

# A Simple, Rapid Method for Extracting Large Plasmid DNA from Bacteria

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## Abstract

We are studying the lateral transfer of transmissible antibiotic resistance plasmids among stream bacteria impacted by fecal runoff from poultry and cattle. Such plasmids are typically large (ca. 40–100 kb) and occur in low copy numbers in the cell and have therefore typically been difficult to isolate and therefore to study. Traditional protocols, based upon variations of the standard alkaline-lysis method, are long (ca. 1 1/2 to 2 days) and difficult. Commercial kits designed for the isolation of Bacterial Artificial Chromosomes (BACs) can be used and are an improvement; however, these are expensive and still require hours of sustained effort. We have adapted a method published by Rondon *et al.* (1999), originally designed for the isolation of BAC DNA, for the rapid isolation of large plasmid DNA. In this method, lysis and alkaline denaturation steps are combined, incubation steps are vastly reduced, proteins are removed via a simple ammonium acetate/chloroform step, and the DNA precipitated using a polyethylene glycol/NaCl step. No ethanol precipitation is required. If additional purification is required, extracted DNA can be further processed through a Qiagen Plasmid Mini or Midi column (Qiagen Inc., Valencia CA). The method is rapid (under 1 hour), easy, very inexpensive and has been reliably used by undergraduate students to isolate large (up to 200 kb) native plasmids from a variety of both Gram-positive and Gram-negative genera including *Shigella*, *Klebsiella*, *E. coli*, *Pseudomonas*, *Bacillus*, *Streptococcus*, *Staphylococcus*, and *Enterococcus*, as well as BACs from *E. coli*. The protocol is simple and reliable enough to be used for the rapid large-scale visualization of native plasmids and we have used it to visualize and isolate DNA from hundreds of multidrug resistance plasmids exogenously captured from stream sediments, soils, and beach sands.

## Introduction

Although some plasmids are small (e.g. 2–10 kilobases) and replicate solely in tandem with their host cells, many plasmids encode their own machinery for mobilization and self-transmission to other host cells. Such plasmids – referred to as “mobilizable” or “self-transmissible”, depending upon the functions they encode – are typically large (e.g. 30–200 kb) and occur in low copy numbers in the cell. Because of their large size and low copy number, such plasmids have typically been difficult to isolate and, therefore, to study. Most large plasmid isolation protocols rely upon the typical alkaline-lysis method, which separates plasmid DNA from chromosomal DNA based upon its ability to rapidly reanneal after denaturation at high pH. Such protocols (e.g. Anderson & McKay, 1983) are typically long (e.g. one and a half to two days), involved, and difficult, with low yields of plasmid DNA. Commercial kits (e.g. the Qiagen Midi plasmid kit; Qiagen Sciences, MD) designed for the isolation of BACs (Bacterial Artificial Chromosomes) have been successfully modified for the isolation of large, low copy plasmids. However, these are expensive and still take most of a day’s effort in the laboratory. We have successfully adapted and modified a method published by Rondon *et al.* (1999), originally intended for BAC isolation, for the isolation of large, native plasmids. The method is rapid (under 1 hour), reliable, and very inexpensive and has been successfully used to isolate large plasmids from a wide variety of both Gram-positive and Gram-negative bacteria.

Another rapid large plasmid isolation protocol has recently been published by Williams *et al.* (2006). This excellent protocol is designed specifically for isolating plasmid DNA with little or no contaminating chromosomal DNA, in order to clone and sequence plasmid DNA. By contrast, the protocol outlined here is much faster and even less expensive than is the Williams protocol, but there is often some chromosomal DNA. Thus, although we have used the protocol for preparing plasmid DNA for cloning and sequencing, it is more appropriate for rapid, large-scale screening, restriction digestion, and PCR amplification-based studies.

