Western Blotting!

Allows visualization of specific protein bands on a gel.

Detection level

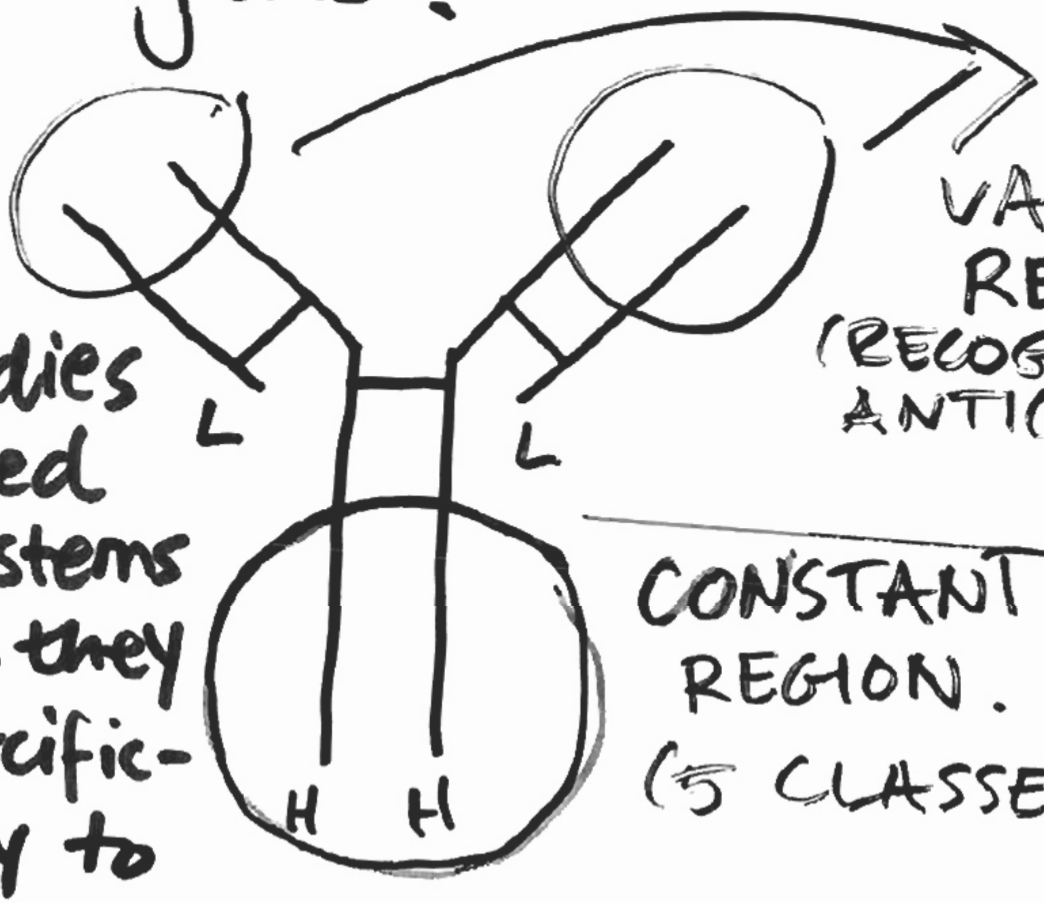
up to 10^{-15} g Western

$\sim 10^{-6}$ g Coomassie

Westerns are extremely sensitive methods of protein detection

Antibodies

Proteins made by the immune system of animals against foreign antigens.



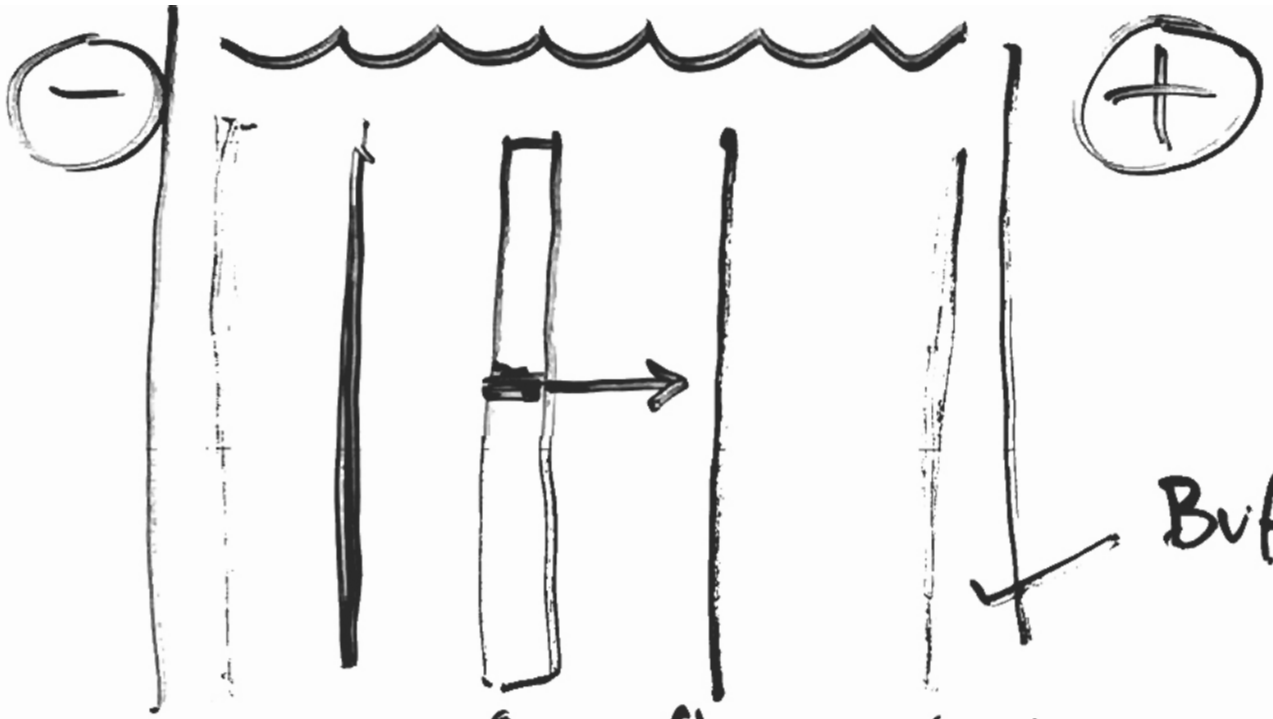
VARIABLE REGION (RECOGNIZES ANTIGEN)

