**pU6-sgRNA-cloning by annealing of 2 primers**

**1. Digestion of the plasmid**

- Digestion of the pU6-universal plasmid with BsaI-HF in cutsmart buffer

(pBM010\_U6-universal\_TUB1-Cas9-T2A-mNeonGreen from Lourido’s lab)

- Check the digestion on agarose gel

- Purification on column and quantification

* Plasmid stock at 100 ng/ul
* No need to dephosphorylate because the 2 sites are not compatible

# **2. Hybridation of the 2 primers**

- Mix:

10 ul F (stock standard =100uM)

10 ul R

5 ul 10x annealing buffer

25 ul H2O

50 ul

10x annealing buffer: 50mM NaCl, 1 mM EDTA, 10 mM Tris pH8

- Incubate 5 minutes at 95°C

- Let the mix coming back to RT slowly

- Keep the primers on ice and make a 1/50 dilution for the ligation

**3. Ligation and transformation**

- Mix:

100 ng of vector = 1uL

Annealed primers = 1uL of the 1/50 dilution

T4 DNA ligase master mix Anza = 5 uL

H20 13 uL

Total volume = 20uL

- Incubate 2 hours at RT

- Transformation as usual in Stellar bacteria with 2,5 ul of the ligation reaction, plate on Ampi

**4. PCR screen on colony**

- screen with specific fwd primer and 3979, around 550 bp