## 1. Outline of plasmid extraction protocol

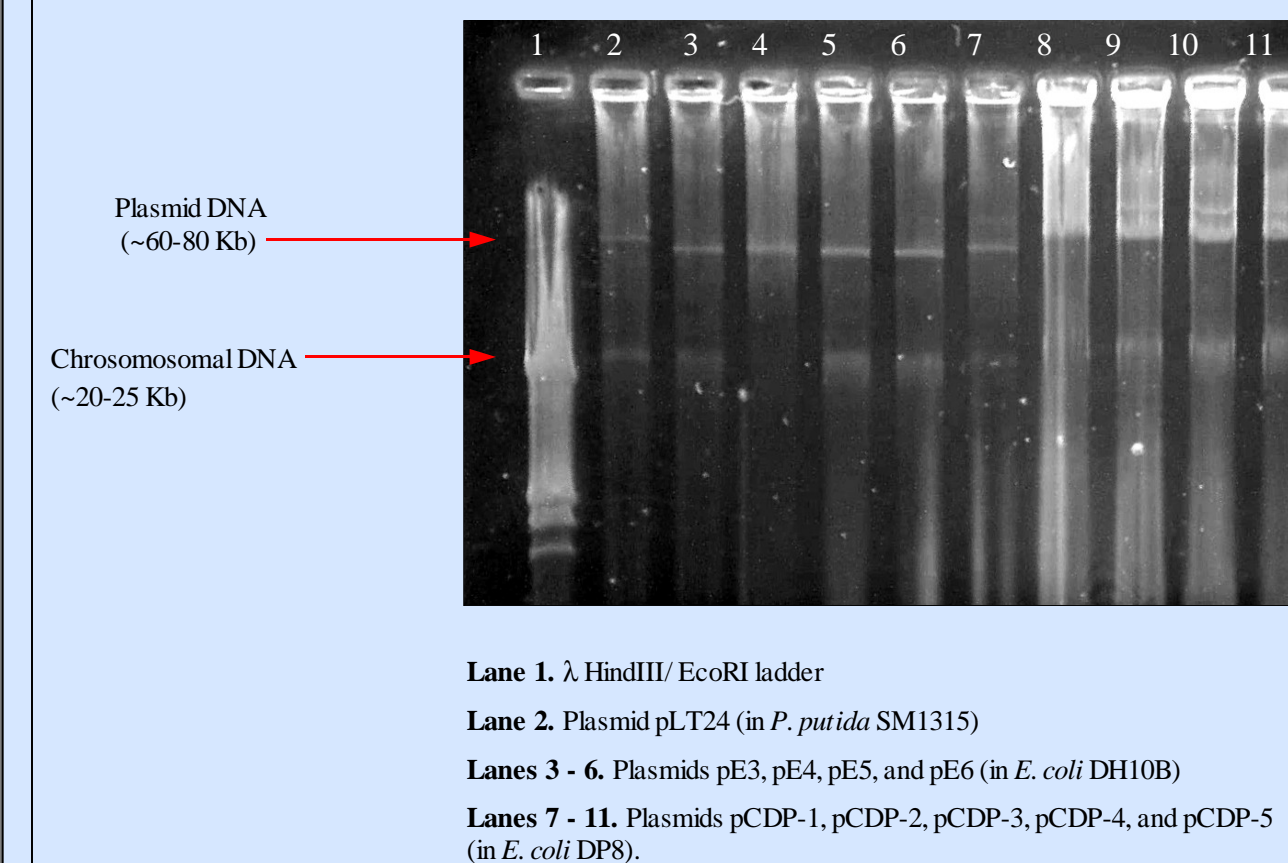
1. Grow strains in broth overnight
2. Pellet 1.5 - 2 ml of cells by centrifugation. Remove supernatant.
3. Resuspend by vortexing in 100  $\mu$ L resuspension buffer (50 mM glucose/10 mM EDTA/10 mM Tris-Cl, pH 8.0).
4. Add 200  $\mu$ L lysis solution (0.2 M NaOH/1% sodium dodecyl sulfate [SDS]). Mix by inversion. Incubate 5 minutes at room temperature.
5. Add 150  $\mu$ L 7.5 M ammonium acetate and 150  $\mu$ L chloroform. Mix by inversion. Chill on ice 10 minutes. Centrifuge 10 minutes.
6. Transfer supernatant to 200  $\mu$ L precipitation solution (30% polyethylene glycol 8000/1.5 M NaCl). Mix by inversion. Chill on ice 15 minutes.
7. Centrifuge to pellet DNA. Remove supernatant. Resuspend in TE or water.

