

## Restriction Digestion Protocol

Check the compatibility of two restriction enzymes and decide which digestion buffer to use (look at the NEB catalog)

Double digest (digest with 2 enzymes at once)

| Vol (uL)                    | Reagent              |
|-----------------------------|----------------------|
| 16                          | ddH <sub>2</sub> O   |
| 7.5                         | Digestion Buffer 10X |
| 7.5                         | BSA 10X              |
| 40                          | DNA                  |
| 2                           | Enzyme 1             |
| 2                           | Enzyme 2             |
|                             |                      |
| 75 ul Total                 |                      |
| Incubate at 37C for 2 hours |                      |

For single digest: alter recipe, perform digest with one enzyme  
Then perform PCR cleanup procedure, elute with 40 uL  
Then can digest DNA with second enzyme