MicroGEM Quick-Start Guide

DNA Extraction Using

*prep*GEM Bacteria

Find more information at
www.microgembio.com

or email
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Bacteria

This quick start guide provides a number of efficient methods to suit a variety of bacterial morphologies and substrate types. These methods can be further optimised and adapted to suit your sample type. We are available to provide help with developing a custom method. Please contact us at info@microgembio.com.

We recommend that you visit: www.microgembio.com for more information.

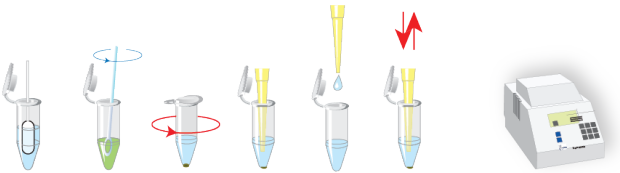
QC of MicroGEM reagents

Microbial DNA in reagents is a well known problem for microbiologists. MicroGEM goes to great lengths 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.
- The **WASH+** buffer is provided at 5x concentration. This needs to be diluted to 1x using DNAase-free water prior to use in protocols. Aliquots of 1x stocks can be made and stored at room temperature or at 4˚C for long-term storage.

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

Removal of Inhibitors

The **WASH+** buffer is a proprietary formulation designed to reduce problems caused by inhibitory polysaccharides and polyphenols in the sample. 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)
- removal of inhibitory tannins, humic substances and polysaccharides from soil and stool

Proceed with a method outlined below

Colonies and Biofilms

Cells from colonies can be suspended directly into the extraction mixture. **Do not be tempted to pick up too much of the colony.** A pre-wash in **WASH+** buffer is recommended for biofilms and high exo-polysaccharide producers.

1. Pipette 400 µl of 1x **WASH+** buffer into a 1.5 ml Eppendorf tube.
2. Lift a small amount of colony with a sterile loop or pipette tip (up to 2 mm^2) or a biofilm and resuspend in **WASH+** buffer.
3. Vortex vigorously to disperse cells.
4. Centrifuge the cells at >10,000 r.c.f. for 5 minutes.
5. Remove **ALL** of the supernatant and discard.
6. Resuspend the pellet in the extraction mix below:

Liquid Cultures, Sputum and Swabs

Liquid Cultures and Sputum

The amount of culture to add is dependent on the density. Using 20 µl of log-phase culture yields good results. This can be optimised for your sample.

1. Pipette 20 µl of log-phase culture or 20-100 µl of sputum in a 1.5 ml Eppendorf tube.

2. Add 400 µl of 1x **WASH+** buffer.
3. Vortex vigorously to disperse cells.
4. Centrifuge the cells at >10,000 r.c.f. for 5 minutes.
5. Remove **ALL** of the supernatant and discard.

6. Resuspend the pellet in the extraction mix below:

Swabs

1. Wash swab for 30 sec in 400 µl 1x **WASH+** buffer in a 1.5 ml Eppendorf tube using a rolling action. Squeeze the swab head against the wall of the tube to extract as much liquid as possible before discarding.

2. Vortex vigorously to disperse cells.
3. Centrifuge the cells at >10,000 r.c.f. for 5 minutes.

4. Remove **ALL** of the supernatant and discard.
5. Resuspend the pellet in the extraction mix below:

Extraction

Extraction Mixture

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**)

Extraction Incubations

Incubate at:

37°C for 15 min (**Omit for Gram -ves**)
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

Soil and Stool

Extracting DNA from soil samples is complicated due to the release of humics. This method relies on a short differential sedimentation of solids in a proprietary buffer.

Differential Sedimentation Step

1. Add up to 50 mg of soil or stool to a 1.5 ml tube.
2. Resuspend in 500 µl of 1x **WASH+** buffer.
3. Vortex vigorously for 1 min to disperse cells.
4. Centrifuge at 200 rcf for 30 seconds.
5. Transfer the supernatant to a new tube.
6. Centrifuge at full speed for 2 min.
7. Carefully pipette away all of the **WASH+** buffer.
8. Resuspend pellet in 100 µl of water.

Extraction

1. Make up the following extraction cocktail:

78 µl	DNA-free water
10 µl	10x **GREEN+** Buffer
10 µl	*Enhancer*
1 µl	*prep*GEM
1 µl	Lysozyme
50 µl	Cell suspension

2. Incubate at:

   37°C for 15 min
   75°C for 10 min
   95°C for 2 min

3. Centrifuge at full speed for 2 min.

The supernatant contains the DNA. Use 1 µl or less for PCR/qPCR

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:

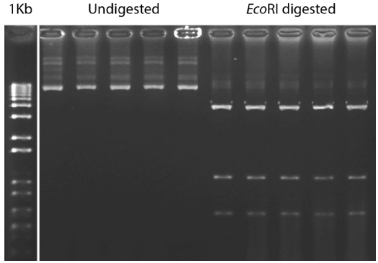
   5 µl 10x **GREEN**+ Buffer
   44 µl DNA-free water
   1 µl *prep*GEM

2. In a thermal cycler, incubate at:

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

3. Centrifuge for 5 minutes at 20,000 r.c.f.
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. 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 at www.microgembio.com