## Notes on the protocol

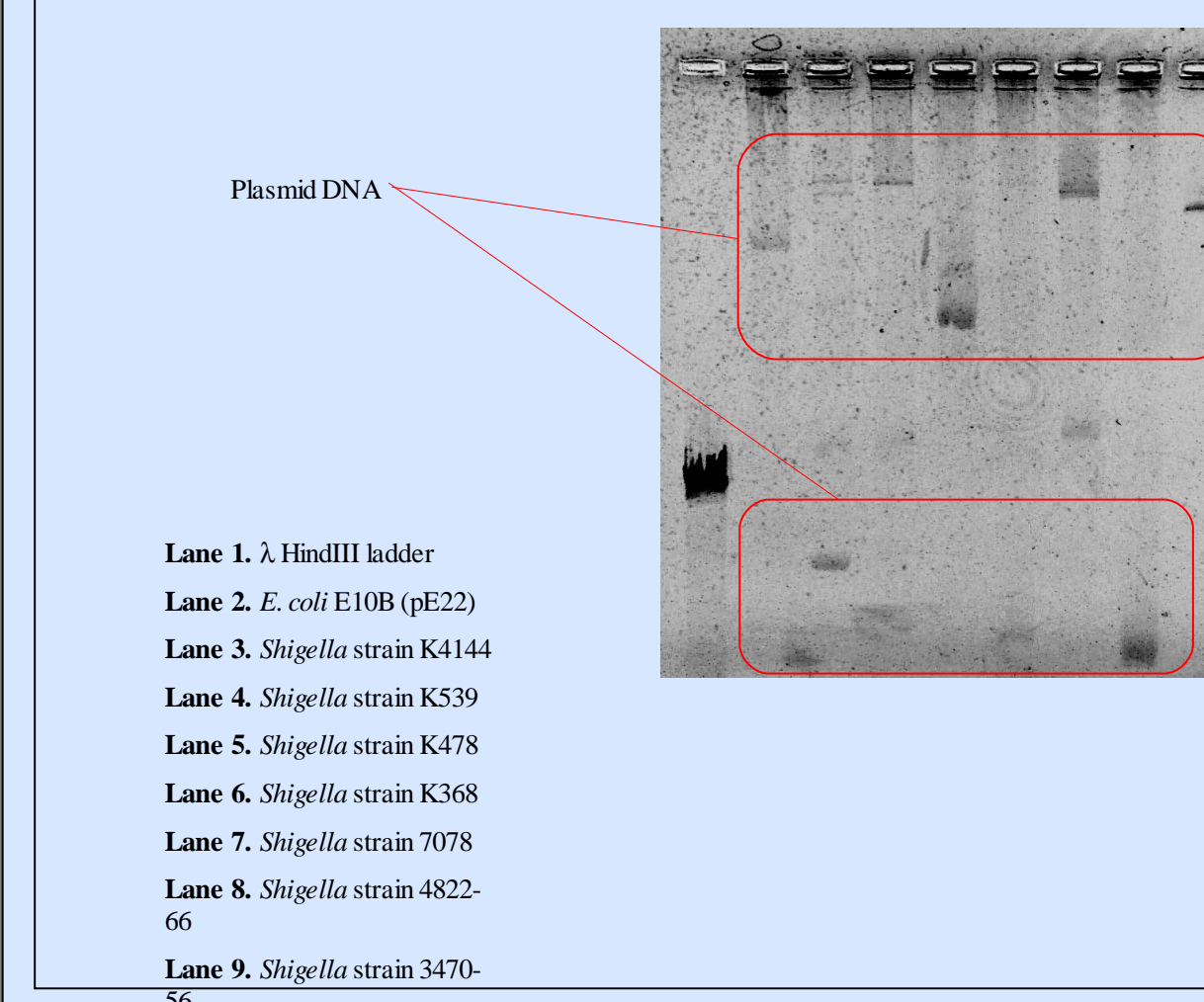
- Steps 1 and 2. We have successfully scaled this protocol up to 100 ml (centrifuge two 50 ml tubes of cells).
- Step 3. For Gram positives, incubation with fresh lysozyme added to the resuspension buffer is recommended.
- Step 4. This step both lyses the cells and denatures the DNA. The lysis solution should be prepared fresh from NaOH pellets and SDS stock solution. For all post-lysis steps, mix by gentle inversion of the tube four or five times.
- Step 5. The chloroform needs to be added quickly after the ammonium acetate. An optional step to clean up the DNA from residual proteins is to add the supernatant after this step to 600  $\mu$ l 1:1 phenol:chloroform.
- Step 6. The precipitation solution should be in a fresh tube.
- Step 7. Dissolve the pellet at 4°C for two hours to overnight. Extended storage recommended at -20°C. We normally dissolve the pellet initially in 50-100  $\mu$ l TE; if after dissolving overnight there is significant undissolved DNA in the wells of an agarose gel, then more TE should be added.

## 2. Plasmids are easily visualized using standard agarose gel electrophoresis

**Figure 1.** Agarose (0.7%) gel electrophoresis of plasmid extracts from *Pseudomonas* and *E. coli* strains containing captured tetracycline resistance plasmids. Plasmids were captured directly from freshwater stream sediments contaminated with poultry and cattle feces using a protocol developed in our laboratory.

