MicroGEM Quick-Start Guide

DNA Extraction Using

PDQeX

Bacteria

MicroGEM

Going to extremes for
effortless DNA extraction

Find more information at
www.microgembio.com

or email
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About this Guide

This Quickstart 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**

Preparation and Storage of 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. Some of the powder might dislodge from bottom of the tube during shipping. It is recommended to spin this down before resuspending.
- 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.
- **GREEN+** Buffer and *Enhancer* need to be stored at 4˚C.

Precautions

1. Do not load the PDQeX machine if the control screen indicates a temperature above 35° C.
2. Ensure the collection drawer and heating block are clean and DNA-free.
3. Ensure the collection drawer is inserted as far as possible, and that it is straight.
4. If fewer than 24 reactions are planned, make sure that the PCR tubes are placed in drawer wells corresponding to the channels to be used in the heating block.

MicroGEM Reagent QC

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 published literature about how to reduce the background signal you may get from your PCR or qPCR reagents.

Removing Inhibitory Polysaccharides and Polyphenols

PDQeX Bacteria comes with **WASH+** buffer. Some samples require a pre-wash step. Typically, these samples are:

- Bacteria producing large amounts of polysaccharide
- Capsulated bacteria
- Samples presented in mucous (for example sputum, throat or vaginal swabs)
- Samples in a matrix of inhibitory tannins, humic substances and polysaccharides. For example soil and stool.

The **WASH+** buffer is a proprietary formulation designed to reduce these problems. It is a blend that binds inhibitors, breaks down mucus and is osmotically buffered. Osmotic buffering is critical when mixed samples are presented composed of fragile and robust bacteria.

Important Technical Tips

- DNA extracted using the PDQeX Bacteria kit is suitable for Whole-Genome Sequencing and many types of genotyping including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.
- The amount of starting material to use in extractions depends on the sample type. It is recommended that you optimise this by testing different amounts of your specific sample. For PCR-based applications, a tiny amount would suffice. For applications requiring high DNA yields like Whole-genome sequencing, ~2 mm^2 of colony or 20 µl of log-phase liquid culture is sufficient for most samples.
- The methods provided can be further optimised for different samples. These methods are designed to limit the effect of inhibitors. If inhibition remains a problem, adding 2.5 µl of the *Enhancer* to a 25 µl PCR mastermix might improve results, especially for stool and soil samples. Try different amounts of extract in your PCR - sometimes less is better. Using 1 µl or less in 25 µl PCRs is often sufficient for bacterial amplifications.
- 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 PDQeX Bacteria can be quantified using a qPCR or by using fluorescent dyes like Pico Green, iQuant, Qubit assays or the like. Nanodrop is incompatible with PDQeX reagents.
- 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.

Colonies, Biofilms, Liquid Cultures, Sputum and Swabs

Completely thaw *prep*GEM and *Lysozyme*, and mix by gently inverting the tubes. Remove **GREEN+** and **WASH+** buffers from the refrigerator and mix.

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, it might clog the PDQeX cartridge (Refer to 'Important technical tips' section for recommendations on amount of sample to use).

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 1.5ml Eppendorf tube.
2. Lift a small amount of colony with a sterile loop or pipette tip 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 and Sputum

The amount of culture to add is dependent on the density (Refer to 'Important technical tips' section for recommendations on amount of sample to use)

1. Pipette up to 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 of 1x **WASH+** buffer in a 1.5ml Eppendorf tube using a rolling action. Before discarding, squeeze the swab head on the wall of the tube to extract as much of the liquid as possible.
2. Vortex vigorously to disperse cells.
3. Sediment the cells by centrifugation 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:

Preparing the extraction mixture

For each extraction, make up:

86 µl	DNA-free water
10 µl	10x **GREEN+** Buffer
2 µl	*prep*GEM
2 µl	*Lysozyme* (Can be omitted for Gram negatives)

PDQeX extraction

1. Dispense 100 µl of the extraction mix with sample into each PDQeX cartridge.
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the appropriate program below for you sample:

Gram Neg Bacteria:

75°C	10 mins
95°C	2 mins

Gram Pos Bacteria:

37˚C	10 mins
75°C	10 mins
95°C	2 mins

- **Times may be adjusted by internal laboratory optimisation.**
- **Changes to the default temperatures are not recommended.**

Soil and Stool

Completely thaw *prep*GEM and *Lysozyme*, and mix by gently inverting the tubes. Remove **GREEN+** and **WASH+** buffers from the refrigerator and mix.

Extracting DNA from soil samples for PCR and qPCR is complicated because of the release of humics. This method uses a short differential sedimentation of solids in the **WASH+** buffer. This buffer is designed to dislodge bacteria from biofilms but it is isotonic to protect more fragile species.

Differential sedimentation step

1. Add up to 10 mg stool or up to 20 mg soil to a 1.5 ml tubes (this can be optimised for your sample).
2. Resuspend in 500 µl of 1x **WASH+** buffer.
3. Vortex vigorously for 1 min to disperse cells.
4. Centrifuge gently at 200 r.c.f for 30 seconds to sediment the solids but leave a suspension of cells.
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 water.

Preparing extraction mixture

For each extraction, make up:

10 µl	10x **GREEN+** Buffer
10 µl	*Enhancer*
2 µl	*Lysozyme* (10 mg / ml)
2 µl	*prep*GEM
76 µl	Water
50 µl	Cell suspension

PDQeX extraction

1. Dispense the above extraction mix with sample into each PDQeX cartridge.
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes

4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the appropriate program for you sample (below):

Soil and Stool:

37˚C	15 mins
75°C	15 mins
95°C	2 mins

- **Times may be adjusted by internal laboratory optimisation.**
- **Changes to the default temperatures are not recommended.**