65°C -ן HBW מחמם

Experienced User Protocol

All spins at ≥12,000 × g, except as noted.

1 Harvest & lyse bacteria

5 minutes at 5000 rpm

- ❒ Pellet cells from 1–5 ml overnight culture ~~4 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. and move to eppendorf.
- ❒ 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.* 10 min!

3 Prepare binding column

- ❒ Add 500 ml Column Preparation Solution to binding column in a collection tube.
- ❒ *Spin at ≥12,000 × 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.*

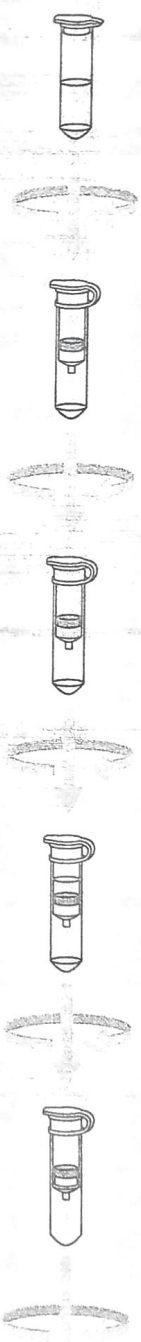
6 Elute purified plasmid DNA

- ❒ Transfer column to new collection tube.
- ❒ Add 100 µl Elution Solution. *Spin 1 minute.* → Add 50µl HBW/UPW
- * *If a more concentrated plasmid DNA prep is required, reduce the elution volume to a minimum of 50 µl.*

after adding 50µl hot HBW, wait 2 min.
and then:

(1) according to noa:
- centrefuge for 1 min at 2,000 xg
- " 17,000 xg

(2) according to sara:
- centrefuge for 2 min at 17,000 xg



Bacterial culture

Pure Plasmid DNA