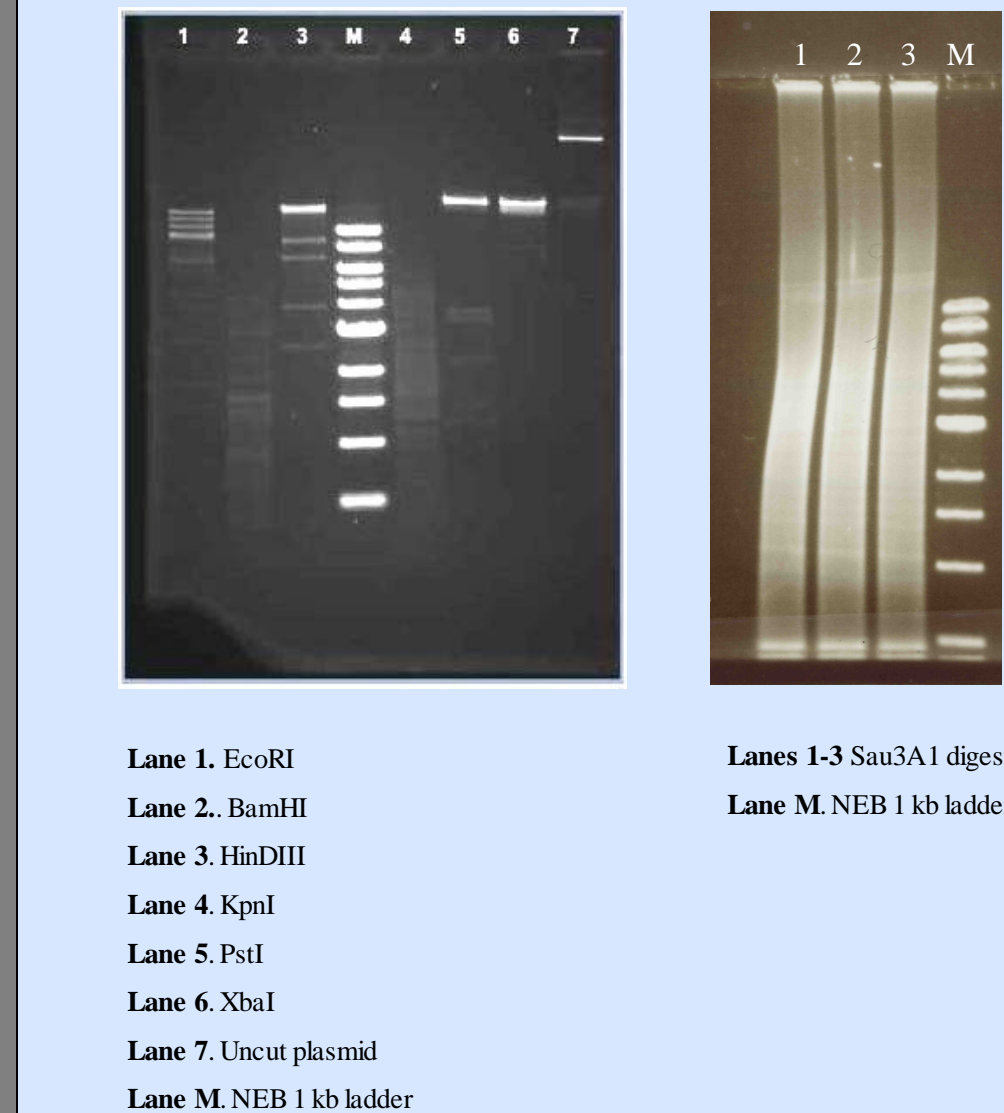


**Figure 2.** Agarose (0.7%) gel electrophoresis of plasmid extracts from seven *Shigella* strains, with an *E. coli* positive control. Note the presence of multiple plasmids in most lane. The largest band seen in most lanes is probably the 200 kb *Shigella*-specific virulence plasmid (Escobar-Paramo, 2003).



## 3. Plasmid DNA is normally restriction digestible without additional purification

**Figure 3.** Agarose (0.7%) gel electrophoresis of restriction digestions of the multiple antibiotic resistance plasmid pL1-9 captured from poultry litter. (A) Six-base cutter digestion. (b) 4-base cutter digestion used for cloning fragments for genomic sequencing.

