ZyGEM Quick-Start Guide

DNA Extraction Using

*prep*GEM™ Bacteria

Find more information at
www.zygem.com

or email
info@zygem.com

Bacteria

No single method will suit all microorganisms. One option is to create a single, complex method to cover all bases, but that results in unnecessarily complexity, cost and wastage when working with easier species.

We have developed, a number of efficient methods to suit a variety of bacterial morphologies and substrate types and these can be downloaded from our website (see links below). This is a growing list and we would like to hear from you if you have adapted one of our methods to suit your sample types. Remember too, we are always available to help provide you with a custom method. We recommend that you visit our web pages at: www.zygem.com for more information.

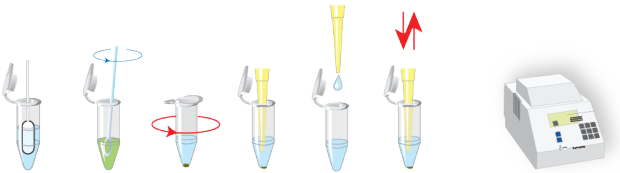
QC of ZyGEM reagents

Microbial DNA in reagents is a well known problem for microbiologists. ZyGEM goes to great lenghts to minimise this problem. Our reagents are made from certified DNA-free chemicals and solutions and all buffers and enzymes are treated with DNAse and UV before shipment. Be aware however, that we have no control over the reagents of other vendors. If you are using universal primers in a PCR (for example 16S rRNA gene primers) you should look at the literature about how to reduce the background signal you may get from your PCR reagents.

Preparation of the Reagents

- 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 and *prep*GEM are best stored in small aliquots at -20°C.

Bacterial method overview



	Resuspend cells in sufficient water.	Resuspend cells in WASH buffer	Centrifuge	Remove ALL residual liquid	Add 1 µl lysozyme to 50 µl ZyGEM reagents	Resuspend in 50 µl ZyGEM reagents	37°C	75°C	95°C
Capsuled Bacteria		■	■	■	■	■	10	10	2
Biofilms		■	■	■	■	■	10	10	2
Mucosal swabs		■	■	■	■	■	10	10	2
Swabbed surfaces	■		■	■	■	■	10	10	2
Gram +ve					■	■	10	10	2
Gram -ve						■		5	2
Mycoplasmas						■		5	2

Capsuled Bacteria biofilms and sputum

The WASH buffer is a proprietary formulation designed to reduce problems caused by polysaccharide. A pre-wash in this buffer is recommended for:

- bacteria producing large amounts of polysaccharide
- capsulated bacteria
- samples presented in mucous (for example sputum, throat or vaginal swabs)

In general, this step involves washing the cells (or swab) in 1x **WASH** Buffer (provided as a 5x solution) followed by a 5 minute centrifugation. The pellet should be drained thoroughly (it is important to remove all of the wash buffer) and then resuspended in 100 µl of the extraction cocktail.

For capsuled bacteria, sputum, mucosal swab and biofilms

1. Pipette 20 - 100 µl of culture / sputum into a 1.5 ml tube and add 400 µl of ZyGEM 1x **WASH** buffer.
2. Vortex vigorously to disperse the cells.
3. Centrifuge the cells at >10,000 r.c.f. for 5 minutes.
4. Remove **ALL** of the supernatant and discard.

Proceed with a method outlined below

Colonies and Liquid cultures

Colonies: 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 10x **GREEN** Buffer
   1 µl *prep*GEM
   1 µl lysozyme (**Omit lysozyme for Gram negatives**)
2. Lift a small amount of the colony with a sterile loop and mix into the extraction cocktail.

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

1. Assuming that you are using 20 µl of culture
   For each extraction make up:
   68 µl DNA-free water
   10 µl 10x **GREEN** Buffer
   1 µl *prep*GEM
   1 µl lysozyme (**Omit lysozyme for Gram negatives**)
2. Add 20 µl of a log phase culture and mix.

Extraction incubations

3. Incubate at:
   37°C for 15 min (**Omit step for Gram negatives**)
   75°C for 10 min
   95°C for 2 min

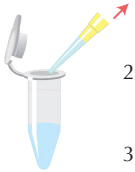
A thermal cycler can be used for this procedure

THE DNA IS NOW READY FOR USE

Swabs

WASH buffer is provided for mucosal swabs

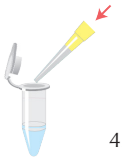
1. Wash the swab for 30 seconds in 400 µl of water by pressing against the side of the tube below the liquid. Before discarding the swab, squeeze the swab head on the wall of the tube to extract as much of the liquid as possible.



