**Restriction enzyme protocol**

Before you begin:

- Check what NEB 10x buffer(s) you need for the reaction:

https://www.neb.com/tools-and-resources/interactive-tools/enzyme-finder

- If you use **2 restriction enzymes**, check for % activity in NEBuffer:
  https://www.neb.com/tools-and-resources/interactive-tools/enzyme-finder

- Remember **to take the enzyme out of the freezer just before you are about to use it,** and to immediately put it back in!

- According to amount of DNA digested, add the following amounts:

| | |
|---|---|
| DNA: | Depends on the application:<br>• Checking miniprep by cutting: around 500ng<br>• Cutting from gel – depending on fragment size (ask your mentor) |
| restriction enzyme: | Known rule: 1 u of enzyme cut 1ug of DNA in 1 hour.<br>In practice: 0.5ul of enzyme. |
| Buffers: | Check NEB website for the specific buffer<br>1.1=1+BSA (X10)<br>2.1=2+BSA (X10)<br>3.1=3+BSA (X10)<br>Cut smart=4+BSA (X10) |
| H2O (mbw) | To minimal volume possible (usually 20ul) |

**Incubate for 2 h at 37°C (dry bath). If both enzymes are HF- 15-30 min and then put on ice!**