Preparation of plasmid DNA (by Wizard midi column)

(Day1; **Bacteria culture**)

Colony (LB / Amp plate) → 50 ml of LB (or 2 x YT) / 50-100 μg/ml of Ampicillin (Amp) in 300ml conical flask

↓ Culture O/N (16-24 h)

(Day2; **Midi-prep**)

Bacteria in conical flask

↓ to 50 ml tube

↓ 5000 rpm, 10 min, 4°C

ppt (sup → autoclave)

↓ add 5 ml of Sol. I

↓ suspend by vortex

↓ add 5 ml of Sol. II (Lysis buffer)

↓ invert 7 times (clear cell lysate)

↓ add 5 ml of Sol. III (Neutralization beffer)

↓ invert 5 times (become clouded)

↓ 8000 rpm, 10 min, 4°C

sup

↓ filtered by KIM WIPE

↓ add 6 ml of Midiprep resin (Promega, A7701)

↓ to column (Promega, A7651) & vacuum

↓ add 17 ml of wash buffer & vacuum

column

↓ cut and to 1.5ml microtube

↓ 15000 rpm, 1 min, 4°C

↓ to new 1.5ml microtube

↓ add 300 μl of TE (65°C)

↓ 15000 rpm, 1 min, 4°C

Quantification (1 : 100) & cut check

Sol. I
50 mM Tris-HCl (pH 7.5)
10 mM EDTA
50-100 μg/ml Rnase A

Sol. II
0.2M NaOH
1% SDS

Sol. III
1.32M KOAc (pH 4.8)