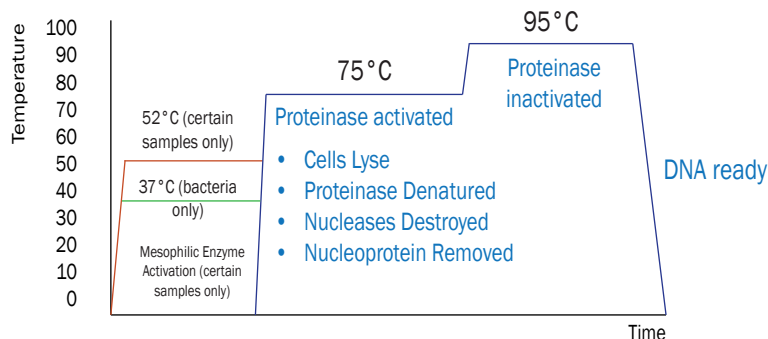




This Quick Start Guide provides methods to extract DNA from a range of sample types.



Principle of the Extraction

MicroGEM extraction products use a unique mixture of thermophilic and mesophilic enzymes. Protocols involve a 75°C step to activate MicroGEM's thermophilic proteinase to lyse cells, denature nucleases, and strip DNA of nucleosomes. Certain protocols in this Quick Start Guide include a initial low temperature step when mesophilic enzymes are required. A final 95°C step shrinks the PDQeX inner tubing, bursting the valve and pushing the extract through a column at the base of the tube to remove the proteinase and inhibitors, resulting in analytics-ready DNA being released from the tube.

Kit Components and Storage Conditions

PDQeX *prepGEM* 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.

- PDQeX *prepGEM* bacteria reagents are delivered at room temperature but on arrival should be stored at 4°C. After tubes have been opened, the *prepGEM* enzyme should be placed at -20°C.
- Lysozyme is provided as a lyophilised powder, to use, resuspend in 100 mM Tris pH 8.0 to the volume specified on the label. Once lysozyme has been rehydrated, it is stable for several weeks at 4°C. If you do not plan to use all of the Lysozyme immediately, it is recommended that you aliquot lysozyme into smaller volumes and store at -20°C immediately after rehydration. The **GREEN+** buffer can remain at 4°C for convenience.
- The **WASH+** buffer is provided at 5x concentration and may appear yellow. This needs to be diluted to 1x using nuclease-free water prior to use in protocols. You may find that the 5x **WASH+** buffer contains some floating crystals, you should warm the 5x **WASH+** buffer to ~35°C to dissolve these fully before diluting to 1x. Aliquots of 1x stocks can be made and stored at room temperature or at 4°C for long-term storage.

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.

General Instructions, Precautions and Technical Tips

- DNA extracted using the PDQeX *prepGEM* Universal 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 mm² 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 *prepGEM* Universal 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.

Sample Preparation and Extraction Procedures

Colonies, Biofilms, Liquid Cultures, Sputum and Swabs

Completely thaw *prepGEM* 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 x g 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 x g 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 1.5 ml 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	prepGEM
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 your 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

Soil and Sperm

Completely thaw *prepGEM* 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 and Sedimentation Step

1. Add up to 10 mg stool or up to 20 mg soil to a 1.5ml 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 x g 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
1 µl	Lysozyme (10 mg / ml)
2 µl	prepGEM
77 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

Troubleshooting

For troubleshooting, please see our guide “MicroGEM Method Optimization and Troubleshooting” using the following link:

https://www.microgembio.com/m_resource/microgem-method-optimisation-and-troubleshooting/

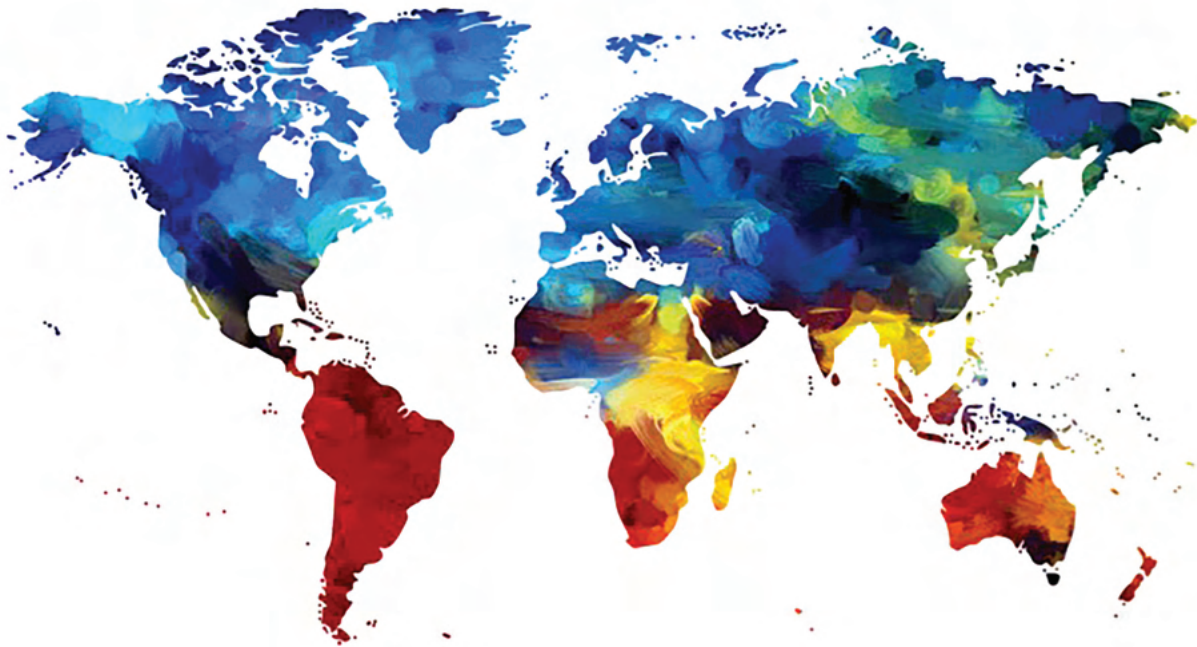
Additional Information

For publications/app notes on your specific sample type, please visit:

<https://www.microgembio.com/resources/>



Committed to minimal packaging, reduced plastic use, and a vibrant, sustainable world.



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At MicroGEM, our goal is to democratize molecular biology, enabling a broader spectrum of users to both employ and benefit from molecular techniques. The first step is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

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