# A rapid method for isolating high quality plasmid DNA suitable for DNA sequencing

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Submitted September 26, 1990

The alkaline-lysis mini-preparation method of isolating plasmid DNA[1] includes several time-consuming steps. Here we provide a shortened protocol in which the volumes and incubation times have been altered (in particular the ethanol precipitation step) and the phenol extraction and RNAse digestion omitted completely.

Recently a mini-prep method has been published requiring the use of caesium chloride and ethidium bromide which then have to be carefully removed[2]. Wong *et al.* describe Sephacryl™ spin columns and state that these may need to be run more than once[3]. Both of these extra procedures add to the expense, and are time-consuming. They are not required in the method we describe here.

Our method yields high-quality DNA that can be readily sequenced by the dideoxy chain termination method (Figure 1 shows a portion of DNA sequence from a recombinant pBluescriptII KS$^+$ plasmid). For practical convenience the method is presented in a simple step-by-step protocol.

1. Inoculate a single 'tooth-picked' bacterial colony into 3 ml of a rich growth medium e.g. Terrific broth (TB)[4], containing antibiotic as appropriate, and incubate with vigorous shaking at 37°C overnight.
2. Decant 2 ml of the overnight culture into an Eppendorf™ tube, and spin in a benchtop centrifuge at 13,000 rpm for 1 minute.
3. Completely remove the supernatant from the bacterial pellet, by aspiration.
4. Carefully resuspend the pellet in 200 μl GTE[1] solution.
5. Add 400 μl of a freshly prepared solution of 0.2 M NaOH/1% SDS, and invert several times before placing on ice for 5 minutes.
6. Add 300 μl of 3 M potassium acetate (pH 4.8), and invert several times before replacing on ice for 5 minutes. Do *not* vortex.
7. Centrifuge at 13,000 rpm for 5 minutes, and remove supernatant to a clean 2 ml Eppendorf™ tube.
8. Add 1 volume (900 μl) of absolute ethanol to the supernatant, vortex briefly, and immediately centrifuge at 13,000 rpm for 5 minutes.
9. Carefully discard the supernatant, and wash the plasmid pellet with 2 ml of 70% ethanol (optional), before a final centrifugation at 13,000 rpm for 2 minutes.
10. Discard the supernatant, and dry the pellet under reduced pressure.
11. Resuspend the DNA pellet in 40 μl of sterile TE. The DNA is then ready for sequencing or restriction enzyme digestion.
12. The DNA is alkali-denatured and the contaminating RNA hydrolysed by incubation of a portion (9 μl of the solution with (1 μl) 2 M NaOH for 10−15 min at 37°C.
13. To anneal the sequencing primer to the denatured template 10 pMoles (1 μl of a 10 μM stock) of primer is added[5] and briefly vortexed prior to neutralisation of the sodium hydroxide with 3 M potassium acetate (3 μl) and the DNA ethanol

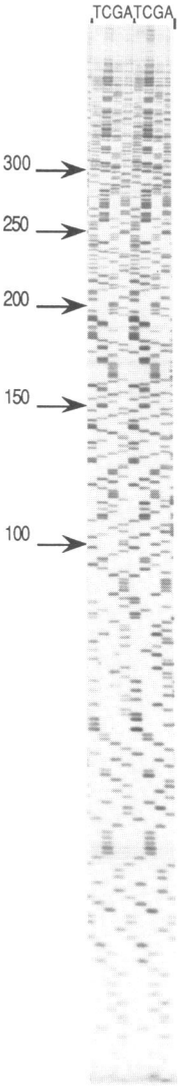


**Figure 1.** Double stranded DNA sequencing performed on two plasmid templates prepared using the method described here. Electrophoresis was performed using standard gel without a buffer gradient but none-the-less 300 bp of DNA sequence can be determined from this gel.

precipitated (75 μl). Following centrifugation at 13,000 rpm for 10 minutes, the pellet is washed with 100 μl of 75% ethanol prior to drying.

The remainder of the protocol is as described in the Sequenase™ (USB) protocol. A typical yield from the purification of the commonly used pUC based plasmids, grown in *E.coli* strains (e.g. DH5α, NM522, TG1) is around 8 μg. Thus there is ample DNA for several sequencing reactions.

This mini-preparation method is rapid and convenient and is in routine use in this laboratory. It has been used for the sequencing of repetitive and G-C as well as A-T rich templates. It produces DNA within 30 minutes from pelleting the overnight bacterial culture and is an excellent template for DNA sequencing.

## REFERENCES

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