# Single Digest

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|---|---|
| **Rationale:** | |
| **Special Observations:** | |
| **Results:** | |
| **Interpretation:** | |

**Experiment Date:**
**Experiment Time:**

Source: NEB

**Primary Experimenter (contact):**
**Other Experimenters:**

Assembled: 6/27/2012

| Reagent | Details | Quantity |
|---|---|---|
| Sterile $H_2O$ | | Up to 50 µL |
| *10X NEB Buffer | See: Enzyme Chart to choose buffer | 5 µL |
| **100X BSA | See: Enzyme Chart to decide if needed | 0.5 µL |
| 1-10 µg DNA (Or 200 ng for minimal gel visualization) | (name)→ | Var. |
| Restriction enzyme | (enzyme)→ | 1 µL |

## 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
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**NOTE:**

- BSA does not inhibit any restriction enzyme

- [ ] **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**
    - $(?\ uL\ H2O) = 50 - (?\ \mu L\ DNA) - 6\ \mu L - (0.5\ ul\ if\ using\ BSA)$
- [ ] **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 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.**
    - Do not vortex the reaction.
- [ ] **Incubate 1 hour in water bath**
    - Use optimal reaction temperature
- [ ] **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)