ALKALINE LYSIS MINI PREP PROCEDURE
(Procedure from Maniatis cloning manual)

1. Inoculate a 5 ml culture of LB/amp (50-100 µg/ml) with a single bacterial colony. Place tube in 37°C shaker overnight.

2. Fill an eppendorf tube with approximately 1.5 ml of the culture and microfuge 1 minute. Remove the supernatant and add another aliquot of culture to the tube. Again, microfuge 1 minute and remove the supernatant. Repeat until the entire 5 ml culture is spun down in one tube.

3. Resuspend pellet in 100 µl of solution I:

\[
\text{Solution I} \\
\begin{align*}
25 \text{ mM Tris pH} & \ 8.0 \\
10 \text{ mM EDTA pH} & \ 8.0 \\
50 \text{ mM Glucose} & \\
\text{dH}_2\text{O} & \\
\end{align*}
\]

100 ml: 1 M Tris 25.0 ml
0.5 M EDTA 2.0 ml
1 M Glucose 5.0 ml
dH\text{O} 68.0 ml

4. Add 20 µl 10 mg/ml lysozyme solution:

1 ml: 0.01 g lysozyme
Fill to 1 ml with 0.250 M Tris pH 8.0
Mix well and let the tube stand at room temp 2 minutes.

5. Add 200 µl of Solution II:

\[
\text{Solution II} \\
\begin{align*}
50 \mu l & \ 20\% \ SDS \\
20 \mu l & \ 10 \ N \ NaOH \\
930 \mu l & \ dH_2O \\
\end{align*}
\]

Mix and ice 5 minutes.

6. Add 150 µl Solution III: (3 M K+, 5 M Acetate)

\[
\text{Solution III} \\
\begin{align*}
5 \text{ M KOAc} & \\
glacial acetic acid & \\
dH_2O & \\
\end{align*}
\]

100 ml: 60 ml
11.5 ml
28.5 ml

\[
5 \text{ M Potassium acetate} \\
100 \text{ ml: 49.075 g potassium acetate} \\
\text{Fill to 100 ml with dH}_2\text{O}
\]

IV.A.1
7. Vortex gently to form small white clumps. Ice 5 minutes.

8. Microfuge 5 minutes in cold microfuge.


10. Transfer aqueous (upper) phase to a new tube. Add 1 ml room temp EtOH. Mix well and stand at room temp for 2 minutes.

11. Microfuge 5 minutes in cold microfuge. Pour off EtOH and let pellet dry completely.

12. Resuspend pellet in 50 µl of TE/RNase (20 µg/ml):
    1 ml: 20 µl 10 mg/ml RNase
    980 µl TE

13. Place tube at 37°C for 30 minutes.

14. Restriction digest of mini-prep DNA:
    10 µl DNA (approximately 1 µg)
    2 µl enzyme buffer
    1 µl enzyme (approx. 10 units)

    Incubate for 2-12 hours at 37°C (for most enzymes--check appropriate temperature before incubation).

15. Prepare a 1% agarose gel with 0.2% EtBr
    100 ml: 1 g agarose
    100 ml 1xTBE
    --boil to dissolve agarose--
    20 µl 10 mg/ml EtBr

    Add 2 µl gel loading dye and microfuge the tube approx. 5 seconds. Load 10 µl sample.