2. Sediment the cells by centrifugation at 10,000 r.c.f for 5 minutes.

3. For each extraction make up:

   88 µl DNA-free water
   10 µl 10x **GREEN** Buffer
   1 µl *prep*GEM
   1 µl lysozyme (**Omit for Gram -ves**)

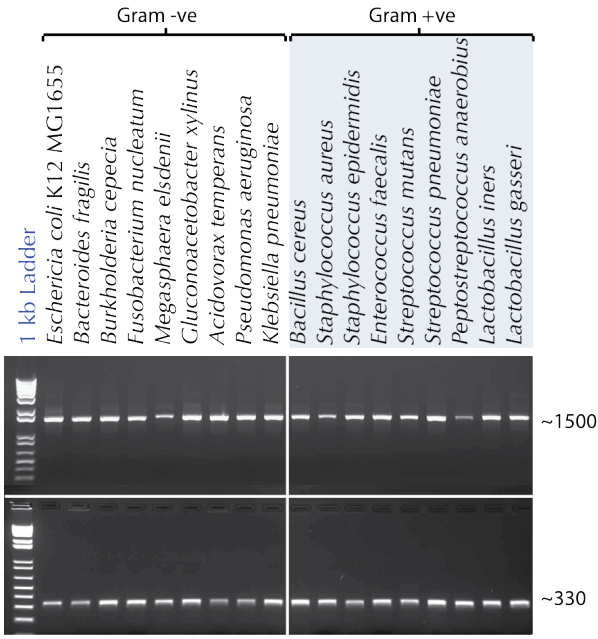


4. Resuspend the cells in the solution and in a thermal cycler, incubate at:

   37°C for 15 min (**Omit for Gram -ves**)
   75°C for 10 min
   95°C for 2 min

THE DNA IS NOW READY FOR USE

Typical Results



PCR products visualized by electrophoresis on a 1% agarose gel. All DNA templates were generated using *prep*GEM Bacteria.

Rapid plasmid prep from E. coli

1. Grow overnight cultures to an OD600 of approximately 1-1.5.
2. Transfer 200 µl of the culture to a thin-walled 200 µl PCR tube
3. Centrifuge at full speed for 2 minutes and pipette away the medium from the pellet.

For colonies, scrape a colony of approximately 4 mm diameter and use in the extraction below.

Extraction

1. Add to the pellet and mix:

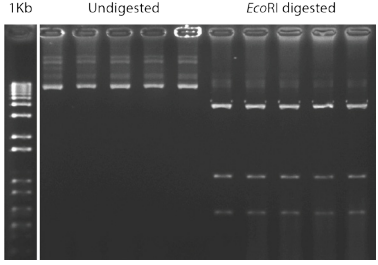
   5 µl 10x **GREEN** Buffer.
   44 µl DNA-free water
   1 µl *prep*GEM

2. In a thermal cycler, incubate at:

   75°C for 5 min
   99°C for 5 min
   4°C 2 for min (**Rapid cooling step should be included**)

3. Centrifuge for 5 minutes at 20,000 rcf.
4. Discard the pellet. The supernatant contains the plasmid.

Typical results for high copy number plasmids extracted using *prep*GEM Bacteria



Technical Tips

- *prep*GEM is a preparative method for DNA extraction. The method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.
- There is no concentration step in the procedure and so the concentration of the extract is dependent on: 1) The quality of the sample; 2) In the case of swabs, the type of swab and the volume of water used to wash the swab; 3) The extraction volume (which in some cases can be scaled).
- 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 standard fluorescent chelating dyes are to be used for normalising samples, then we recommend taking a sample of the extract before the 95°C step (See ZyGEM Application Note 109). Alternatively, you can generate a standard curve using a previously-made extract that has been quantified.
- 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.
- For long term storage of the extracted DNA, add TE buffer to 1x (10 mM Tris, pH 7.5, 1 mM EDTA) and store at -20°C.

The *prep*GEM reagents are stable at room temperature, but after tubes have been opened and for longer term storage, the enzymes should be stored at -20°C and the buffers at 4°C.

More information is available on our website at www.zygem.com