Double Digest

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:
Primary Experimenter (contact):
Other Experimenters:

Source: NEB

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 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
***Can be scaled up, but use 1 ul of each enzyme for each ug of DNA (2-3 units of enzyme). Increase incubation time for larger amounts.

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

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

Calculate H2O volume to use

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

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

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 at least 1 hour in water bath

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

Stop reaction by heat killing (check chart for temperature)

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