CONSTANT REGION. (5 CLASSES)

Antibodies are used in Westerns because they bind specifically only to proteins which they recognize.

Nitrocellulose membrane

Used (amongst other things) in Western Blotting to allow transfer and detection of your favorite protein. has <u>really</u> <u>high</u> affinity for proteins



Setup of Western transfer

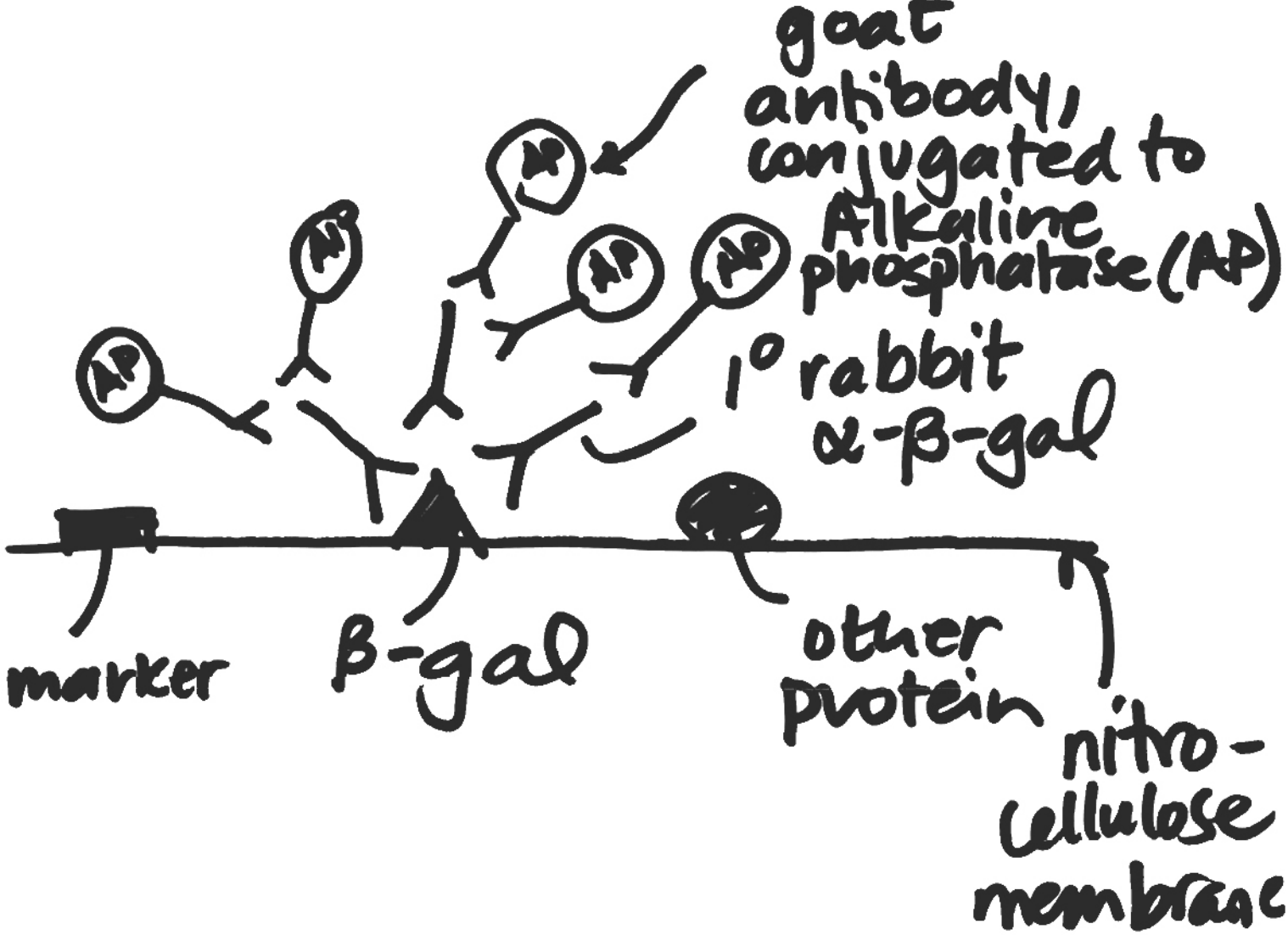
* Note that there should be <u>no space</u> between gel by membrane!

After Western transfer, you need to do the following:

1. Block with non-specific milk protein (necessary to cover all empty spaces on Western so that antibodies do not bind everywhere!
2. Add primary antibody which binds directly to β-gal. (from rabbit)
3. Add secondary antibody (from goat) that binds to primary antibody

Secondary antibody is useful to detect any band that is bound to <u>any</u> rabbit antibody; it also allows for amplification of signal.

- Primary anti-β gal binds to β-gal ONLY
- Secondary antibody binds to constant region of primary α-β-gal antibody.



Alkaline phosphatase

↳ removes phosphate

AP + BCIP → BCI + P

+NBT → purple precipitate