Single Digest

| Rationale: |  |
| Special Observations: |  |
| Results: |  |
| Interpretation: |  |

**Experiment Date:** Source: NEB

**Experiment Time:**

**Primary Experimenter (contact):**

**Other Experimenters:** Assembled: 6/27/2012

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Details</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile H2O</td>
<td></td>
<td>Up to 50 µL</td>
</tr>
<tr>
<td>*10X NEB Buffer</td>
<td>See: Enzyme Chart to choose buffer</td>
<td>5 µL</td>
</tr>
<tr>
<td>**100X BSA</td>
<td>See: Enzyme Chart to decide if needed</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>1-10 µg DNA (Or 200 ng for minimal gel visualization)</td>
<td>(name)</td>
<td>Var.</td>
</tr>
<tr>
<td>Restriction enzyme</td>
<td>(enzyme)</td>
<td>1 µL</td>
</tr>
</tbody>
</table>

**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
☐ Turn on water bath
  o Check enzyme chart for reaction temperature

☐ Calculate DNA volume to use
  \[
  (? \mu L \ DNA) = \frac{1000 \text{ ng}}{\text{DNA sample concentration} \ \text{ng} \ \mu L}
  \]

☐ Calculate H2O volume to use
  \[
  (? \mu L \ H2O) = 50 - (? \mu L \ DNA) - 6 \mu L - (0.5 \mu L \text{ if using BSA})
  \]

☐ Add (? \mu L \ H2O) to reaction tube
☐ Add 5 \mu L 10X NEB buffer to reaction tube
☐ IF REQUIRED, add 0.5 \mu L 100X BSA to reaction tube
☐ Add (? \mu L \ DNA) to reaction tube
☐ Add 1 \mu L restriction enzyme 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.
  o Do not vortex the reaction.

☐ Incubate 1 hour in water bath
  o Use optimal reaction temperature

☐ Stop reaction
  o If further manipulating DNA NOT required, do DNA gel electrophoresis with a loading dye that includes EDTA
  o If further manipulation required, heat inactivate (See: Enzyme Chart)