Western Blotting!

Allows visualization of specific protein bands on a gel.
Detection level up to $10^{-9}$ g

Western ~ $10^{-6}$ g Coomassie

Westerns are extremely sensitive methods of protein detection
Antibodies

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

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 really high affinity for proteins.
Setup of Western transfer

*Note that there should be no space between gel & 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 any 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.

2° α-rabbit goat antibody, conjugated to Alkaline phosphatase (AP)
1° rabbit α-β-gal

marker β-gal other protein nitrocellulose membrane
Alkaline phosphatase

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\text{AP} + \text{BCIP} \rightarrow \frac{\text{BCI} + P}{\text{NBT}} \rightarrow \text{purple precipitate}
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