Gel Purification Protocol

1) Pool PCR products from multiple reactions
    - Anywhere from 4-12 reactions
    - “Can I gel purify 1 reaction?” Sure, but expect low yield…good luck downstream!

2) Add 0.5-1uL of DpnI to pooled reaction per 100 uL of PCR product -> 37°C for 1+ hours (Overnight is fine)

3) Desalt/concentrate digested PCR product with PCR Cleanup Protocol (Silica column cheat sheet)
    - Just concentrate PCR product and run on gel? -> Smear on agarose due to concentrated salt/buffer
    - Large volume difficult to load on low-melt agarose gel -> Diluted in gel volume
    - Silica column desalts AND concentrates pooled PCR product (Better than G50 resin, trust)

4) Elute in 35-50 uL if you don’t have a Centrivap, elute in 100 uL and concentrate to 35 uL if you do

5) Load onto low-melt agarose with a blue-light reactive dye
    - Ethidium bromide + UV -> Damages DNA -> Longer DNA fragments = More damage/time
    - Don’t have a gel comb that will fit 35-50uL? Tape a few lanes together

6) Cut out desired band under blue light and purify with silica column (Silica column cheat sheet)

7) Elute in 35-50 uL if you don’t have a Centrivap, elute in 100 uL and concentrate to 35-50 uL if you do

8) Determine concentration of fragments with spectrophotometer:
    - **Less than 10ng/uL?** Dicey, but doable. Consider additional PCR reactions to bulk up concentration
    - **10-30ng/uL?** Good enough depending on size of fragments
    - **30-100ng/uL?** Comfortable amount for most applications
    - **>100ng/uL?** You’re laughing! Enough for repeats!

MVP <3 I luv u