



Antarctica - the source of prepGEM® (MacKay Glacier, Ross Sea Region)

Efficient, closed-tube DNA extraction using prepGEM® Bacteria

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Introduction

There is a pressing need for a simple, universal microbial DNA extraction method to support nucleic acid-based detection and identification of bacteria.

Morphological diversity is exceptionally broad for microbes. Different species have different cell walls and can produce a wide variety of PCR inhibitors. Furthermore, lysis-resistant species create a special challenge for DNA extraction, while rigorous methods (for example bead-beating) can fragment the DNA from more fragile organisms.

Many kit-based and 'homebrew' DNA extraction strategies have separate methods for different morphotypes, but this strategy is unsuitable for mixed microbial communities or when the makeup of a sample is unknown.

The prepGEM® Bacteria kit from ZyGEM uses a thermophilic proteinase in combination with lysozyme. These enzymes can be used in tandem because the proteinase is inactive at the temperature where lysozyme is optimally active. After the lysozyme has completed its task, the proteinase is activated by raising the temperature whereupon it completes lysis of the cells, digests nucleases and destroys the lysozyme. In addition to the two enzymes, the buffer used in the extraction contains proprietary agents that enhance cell lysis whilst having little effect on downstream PCR applications.

A key advantage of controlling a two-step reaction by temperature is that the tube remains closed. Hence, the risk of contamination is greatly reduced and the method is easily adapted for automation.

To evaluate the prepGEM Bacteria kit, a range of diverse bacteria were selected to test extraction efficiency. In addition, we demonstrate the use of the kit for low volume DNA extraction from low cell numbers.

1. Extraction efficiency with different species

Methods

Preparation of cultures and DNA extraction

Eighteen bacterial species were grown on solid media using growth conditions recommended for the species [1]. A loopful of cells was lifted from the plate and the weight determined. This sample was then resuspended in water to a normalised density of 10 ng / 20 µl.

DNA was extracted using the procedure recommended with the prepGEM Bacteria Kit:

- 1 µl prepGEM
- 1 µl Lysozyme
- 10 µl Buffer GREEN
- 78 µl H₂O
- 20 µl culture suspension (equivalent to 10 ng wet-weight)

The tubes were then incubated at 37°C, 75°C and 95°C for 15 minutes each.

Polymerase Chain Reaction

Universal 16S rRNA gene (~ 330 bp)	
16S_1061F:	GTCGTCAGCTCGTGTGTGA
16S_1388R:	GCCCGGGAACGTATTAC
Universal 16S rRNA gene (~ 1500 bp)	
1SS_10F:	AGRGTGGATCMTGGCTCAG
16S_1510R:	GKTACCTGTTACGACTT

PCR was performed using 5 µl of extract in a 25 µl reaction containing the following: 1 x Buffer II, 2.5 mM MgCl₂, 0.2 µM primers (above), 0.1 mM dNTPs, 0.1 mg/ml BSA, 0.1 U AmpliTaq® (Applied Biosystems).

PCR cycling conditions were: 95°C 2 mins, followed by 30 cycles of 95°C 30 sec, 60°C 30 sec, 72°C 45 sec, and a final extension of 72°C for 10 minutes.

Amplicons were visualised by agarose gel electrophoresis.

Results

Figure 1 shows that appropriate PCR products were generated for each species. Although it is not possible to quantitatively assess relative efficiencies by this method, it is clear that prepGEM Bacteria is capable of extracting DNA from Gram positive, Gram negative and polysaccharide secreting species with a single procedure.

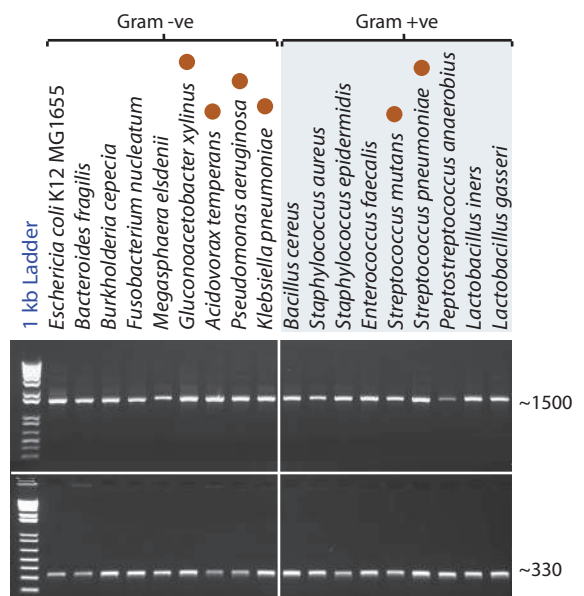


Figure 1: Agarose gels of PCR amplicons generated using two different primer pairs and DNA extracted with prepGEM Bacteria from 18 different bacterial species. Gram +ve and -ve species are indicated. ● Indicates species known to cause problems in DNA extraction due to the secretion of polysaccharides.

