Restriction (Double) Digests (25 µL):

(**Note:** Miniprep must produce at least ~12.5 ng/µL for a 250 ng digest)

1. Label 0.2 mL PCR tubes for each reaction
2. Determine the appropriate restriction enzymes for each reaction
   - EcoRI & SpeI (e.g. promoter/upstream biobrick)
   - XbaI & PstI (e.g. reporter/downstream biobrick)
   - EcoRI & PstI (e.g. backbone/destination vector)
   - EcoRI & AgeI (e.g. upstream insert for fusions)
   - NgoMIV & AgeI (e.g. downstream insert for fusions)
3. Use NEB double digest finder to choose appropriate buffer for reaction
   - 10x Cutsmart Buffer works for most reactions
4. Calculate volume of DNA for reaction
   - For a 250 ng digest: $\mathrm{V} = {}^{250\ \mathrm{ng}}\Big/_{\mathrm{Conc.}\left({}^{\mathrm{ng}}/_{\mu\mathrm{L}}\right)}$
   - For a 500 ng digest: $\mathrm{V} = {}^{500\ \mathrm{ng}}\Big/_{\mathrm{Conc.}\left({}^{\mathrm{ng}}/_{\mu\mathrm{L}}\right)}$

(**Recommendation:** Use 250 ng for screening purposes, 500 ng for gel purification and subsequent ligation reactions)

5. Add each component to 0.2 mL PCR tube
   - DNA - V µL
   - ddH_2O - (21.5 - V) µL
   - Reaction Buffer - 2.50 µL
   - RE1 - 0.50 µL
   - RE2 - 0.50 µL

(**Note:** Keep restriction enzymes as cold as possible)

6. Give the tubes a light spin to collect solution at the bottom
7. Incubate in thermocycler (w/ heated lid)
   - 1 hr. @ 37° C
   - 20 min. @ 80° C (heat inactivation cycle)
8. Store @ -20° C