Extracting plasmid DNA from Agrobacterium with the Bioline ISOLATE II Plasmid Mini Kit

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Abstract

To extract plasmid DNA from *Agrobacterium*, to confirm the correct plasmid has been taken-up, this protocol allows you to directly perform a mini-prep on transformed *Agrobacterium*, digest this DNA and visualize it on a gel. This is without the need of rescuing the plasmid in *E. coli* or genotyping with PCR. This protocol using the commercially available Bioline ISOLATE II Plasmid Mini Kit, designed for use with *E. coli*. The standard protocol this is modified from is available here: https://www.bioline.com/au/downloads/dl/file/id/1219/isolate_ii_plasmid_mini_kit_protocol.pdf

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Before start

Ensure RNase A has been added to Resuspension Buffer P1. Pre-warm the required amount of PW1 to 50-55 ºC before starting the extration. Ensure Ethanol has been added to PW2.

Materials

ISOLATE II Plasmid Mini Kit BIO-52057 by Bioline

Protocol

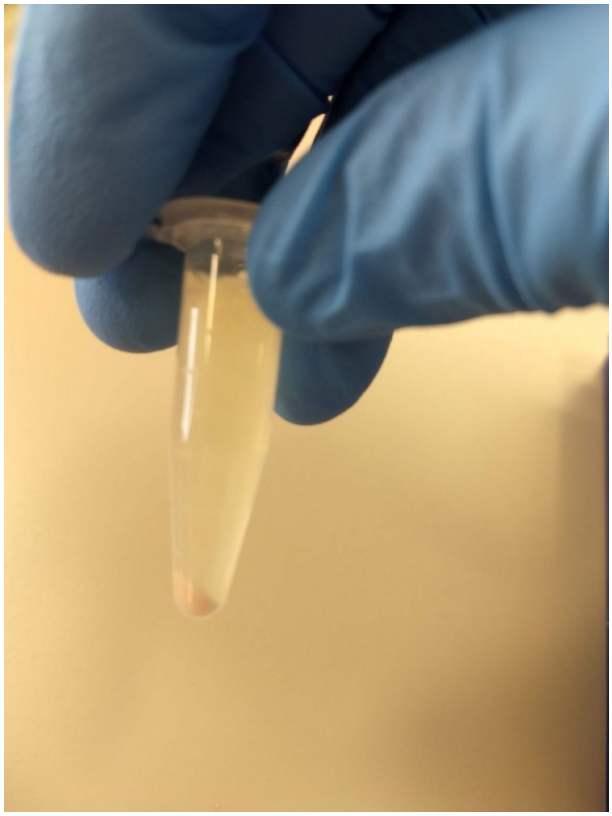
Harvest bacterial cells

Step 1.

Pellet 3 ml of a saturated Agrobacterium LB culture with appropriate antibiotics for 30s at 11,000 x g.

EXPECTED RESULTS

Typical pellet after spinning down 3 ml of 24 hour grown Agrobacterium.



NOTES

I grew Agrobacterium in 5 ml of LB with appropriate antibiotics for 24 hours at 29 ºC.

Spinning the 3 ml down can be done in two steps of 1.5 ml.

Harvest bacterial cells

Step 2.

Discard supernatant and remove as much liquid as possible.

Lyse cells

Step 3.

Add 250µl Resuspension Buffer P1 and resuspend cell pellet by pipetting up and down.

Lyse cells

Step 4.

Add 250µl Lysis Buffer P2. Mix gently by inverting tube 6-8 times. Incubate at room temperature for up to 5 min or until lysate appears clear.

Note: Do not vortex to avoid shearing genomic DNA

DURATION
00:05:00 : Lysis

Lyse cells

Step 5.

Add 300µl Neutralization Buffer P3. Mix thoroughly by inverting tube 6-8 times.

Note: Do not vortex to avoid shearing genomic DNA

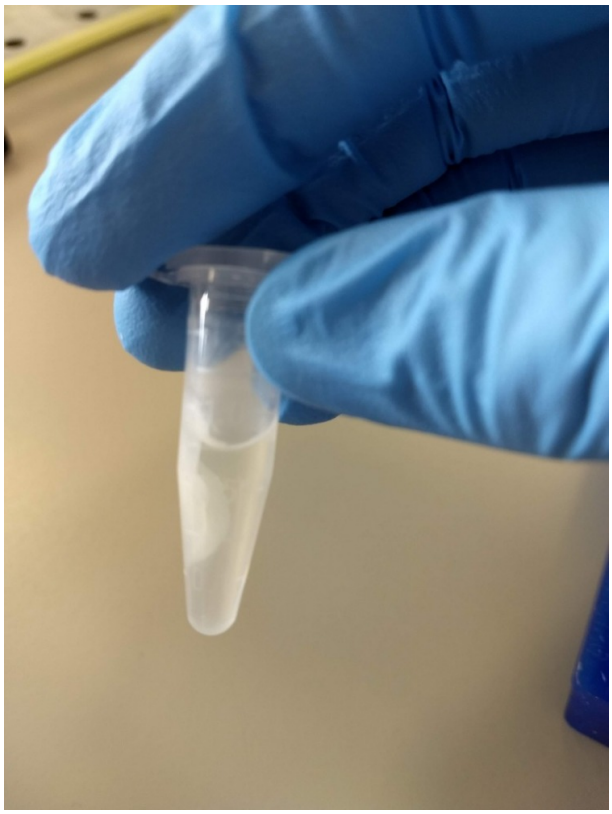
Clarification of lysate

Step 6.

Centrifuge 10 min at max speed at room temperature.

EXPECTED RESULTS

This is the very snot-like pellet. Ensure you do not disturb it when you take the supernatant.



NOTES

The max speed on the centrifuged I used was 20817 x g but faster is probably not a bad thing.

The pellet is very glopy (snot-like) so a long, fast spin at this stage is vital to being able to get the supernatant.

Bind DNA

Step 7.

Bind DNA Place ISOLATE II Plasmid Mini Spin Column in a 2ml Collection Tube. Pipette (do not decant) a maximum of 750µl (usually less) of clarified sample supernatant onto column.

Centrifuge 1 min at 11,000 x g and discard flow-through.

Wash silica membrane

Step 8.

Add 500µl Wash Buffer PW1 preheated to 50°C. Centrifuge 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.

NOTES

The use of PW1 is considered optional for some E. coli strains but is essential for extracting plasmid from Agrobacterium as it contains many nucleases, which PW1 is used to remove.

I actually warmed it up to 55 ºC, but I am sure the manual's recommended 50 ºC will work just the same.

Wash silica membrane

Step 9.

Add 600µl Wash Buffer PW2 (supplemented with ethanol). Centrifuge 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.

Dry silica membrane

Step 10.

Centrifuge 2 min at 11,000 x g, to remove residual ethanol. Place ISOLATE II Plasmid Mini Spin Column in a 1.5ml microcentrifuge tube.

Elute DNA

Step 11.

Add 30-50µl Elution Buffer P, nuclease-free water or TE buffer directly onto center of silica membrane. Incubate at room temperature for 1 min.

DURATION

00:01:00 : Elution incubation

Elute DNA

Step 12.

Centrifuge 1 min at 11,000 x g.

Restriction enzyme digestion

Step 13.

The DNA is now very dilute so it would be a good idea to use 20 µl in a typical restriction enzyme digest. This is usually much less than you would use from a prep from E. coli.

Warnings

Please check the MSDS information for the ISOLATE II Plasmid Mini Kit: https://www.bioline.com/au/downloads/dl/file/id/2807/isolate_ii_plasmid_mini_kit_msds.pdf