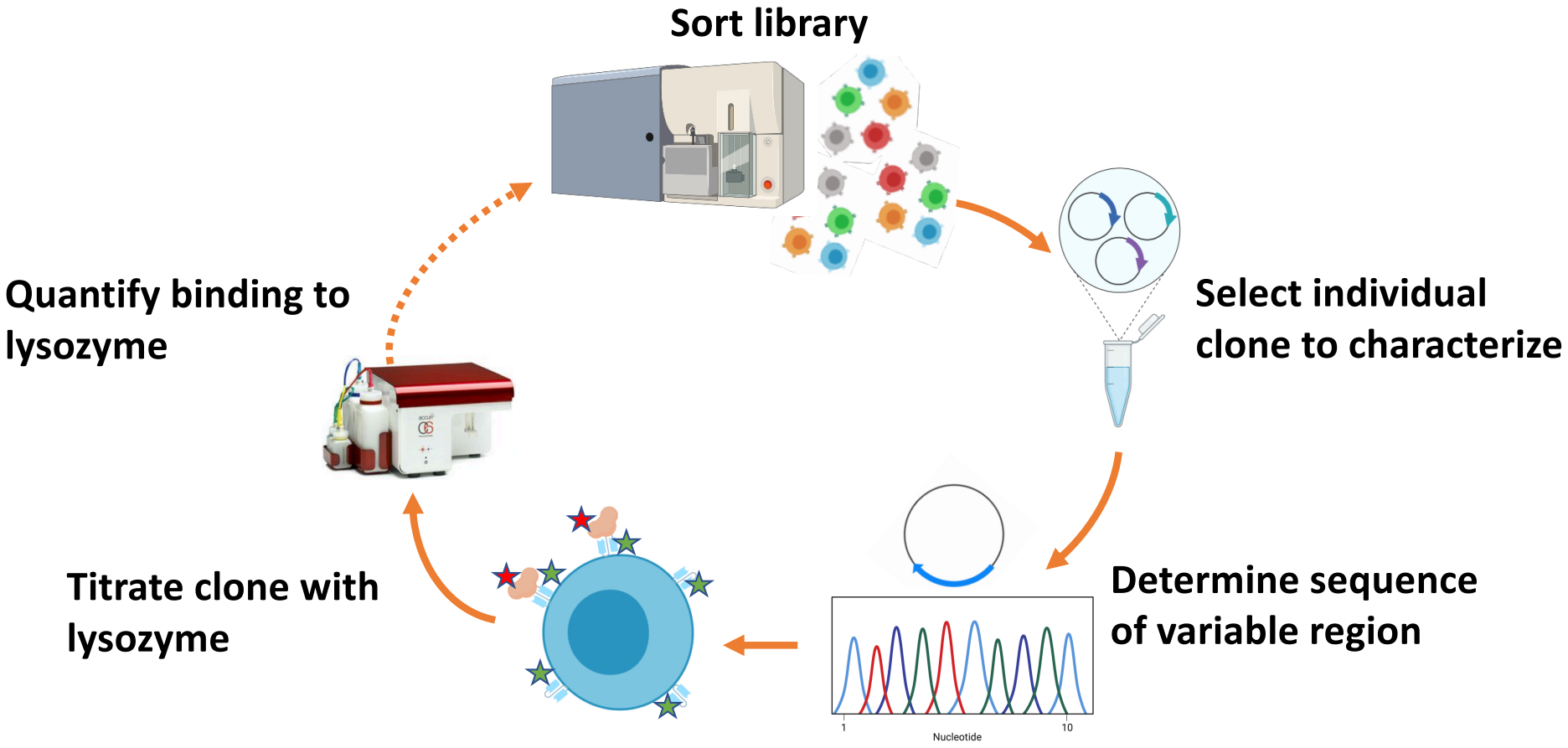
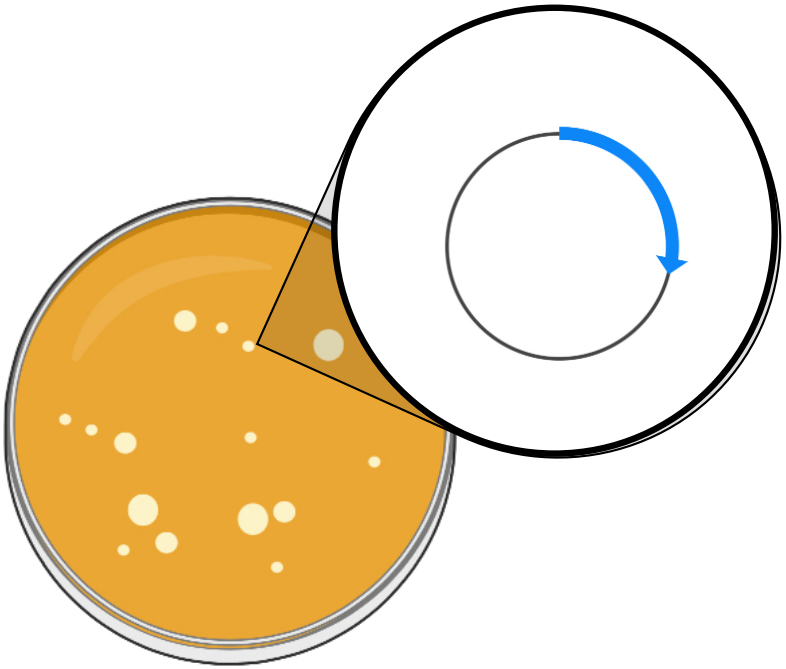
Mod 3 Workflow: Selection and characterization of lysozyme binding scFvs