2. DNA extraction from small volumes

Methods

Preparation of culture and DNA extraction

Escherichia coli MG1655 was grown overnight in LB media and the cell density determined. A 1/10 dilution series was made in sterile water and a DNA extraction performed in 10 µl volume with all reagents in proportion to the method outlined in Experiment 1. (0.1 µl *prepGEM*[®], 0.1 µl lysozyme, 1 µl Buffer GREEN, 5 µl cell dilution, 3.8 µl water). Six replicate extractions were performed for each dilution.

The tubes were incubated in a thermal cycler as recommended in the guide (37°C, 75°C, 95°C for 15 minutes each).

Polymerase Chain Reaction

5 µl of the extraction was added to 15 µl qPCR mix (Invitrogen Platinum SYBR master mix, 0.2 mM primers).

Escherichia coli leuB gene (159 bp)

EcleuB247F:	CATTACCACCAGACCAGCA
EcleuB405R:	CAGGATGTCGAAGCCGTTTG

A qPCR was performed using the following cycling conditions: 95°C 2 mins followed by 40 cycles of 95°C 30 sec, 60°C 30 sec, 72°C 30 sec.

Discussion

Experiment 1 demonstrates the broad applicability of the *prepGEM*[®] method for extracting DNA. Despite the morphological diversity of the panel of organisms chosen, all species yielded DNA that was amenable to the PCR.

The method has a number of advantages (see side panel) and having broad utility means that it is ideally suited for analysing mixed populations and samples containing unknown organisms.

Experiment 2 shows how the method can be scaled for small samples containing low cell numbers. There is excellent linearity from 10⁵ cells down to less than 10 cells, and variation in the C_T values within replicates is small when a constant number of cells are supplied. Increased deviation is only seen when low cell densities are used and the number of cells per tube cannot be accurately determined.

The ability to process low cell numbers in low volumes means that many samples can be used directly without a concentration step by centrifugation or filtration. Furthermore, the closed-tube procedure makes it less likely to suffer from extraneous contamination - a serious problem for microbiologists and clinicians using generic 16S rRNA primers or performing direct DNA sequencing [2]

Results

Figure 2 shows a graph with C_T values plotted against estimated cell numbers.

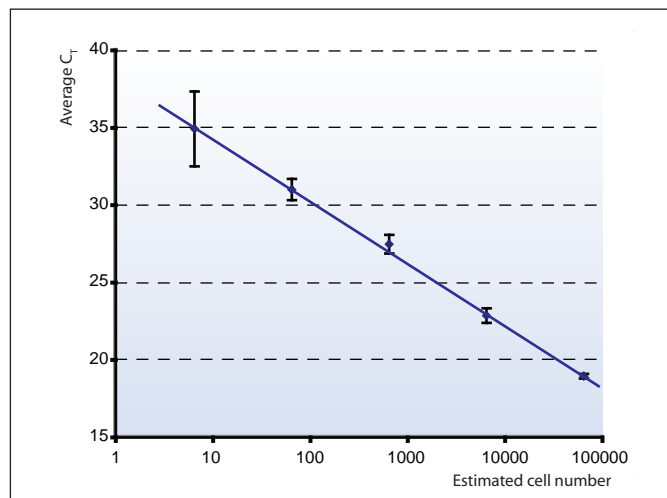


Figure 2: Graph plotting the average observed C_T values (n = 6) against number of *Escherichia coli* cells extracted. The X axis is displayed on a logarithmic scale. Error bars are one standard deviation each side of the mean.

prepGEM[®] Bacteria - Advantages

- Simple, universal method
- Effective on a broad range of bacterial species
- Closed-tube
 - Protects the sample from contamination
 - Protects staff from aerosols
- Single tube / reduced pipette tips
 - Reduces costs
 - Less plastic waste
- DNA suitable for all polymerase-based applications

References

- [1] http://www.cabri.org/CABRI/srs-doc/dsmz_bact.info.html
- [2] Grahn N., Olofsson M., Ellnebo-Svedlund K., Monstein H.-J., Jonasson J (2003) Identification of mixed bacterial DNA contamination in broad-range PCR amplification of 16S rDNA V1 and V3 variable regions by pyrosequencing of cloned amplicons. FEMS Microbiology Letters 219:87-91

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