Experienced User Protocol

All spins at ;12,000 3 g, except as noted.

1 Harvest & lyse bacteria

- Pellet cells from 1–5 ml overnight culture *1 minute* (1 ml from TB or 2xYT; 1–5 ml from LB medium). Discard supernatant.
- Resuspend cells in 200 µl Resuspension Solution. Pipette up and down or vortex.
- Add 200 µl of Lysis Solution. Invert gently to mix. Do not vortex. Allow to clear for :5 minutes

* *Prior to first time use, be sure to add the RNase A to the Resuspension Solution.*

2 Prepare cleared lysate

- Add 350 µl of Neutralization Solution (S3). Invert 4–6 times to mix.
- *Pellet debris 10 minutes at max speed.*

3 Prepare binding column

- Add 500 ml Column Preparation Solution to binding column in a collection tube.
- *Spin at ≥12,000 x g, 1 minute.* Discard flow-through.

4 Bind plasmid DNA to column

- Transfer cleared lysate into binding column.
- *Spin 30",1 minute.* Discard flow-through.

5 Wash to remove contaminants

- *Optional (EndA$^+$ strains only):* Add 500 µl Optional Wash Solution to column. *Spin 30", 1 minute.* Discard flow-through.
- Add 750 µl Wash Solution to column. *Spin 30", 1 minute.* Discard flow-through.
- *Spin 1 minute* to dry column.

* *Prior to first time use, be sure to add ethanol to the concentrated Wash Solution.*

6 Elute purified plasmid DNA

- Transfer column to new collection tube.
- Add 100 µl Elution Solution. *Spin 1 minute.*

* *If a more concentrated plasmid DNA prep is required, reduce the elution volume to a minimum of 50 µl.*