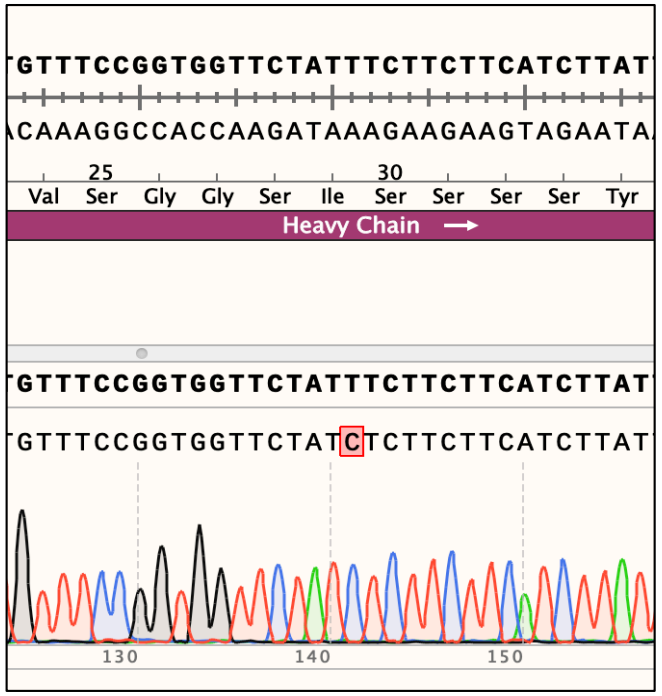


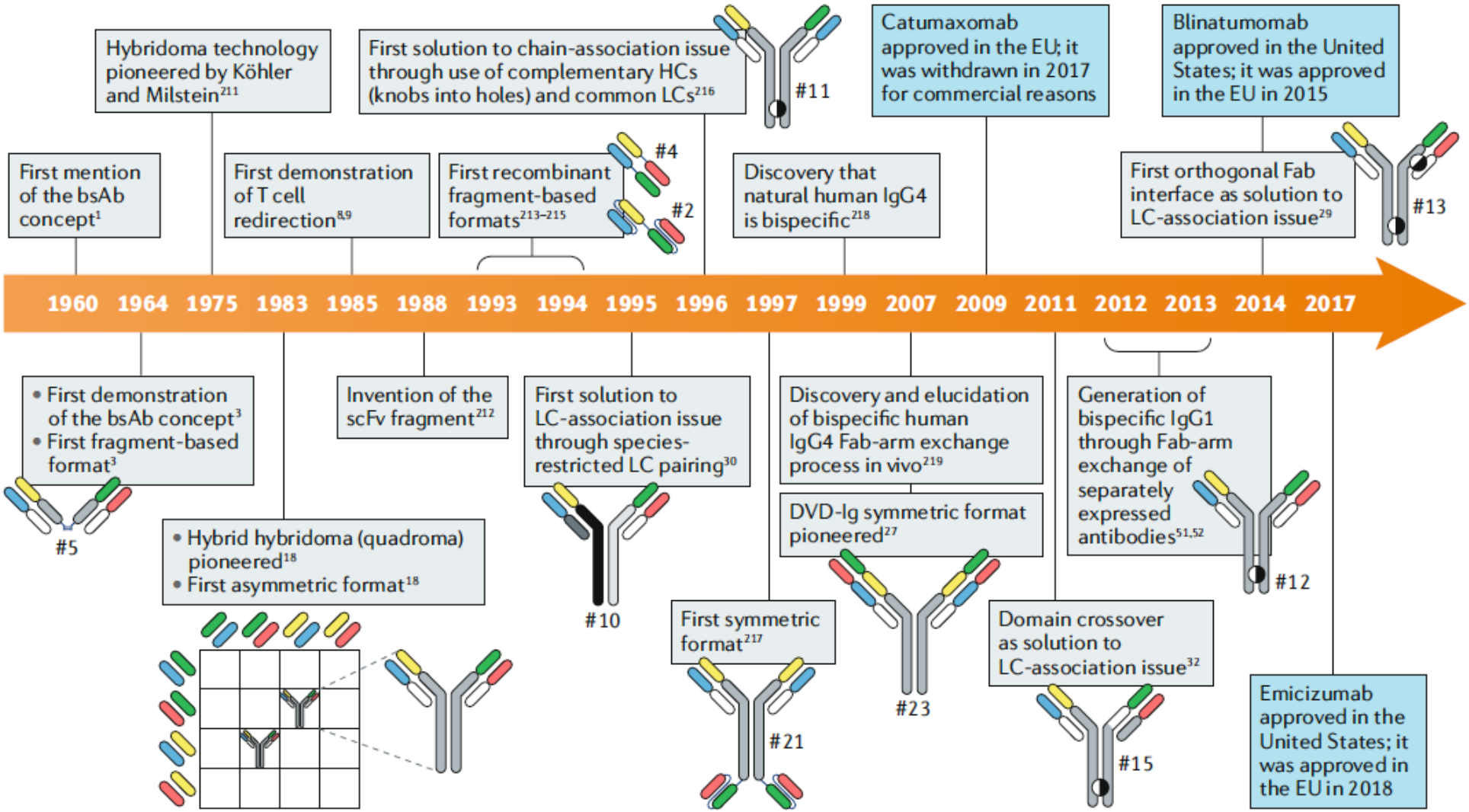
Today in “lab”

1) Set up sequencing reaction for purified plasmid DNA



2) Align clone sequencing results to plasmid Ab31375





Labrijn et al. Bispecific antibodies: a mechanistic review of the pipeline. Nature Reviews Drug Discovery. (2019)