The DNA extraction with alkaline extraction

1.5 ml tube

↓

＋1.5 ml culture

↓

Centrifuge　13000 rpm 1min 4℃

↓

Collect the supernatant and cast aside

↓

＋Soln 1　200μl　mix by voltex

↓

＋soln 2　200μl

↓

Gently mix by inverting the tube

↓

＋soln 3　200μl

↓

Gently mix

↓

On ice 5min

↓

Centrifuge　13000 rpm 10 min 4℃

↓

Collect the supernatant and　add to the new tube

↓

＋phenol、chloroform、isoamylalcohol　500μl

↓

Mix by voltex

↓

Centrifuge　13000 rpm 5 min 4℃

↓

Take only the only water layer

↓

add to the new tube

↓

＋100　% Etoh 400 μl

↓

Mix by vortex

↓

Centrifuge　13000 rpm 20 min 4 ℃

↓

Throw away the supernatant to alcohol waste bin

↓

＋70　% Etoh 500 μl

↓

Gently mix

↓

Throw away the supernatant to alcohol waste bin

↓

65 ℃ to dry

↓

＋TE buffer 50 μl

↓

＋RNase A (0.5 μg/μl)

↓

37 ℃　20 min

↓

Voltex and Tabletop small centrifuge

↓

4 ℃　preserve Refrigerator

TE buffer 1ml (dH_2O 985 μl , 1M Tris-HCl (pH8.0) 10 μl , 200mM EDTA 5 μl)

Soln 1　1 ml (dH_2O 900 μl , 1M Tris-HCl (pH8.0) 50 μl , 200mM EDTA 50 μl)

Soln 2　1 ml (dH_2O 876 μl , 5M NaOH　24 μl , 10%SDS 100 μl)

Soln 3　1 ml (dH_2O 290 μl , 5M potassium acetate , 600 μl , acetic acid 110 μl)

RNase A (0.5 μg/μl) 1 μl

1M Tris-HCl　60 μl：　3￥ ≒ 0.03 $

200mM EDTA　55 μl：　0.396￥ ≒ 0.003 $

5M NaOH　24 μl：　0.028￥≒0.0002 $

10%SDS　100 μl：　1.8￥≒0.0149 $

5M potassium acetate　600μl　：　1.65¥≒0.0136$

acetic acid　110μl　：　0.18¥≒0.0015$

RNase A (0.5　μg/μl)　1μl　：　130¥≒1.1$

Tube　×3：　6.5¥≒0.054$

TOTAL　144¥≒1.22$