

PCR cleanup

If your PCR reaction worked, you need to clean it up to prepare it for sequencing (If it didn't work, work with someone that has PCR product).

Why clean up? Right now, your PCR reaction has a lot of free nucleotides floating around that didn't get incorporated into your PCR product. There are also some primers floating around that haven't been used yet, as well as unwanted salts. You need to get rid of these before going to the DNA sequencing step. This Wizard cleanup kit will get rid of everything except the DNA fragment that you want to sequence.

- Add 20ul of **Membrane Binding Solution** to the PCR product that remains in the tube. Mix by pipetting up and down slowly.
- Add the solution to the filter tube. (you should have a filter sitting in a larger bottom tube)
- Centrifuge the filter tube (while it is sitting in the bottom tube) for 1 minute
- Discard the liquid that flows into the bottom tube (put the liquid in the sink), but don't throw away the bottom tube. Your DNA should be stuck to the filter right now.
- Put the filter tube back into the bottom tube.
- Add 700ul **Membrane Wash Solution** to the filter tube. This solution contains ethanol so that your DNA will not dissolve in the liquid.
- Centrifuge for 1 minute, then discard the liquid in the bottom tube. This step is to wash your DNA. The DNA is still stuck to the filter.
- Add 500ul **Membrane Wash Solution** to the filter tube
- Centrifuge for 1 minute, and discard the liquid that flows through into the bottom tube.
- Put the filter back into the bottom tube
- Centrifuge another 5 minutes. This is to dry the filter and dry your DNA
- Transfer the filter tube to a clean microcentrifuge tube
- Add 25ul **Nuclease-Free Water** to the top of the filter
- Incubate at room temperature for 5 minutes. Remember, DNA is soluble in water. Now your DNA will come off of the membrane and get dissolved in the water.
- Centrifuge 1 minute. Now your clean DNA is at the bottom of the tube. This is what you will use for DNA sequencing next week.