

**MAXIPREP PROTOCOL**

**DATE:**

CULTURES NOT GROWN IN CHLORAMPHENICOL

- 1 INNOCULATE 500ML CULTURE AND INCUBATE @ 37 C O/N ON A SHAKER
- 2 SPIN DOWN CULTURE FOR 15 MIN @ 4000RPM
- 3 POUR OFF MEDIA, LEAVE BOTTLE INVERTED ON A PAPER TOWEL AND ALLOW THE PELLETT TO DRAIN
- 4 RESUSPEND PELLETT IN **18ML OF ICE COLD SOLUTION 1** - VORTEX
- 5 ADD **2ML OF FRESHLY PREPARED LYSOZYME** (10 mg/mL IN 10 mM TRIS.Cl pH 8.0)
- 6 ADD **40ML OF FRESHLY PREPARED SOLUTION 2** ---> MIX BY INVERSION ----> STORE @ RT FOR 5 -10 MIN
- 7 ADD **20ML OF ICE COLD SOLUTION 3** ----> VORTEX GENTLY -----> STORE ON ICE FOR 5 -10 MIN
- 8 SPIN @ 4000 RPM FOR 15 MIN @ 4 C AND PLACE S/N INTO FRESH TUBE
- 9 FILTER THRU 2 LAYERS OFF KIMWIPES INTO A GRADUATED CYLINDER
- 10 ADD 0.6 VOLUMES OF ISOPROPANOL ----> MIX WELL -----> STORE @ RT FOR 10 MIN
- 11 SPIN @ 5000RPM FOR 15 MIN @ RT
- 12 DECANT S/N CAREFULLY -----> RINSE PELLETT AND WALLS W/ 70% ETHANOL ----> RESUSPEND PELLETT IN 70% ETHANOL AND TRANSFER TO EPIE TUBES ----> SPIN DOWN FOR 5 MIN ----> SPEED VAC FOR 5 MIN ----> ADD 3 MLS TE (pH 8.0) AND RESUSPEND

**OPTIONAL            ADD AN EXCESS OF RNASE & INCUBATE @ 37 C FOR 20MIN**  
**-----> PHENOL/CHLROFORM EXTRACTION ----> ETHANOL**  
**PRECIPITATION**

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**CLONES MINIPREPPEP**

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| 1 | 4 |
| 2 | 5 |
| 3 | 6 |