

הנה 300 נא 300N נא - HF נא 100 נא
 Cut Smart נא 100 נא

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: * Checking miniprep by cutting: around 500ng * Cutting from gel – depending on fragment size (ask your mentor)
restriction enzyme:	Known rule: 1 unit of enzyme cut 1ug of DNA in 1 hour. In practice: 0.5ul of enzyme.
Buffers:	Check NEB website for the specific buffer 1.1=1+BSA (X10) 2.1=2+BSA (X10) 3.1=3+BSA (X10) 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!

20ul נא 2ul ← CutSmart x 10 נא
 HF נא 1ul