PROTOCOL

Bacterial Kit

Part Number:	2BPK-100
Version:	1.0
Storage:	Store at room temperature & at -20°C
Batch Number:	Marked on tube
Expiration Date:	3 years from packing date

Protocol

Before you start:

- Prepare 60°C waterbath
- If precipitate has formed in Solution LA incubate the tube/bottle at 60°C until the solution becomes clear
- Thaw Proteinase K and RNase A at room temperature
- Pellet the cells in a microfuge, pour off most of the supernatant and flick or vortex the tube to break up the pellet before continuing

DNA Extraction

- Add 0.5 ml of Solution LA*
- Add 10 µl of RNase A solution**
- Incubate at room temperature for 10 mins
- Add 20 µl of Proteinase K solution**
- Place in a water bath and incubate at 60°C for 10 mins
- Add 30 µl of Solution PA. Vortex briefly or invert the tube to mix (do not place on ice!)
- Spin at 10,000 rpm for 5 minutes in a microfuge. (White precipitate will form)
- Transfer 450 µl of the supernatant into a new tube containing 450 µl of Solution CA, being careful to avoid transferring any debris. Vortex briefly or invert the tube to mix
- Leave on the bench for 5 minutes
- Spin in a microfuge at 13,000 rpm for 7 minutes to pellet the DNA
- Remove the supernatant with a 1 ml pipette
- Re-spin the tube briefly and remove the dregs
- Add 50 µl of 10/1 TE (guide only) or Molecular Grade Water

  NB: The pellet may not be visible.

- Leave for 30 minutes (or overnight) to allow the DNA to rehydrate

TIPS:

*Solution LA and RNase A solution can be pooled and added together

**Refreeze remainder of Proteinase K and RNase A after use

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