ISOLATION OF HIGH-COPY PLASMID DNA FROM *E. COLI*

1 **Harvest bacterial cells**
Pellet 1-5ml of a saturated *E. coli* LB culture for 30s at 11,000 x g.
Discard supernatant and remove as much liquid as possible.

2 **Lyse cells**
- Add 250µl Resuspension Buffer P1 and resuspend cell pellet by vortexing or pipetting up and down.
- 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.
- Add 300µl Neutralization Buffer P3.
Mix thoroughly by inverting tube 6-8 times.

3 **Clarification of lysate**
Centrifuge 5 min at 11,000 x g at room temperature.

4 **Bind DNA**
Place ISOLATE II Plasmid Mini Spin Column in a 2ml Collection Tube (supplied).
Decant or pipette a maximum of 750µl of clarified sample supernatant onto column.
Centrifuge 1 min at 11,000 x g and discard flow-through.

5 **Wash silica membrane**
If plasmid DNA is prepared from host strains containing high levels of nucleases, an extra wash with Wash Buffer PW1 is strongly recommended.
(Optional) Add 500µl Wash Buffer PW1 preheated to 50°C.
Centrifuge 1 min at 11,000 x g.
Add 600µl Wash Buffer PW2 (supplemented with ethanol).
Centrifuge 1 min at 11,000 x g.
Discard flow-through and reuse Collection Tube.

6 **Dry silica membrane**
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 (not supplied).

7 **Elute DNA**
Add 50µl Elution Buffer P directly onto center of silica membrane.
Incubate at room temperature for 1 min.
Centrifuge 1 min at 11,000 x g.

BTP0812V1

ISOLATION OF LOW-COPY PLASMID, P1 CONSTRUCTS OR COSMID DNA FROM *E. COLI*

If this protocol is to be used frequently, an additional P1, P2 and P3 ISOLATE II Plasmid Buffer Set can be ordered separately.

1 **Harvest bacterial cells**
Pellet 5-10ml of a saturated *E. coli* LB culture, for 30s at 11,000 x g.
Discard supernatant and remove as much liquid as possible.

2 **Lyse cells**
- Add 500µl Resuspension Buffer P1 and resuspend cell pellet by vortexing or pipetting up and down.
- Add 500µl 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.
- Add 600µl Neutralization Buffer P3.
  Mix thoroughly by inverting tube 6-8 times.

3 **Clarification of lysate**
Centrifuge 10 min at 11,000 x g at room temperature.

4 **Bind DNA**
Place ISOLATE II Plasmid Mini Spin Column in a 2ml Collection Tube (supplied).
Decant or pipette 750µl of clarified sample supernatant onto column.
Centrifuge 1 min at 11,000 x g and discard flow-through.
Repeat with any remaining clarified sample supernatant.

5 **Wash silica membrane**
An extra wash with Wash Buffer PW1 is recommended to enhance the perfomance of downstream applications.
(Optional) Add 500µl Wash Buffer PW1 preheated to 50°C.
Centrifuge 1 min at 11,000 x g.
Add 600µl Wash Buffer PW2 (supplemented with ethanol).
Centrifuge 1 min at 11,000 x g.
Discard flow-through and reuse Collection Tube.

6 **Dry silica membrane**
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 (not supplied).

7 **Elute DNA**
Add 50µl Buffer P preheated to 70°C directly onto center of silica membrane.
Incubate for 2 min at 70°C.
Centrifuge 1 min at 11,000 x g.

BTP0812V1