Gel Purification of DNA

(**Note:** This purification method works well when proceeding to ligation reactions but not for isolating PCR template as TAE is undesirable in PCR reactions)

1. Prepare a standard agarose gel
    - 0.7-1.0% agarose to ~75 mL of 1x TAE
        - 1.0% = ~0.75 g - small fragments (<1000 bp)
        - 0.7% = ~0.525 g - large fragments (~1000+ bp)
        - 7.5 µL SYBR®Safe stain
2. Load DNA w/ loading dye into the gel
    - 5 µL DNA ladder
    - 75 ng is sufficient for screening purposes
    - Full 500 ng digest is recommended for purification
      (**Note:** It is preferable to leave an empty well between samples for the purposes of purification)
3. Run gel for ~30-40 min. @ 120 V until ladder and bands are fully resolved
    - If purification is desired, prepare materials
        - Puncture a hole in the bottom of a 0.65 mL micro-centrifuge tube with a syringe
          (**Note:** The micro-centrifuge tube serves as a filter)
        - Place the micro-centrifuge tube inside a 1.5 mL Eppendorf tube
          (**Note:** The Eppendorf tube serves as a collection tube)
        - Cut filter paper and dialysis tubing into small rectangles such that they are slightly wider than the loading well and slightly taller than the height of the gel
          (**Note:** Too small will make it difficult to capture all of the DNA, too large will result in less concentrated product)
4. Image the gel to verify length band size is correct
    - Stop here or proceed to purification

5. Use a razor blade or scalpel to make an incision directly below the band for the segment you wish to purify
(**Note:** Make a wide slice to help separate the gel and ease the insertion of filter paper and dialysis tubing. It is okay to cut the gel all the way across.)
6. Insert the filter paper and dialysis tubing such that the filter paper is between the DNA sample and the dialysis tubing
(**Note:** The purpose of the filter paper is to absorb the DNA, the purpose of the dialysis tubing is to prevent the DNA from passing through. Tweezers are useful for this process.)
7. Run the gel for ~ 5 min. @ 120 V
8. Image the gel to ensure that DNA is trapped within the filter paper and dialysis tubing
9. Remove the filter paper and dialysis tubing from the gel and place them in the micro-centrifuge tube
(**Note:** Band should no longer be observable by an imaging device)
10. Spin for 30 s. @ 13,200 RPM to collect the purified sample in the 1.5 mL Eppendorf tube
11. Remove the micro-centrifuge tube and store @ -20° C