Cloning Strategy with Restriction Enzymes

- Digestion: Vector/Backbone
  - X µL 600 ng Vektor
  - 3 µL 10 x buffer (NEB2.1 or Cutsmart)
  - 1 µL 1. Restriction enzyme
  - 1 µL 2. Restriction enzyme
  - Add 30 µL ddH_2O

Digestion: Insert

X µL 100 ng Insert
3 µL 10 x buffer
1 µL 1. Restriction enzyme
1 µL 2. Restriction enzyme
Add 30 µL ddH_2O

- ➔ Incubation 1 h, 37 °C
- ➔ Inactivation 10 min, 37 °C

- Dephosphorylation of Vector/Backbone
  - Add 2 µL of Shrimp Alkaline Phosphatase (Jena Bioscience)

- ➔ Incubation 30 min, 37 °C
- ➔ Inactivation 5 min, 75 °C

- Phosphorylation of Insert
  - Add 3 µL 10x T4 Ligase Buffer (Thermo Fisher) -> contains right amount of ATP
  - Add 1 µL PNK (NEB)

- ➔ Incubation 30 min, 37 °C
- ➔ Inactivation 20 min , 65 °C

- Purification of Vector and Insert

- ➔ Any given Gel and PCR Purification Kit
- ➔ We recommend purification after gel electrophoresis

- Ligation

  Molar ratio: 1:5; Vector: XXX bp; Insert: XXX bp

  Calculation of needed Insert in ng:

  XX ng Insert = 50 ng Vector • 1/5 • (insert (bp) / vector (bp))

  - ◊ X µL 50 ng Vector
  - ◊ X µL XX ng Insert
  - ◊ 2 µl T4 DNA Ligase Puffer (Thermo Fisher)
  - ◊ 1 µL T4 DNA Ligase (Thermo Fisher)
  - ◊ Ad 30 µL ddH_2O

➔ Incubation 1 h, RT
➔ Inactivation 20 min, 80 °C

- Transformation

  We recommend Heat Shock Transformation of chemically competent cells.

  If you prefer transformation with electro competent cells we recommend purifying the ligation product.