Double Digest

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:

Source: NEB

Primary Experimenter (contact):
Other Experimenters:

Assembled: 6/27/2012

Reagent	Details	Quantity
ddH2O		Up to 50 µL
*10X NEB Buffer	(1, 2, 3 , 4?)→	5 µL
**100X BSA	(Used?)→	0.5 µL
1-10 µg DNA (Or 200 ng for minimal gel visualization)	(IDs/details)→	Var.
Restriction enzyme 1	(enzyme)→	1 µL
Restriction enzyme 2	(enzyme)→	1 µL

*See: Enzyme Chart to choose buffer
**See: Enzyme Chart to decide if needed

Please write equations on the front/back of this sheet
Procedure:

Critical Steps:

- Restriction enzymes are expensive! Leave frozen until final step.
- Use small volume tubes
- Carefully label tubes
- All steps on ice
- See: Enzyme Chart to choose reaction temperature

NOTE:

- BSA does not inhibit any restriction enzyme
- If two different incubation temperatures are necessary, choose the optimal reaction buffer and set up reaction accordingly. Add the first enzyme and incubate at the desired temperature, Then, heat kill the first enzyme, add the second enzyme and incubate at the recommended temperature.

☐ **Turn on water bath**

- Check enzyme chart for reaction temperature

☐ **Calculate DNA volume to use**

- $(?\ \mu L\ DNA) = \frac{1000\ ng}{DNA\ sample\ concentration \frac{ng}{\mu L}}$

☐ **Calculate H2O volume to use**

- $(?\ uL\ H2O) = 50 - (?\ \mu L\ DNA) - 7\ \mu L - (0.5\ ul\ if\ using\ BSA)$

☐ **Add (? µL H2O) to reaction tube**

☐ **Add 5 µL 10X NEB buffer to reaction tube**

☐ **IF REQUIRED, add 0.5 µL 100X BSA to reaction tube**

☐ **Add (? µL DNA) to reaction tube**

☐ **Add 1 µL enzyme 1 and 1 µL enzyme 2 to reaction tube**

☐ **Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge.**

- Do not vortex.

☐ **Incubate 1 hour in water bath**

- Use optimal reaction temperature (See: Enzyme Chart to choose temp.)

☐ **Stop reaction**

- If further manipulating DNA NOT required, do DNA gel electrophoresis with a loading dye that includes EDTA

- If further manipulation required, heat inactivate (See: Enzyme Chart)