Digestion of DNA with Restriction Enzymes, Dephosphorylation of Vector and Purification

1) Digestion with NdeI and XmaCI

20 µL vector (~200-500 $^{ng}/_{\mu L}$)

(**OR** 30 µL PCR product after purification on agarose gel)

5 µL buffer #4 (10x, NEB)

23 µL (**OR** 13 µL for PCR product) ddH_2O, mix well

2 µL NdeI (20 $^{U}/_{\mu L}$, NEB), mix well

50 µL

- 18h at 37°C (without shaking)
  (using the PCR machine for this purpose works very well)

- add:

3 µL XmaCI (10 $^{U}/_{\mu L}$, NEB)

0.55 µL BSA (100x, NEB)

0.5 µL buffer #4 (10x, NEB)

0.95 µL ddH_2O, mix well

55 µL

- 18-22h at 37°C (without shaking)
- 20min at 65°C (to denature enzymes)

2) Dephosphorylation of vector with Shrimp Alkaline Phosphatase (SAP)

55 µL digestion mix

7 µL SAP buffer (10x, Roche)

8 µL SAP (1 $^{U}/_{\mu L}$, Roche), mix well

70 µL

- 2.5h at 37°C (without shaking)
- 20min at 65°C (to denature enzyme)

3) Purification of digested vector and PCR product with agarose gels

- vector: usually ~1%
  PCR products: usually ~2%