DNA Extraction from Gram negative bacteria on plates and liquid culture using *prep*GEM® Bacteria

This document contains a method for extracting DNA from Gram negative bacteria. It should be understood that substantial vations may be present from species to species and from sample to sample, and so optimization of the method may be possible.

Kit contents

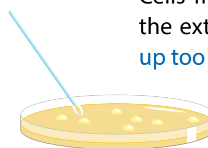
1. *prep*GEM proteinase
   *prep*GEM is highly stable and is delivered to the customer at room temperature. However, once opened, we recommend that the tube be stored at -20°C to reduce the possibility of contamination.
2. GREEN Reaction Buffer (10x Solution)
   Store at +4°C
3. WASH Buffer (5x Solution)
   Store at +4°C . The wash buffer is an osmotically buffered solution. During cold storage, crystals may form. These can be re-dissolved by heating the solution to around 40°C for 5 minutes.
   [NOT NEEDED IN THIS METHOD]
4. Lysozyme powder
   Lysozyme is provided as a lyophilised powder. To use, resuspend in 100 mM Tris pH 8.0 to the volume specified on the label. To reduce the potential for contamination and activity loss, the lysozyme is best stored in small aliquots at -20°C.
   [NOT NEEDED IN THIS METHOD]

Preparation

- Appropriate handling should be used with pathogenic organisms.
- All manipulations should be performed in a PCR or Class II hood.
- Use only certified DNA-free tubes and reagents and wash surfaces likely to come into contact with the samples in 0.05% H hypochlorite bleach. Rinse thoroughly with DNA-free water

Colony Stabs

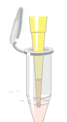
Cells from colonies can be suspended directly into the extraction mixture. Do not be tempted to pick up too much of the colony.



1. For each extraction make up:
   88 µl DNA-free water
   10 µl of 10x GREEN Buffer
   1 µl *prep*GEM

2. Lift a small amount of the colony with a sterile loop and mix into the extraction cocktail.

3. Incubate at:
   75°C for 5 min
   95°C for 5 min



A thermal cycler can be used for this step

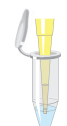
Liquid Cultures

1. For each extraction make up:
   68 µl DNA-free water
   10 µl of 10x GREEN Buffer
   1 µl *prep*GEM

2. Add 10 µl - 20 µl of a log phase culture and mix.

The amount is dependent on culture density. For low-density cultures, it may be necessary to pellet the cells and resuspend the pellet directly in the extraction cocktail

3. Incubate at:
   75°C for 5 - 15 min
   95°C for 5 min



A thermal cycler can be used for this step

Technical tips

- *prep*GEM Bacteria is a preparative method for DNA extraction from Gram -ve and Gram +ve bacteria. The *prep*GEM method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP analysis as well as quantitative, multiplex and end-point PCR.
- The wash step is only required for bacteria that produce large amounts of polysaccharide and for swabs from mucous membranes.
- DNA extracted using *prep*GEM is largely single-stranded because of the 95°C heat step. For accurate yield assessment, a qPCR is recommended.
- If fluorescent chelating dyes are to be used for quantification, then this factor should be taken into consideration and the method calibrated with DNA passed through the ZyGEM extraction process.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.
- Extracts should be stored at -20°C.
- If the extracts are excessively cloudy, centrifuge the sample for 5 minutes at 6000 r.c.f. to remove the debris. Aspirate the supernatant from the sedimented debris and store in a new tube.
- The presence of EDTA in the sample will reduce enzyme performance in the extraction. This problem can be overcome by adding $CaCl_2$ to a final concentration of 200 µM.