Preparation of plasmid DNA (Mini-Prep)

（用途）

インサートのチェックの時に用いる。
（とったPlasmidをシークエンスするときはPCR purification kitで精製）

(Day1; **Bacteria culture**)

Colony (LB / Amp plate) → 2 ml of LB (or 2 x YT) / Amp (50-100 μg/ml) in 15 ml tube

↓ Culture O/N (～18 h)

(Day2; **Mini-prep**)

Bacteria in 15 ml tubes

↓ to 1.5 ml tube

↓ 15,000 rpm, 1 min, 4°C

ppt

↓ add 200 μl of Sol. I

↓ suspend by vortex

↓ add 200 μl of Sol. II (Lysis buffer)

↓ invert 7 times (clear cell lysate)

↓ add 200 μl of Sol. III (Neutralization buffer)

↓ invert 5 times (become clouded)

↓ Add 10 μl of chloroform

↓ 15,000 rpm, 10 min, 4°C

sup (~ 550 μl)

↓ add 550 μl of isopropanol

↓ R.T 5 min

↓ 15,000 rpm, 10 min, 4°C

ppt

↓ add 500 μl of 70% EtOH

↓ vortex

↓ 15,000 rpm, 5 min, 4°C

ppt

↓ air dry

↓ dissolve in 40 μl of TE

Quantification (1: 20) & cut check (5~10 μl)

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)