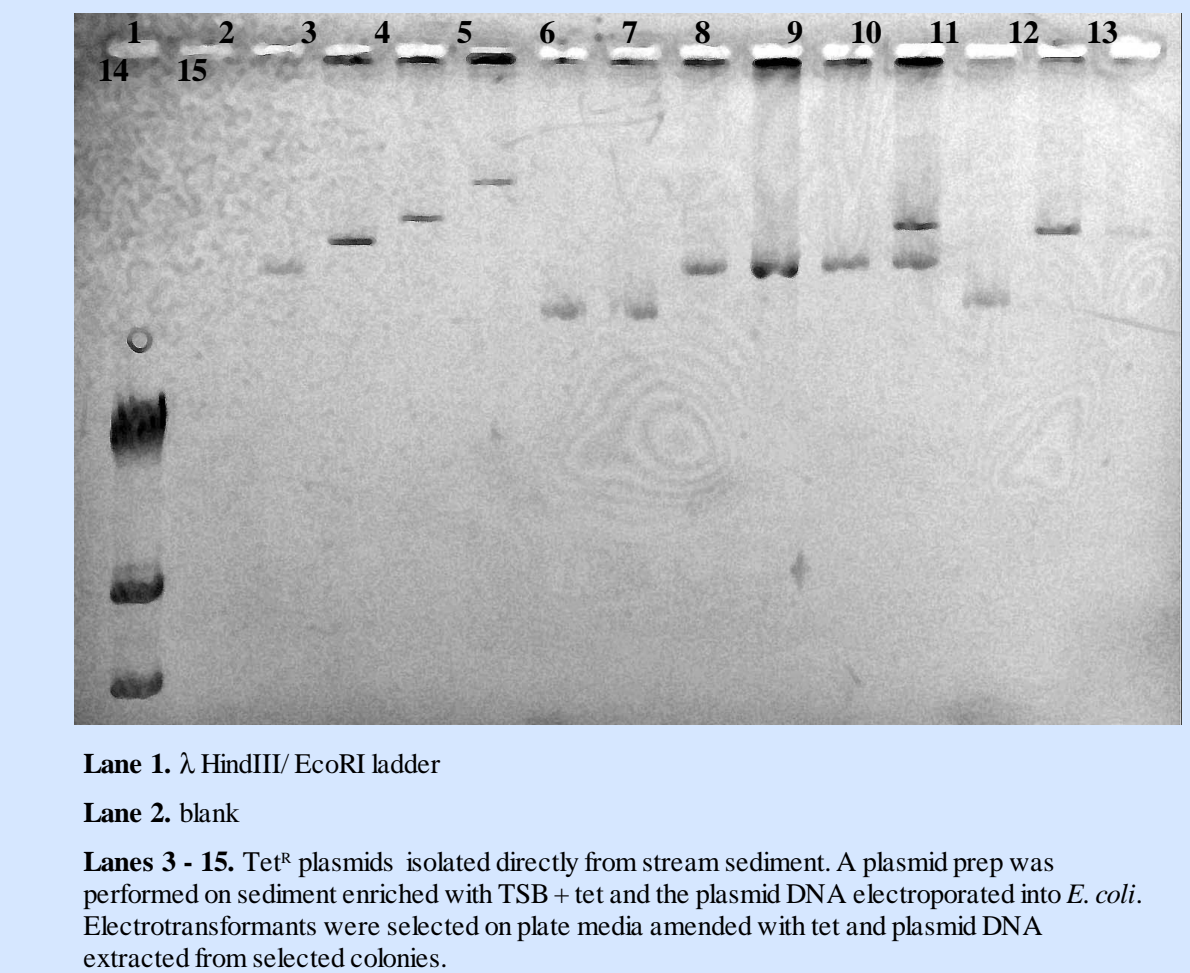


## 4. Plasmids have been successfully isolated from a range of Gram negative and Gram positive genera

- *Escherichia* (incl. native plasmids & BACs)
- *Pseudomonas*
- *Klebsiella*
- *Shigella*
- *Streptococcus*
- *Staphylococcus*
- *Enterococcus*
- *Bacillus*

## 5. Multiple resistance plasmids can be directly extracted from a mixed community of stream sediment bacteria without culturing isolates

**Figure 4.** Agarose gel electrophoresis of plasmid DNA extracted directly from stream sediment enriched in TSB + tetracycline, electroporated into *E. coli*, and selected on TSA + tetracycline plates.



## Conclusions

- We have adapted a protocol, originally developed for BAC isolation, for large plasmid isolation. It is inexpensive, simple, and very fast (under 1 hour).
- It is extremely useful for fast screening (e.g. after exogenous plasmid capture) or as preparation for subsequent restriction digestion or PCR.
- Product is generally pure enough for immediate restriction digestion.
- The protocol can be scaled up.
- We have used the protocol, along with electroporation, to extract resistance plasmids directly from stream sediments, without culturing isolates and without exogenous capture.
- With gel purification, the product can be useful for cloning and sequencing of large, native plasmids such as transmissible antibiotic resistance plasmids. We are currently sequencing a ca. 80 kb multiresistance plasmid captured from poultry litter.

## References

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