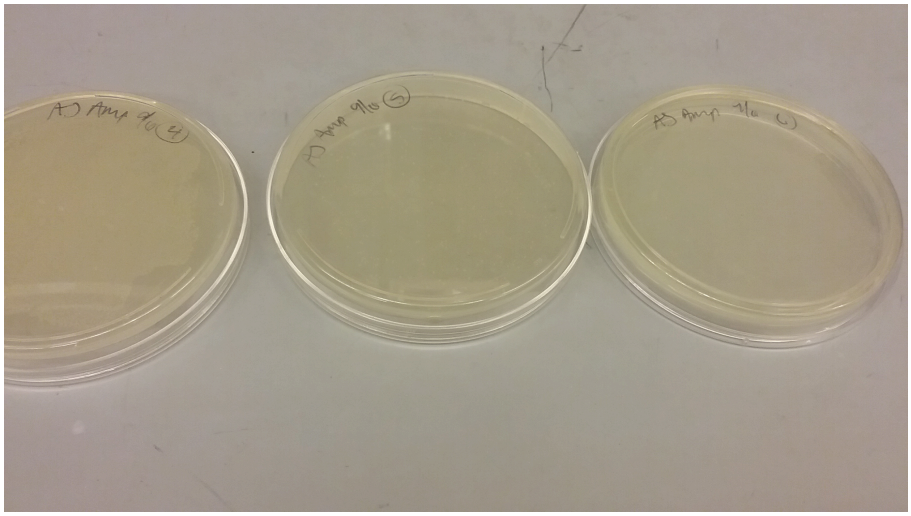
Restriction Digest-Based Cloning
Updated: AJ 22.04.02

Overall protocol found here.

1. Choose compatible enzymes (do that bit in Benchling; you can PCR amplify using compatible primers if you don't have compatible enzymes)
    a. Benchling → Assembly Wizard → Digest and Ligate
2. Digest 1.5-2µg of donor plasmid (containing insert) and 1µg of recipient plasmid (i.e. backbone).
    a. It is also critical that as much of the recipient plasmid as possible be cut with both enzymes, and therefore it is important that the digest go at least 4 hours and as long as overnight.
    b. I have donor at 270ng/uL and recipient at 140ng/uL. 10 uL rxns: 1 uL Cutsmart, 1 uL each enzyme, 7 uL plasmid - this is actually close to perfect.
    c. Also going to run 10 uL rxns with 1 cutsmart, .5 each enzyme, 2 uL plasmid, 6 H2O in case I don't get complete digestion.
    d. I'm working with BamHI-HF and HindIII-HF, so gonna run double digest in Cutsmart at 37 deg for … try 1 hr.
    e. Heat inactivate 80 deg 20 min. (For HindIII.)
3. Ensure you only have linearized vector
    a. Run all your DNA on an agarose gel and gel extract. It's okay if the nanodrop gives a very low reading / lots of 230 signal.
    b. You can't do DPN1 digest here because the DNA you want is also methylated.
4. Ligate insert and vector.
    a. We recommend around 100ng of total DNA in a standard ligation reaction. You ideally want a recipient plasmid to insert *molar* ratio of approximately 1:3. Since the number of base pairs for each varies, it is difficult to calculate this based on DNA concentration alone. One method is to conduct 2 ligations for each plasmid you are trying to create, with varying ratios of recipient plasmid to insert.
    b. T4 ligase - 2 hr at RT or 16 deg O/N. We have in blue ice block. (Ran 100 min)
    c. 10 uL rxn: 1 uL Ligase, 1 uL 10x buffer, etc. Heat inactivate 15 min 65 deg. (oops, skipped this step)
    d. Run cut vector only + ligase control!! Also consider cut vector + no ligase.
5. Transform competent cells (see "Transformation for Subcloning")



4=ligation, 5= digested vector + ligase, 6 = digested vector only
What you can't see in this photo is that the ligation plate is almost overgrown, plate 5 has ~100 colonies, and plate 6 has about 20