



Going to extremes for  
effortless DNA extraction



## PLANT and Fungal DNA EXTRACTION (for use with *phytoGEM* kits)

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# DNA Extraction Protocol

## Reagent Storage:

- *Histosolv*\* -20° C (Limit freeze/thaw cycles)
- *prepGEM* -20° C
- **GREEN+** Buffer. 4° C
- *Enhancer*. 4° C

\* The *Histosolv* is delivered as a dry powder. When your kit arrives add DNA-free water as follows:

Kit size (Rxn)	Code	Volume of water to add
50	PPP0050	0.55 ml
100	PPP0100	1.1 ml
500	PPP0500	5.5 ml
1000	PPP1000	11.0 ml

## PRECAUTIONS

1. Do not load the PDQeX machine if the control screen indicates a temperature above 40° 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.

This QuickStartGguide provides methods for three sample types. Note however, that the *phytoGEM* system can be easily adapted for other sample types. Please contact us at...

**info@microgembio.com**

... for advice from our team on how you can modify the methods for your specific sample.

For more information, visit: [www.microgembio.com](http://www.microgembio.com)

## METHOD 1. Extraction of DNA from storage cards

### READ THE TECHNICAL TIPS AT THE END OF THIS DOCUMENT

#### Sample Types:

- Plant leaves, stems and roots
- Plant pathogens.

#### Method:

1. Completely thaw *prepGEM* and *Histosolv*, and mix by gently inverting the tubes. Remove **GREEN+** buffer and the *Enhancer* from the refrigerator and mix.
2. Prepare the following extraction mixture:

10 $\mu$ l	<b>GREEN+</b> buffer
2 $\mu$ l	<i>prepGEM</i>
10 $\mu$ l	<i>Histosolv</i>
10 $\mu$ l	<i>Enhancer</i>
68 $\mu$ l	DNA-free water
3. Dispense 100  $\mu$ l of master mix into each PDQeX cartridge.
4. Take a sample by crushing the plant onto the storage cards using the crusher tool.

**IF USING MULTI-USE PUNCHES, RINSE THE PUNCH IN DILUTE BLEACH AND THEN ETHANOL. BLOT DRY**
5. Take 1 - 4 punches from your storage card and drop it in the PDQeX cartridge. Make sure that the punches are completely submerged in the reagents.
6. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
7. Load 24-well plate, 8-strip tubes or individual tubes into the collection drawer and put the drawer in place.
8. Insert the PDQeX cartridges into the heating block.

9. 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**

10. Select the "Plant" program.

52 °C      5 mins.

75 °C      5 mins.

95 °C      2 mins.

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

## METHOD 2. Extraction of DNA from homogenised leaves, ground plant tissue

READ THE TECHNICAL TIPS AT THE END OF THIS DOCUMENT

### Sample Types:

- Homogenised plant leaves, stems and roots
- Plant pathogens in homogenised samples

### Method:

1. Weigh 5-10 mg of leaf and place in a 1.6 ml tube. Add 100  $\mu$ l of ultrapure water to this and homogenise using a sterile hand-held homogenising tool, tissue lyser or a mortar and pestle.

Ensure the sample is homogenised completely. For ground bark, woody tissue or roots, make a suspension of 5 - 10 mg ground tissue in 100  $\mu$ l ultrapure water. Prepare and manage on ice or at 4 °C for best results.

2. Prepare the following extraction mixture:

10 $\mu$ l	10x GREEN+ buffer
2 $\mu$ l	prepGEM
10 $\mu$ l	Histosolv
10 $\mu$ l	Enhancer
63 $\mu$ l	Ultrapure water
5 $\mu$ l	Leaf homogenate or tissue suspension

Cut the ends off the pipette tips to help transfer the homogenate. Mix thoroughly. Ensure large pieces of leaf matter are removed, they will clog the extraction cartridges.

3. Mix by vortexing and dispense into a PDQeX cartridge.
4. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.

5. Load 24-well plate, 8-strip tubes or individual tubes into the collection drawer and put the drawer in place.
6. Insert the PDQeX cartridges into the heating block.
7. 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**

8. Select the "Plant" program.

52 °C	5 mins.
75 °C	5 mins.
95 °C	2 mins.

- Times may be adjusted during method optimisation.
- Changes to the default temperatures are not recommended.

## METHOD 3. Extraction of DNA from fungal cultures

READ THE TECHNICAL TIPS AT THE END OF THIS DOCUMENT

### Method:

1. Transfer a small amount of fungal culture into a 1.6 ml Eppendorf tube using an inoculation loop or tease tool (half a loopful of sample is usually sufficient for good DNA yield). Add 200  $\mu$ l of ultrapure water to this and homogenise using a sterile hand-held homogenising tool or tissue lyser. Prepare and manage on ice or at 4 °C for best results.
2. Prepare the following extraction mixture:

10 $\mu$ l	10x GREEN+ buffer
2 $\mu$ l	<i>prepGEM</i>
10 $\mu$ l	<i>Histosolv</i>
10 $\mu$ l	<i>Enhancer</i>
68 $\mu$ l	Fungal homogenate

3. Mix by vortexing and dispense into a PDQeX cartridge.
4. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
5. Load 24-well plate, 8-strip tubes or individual tubes into the collection drawer and put the drawer in place.
6. Insert the PDQeX cartridges into the heating block.
7. 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**

8. Select the "Plant" program.

52 °C	5 min
75 °C	5 min
95 °C	2 min

- Times may be adjusted during method optimisation.
- Changes to the default temperatures are not recommended.

## Important Technical tips

- The device must be kept clean and DNA-free. The UV cycle will assist in removing DNA but the wells and the top and base of the heating block should be cleaned regularly using dilute HClO bleach on a swab. Wipe residual bleach off the surfaces using a second swab soaked with DNA-free water.
- The device produces DNA that can be used for SNIps, STRs, quantitative, multiplex and routine PCR applications.
- OD<sub>260</sub> methods for yield estimation are generally unsuitable for the DNA produced by the PDQeX. For accurate yield assessment, a qPCR or fluorometric assay is recommended.
- For best results prepare and manage samples at 4° C, or on ice before and after extraction.
- For long term storage of the extracted DNA, add TE buffer to 1x and store at -20° C.
- The phytoGEM extraction chemistry and cartridges are designed to remove polyphenols, polysaccharides and other inhibitory compounds from the extract. Nonetheless, some plant samples will have more inhibitory material than others - especially if large amounts of extract are used in the PCR. The following suggestions should be tried if you experience any problems with your PCR:
  1. Make sure that the problem is inhibition and not a low concentration of DNA. Inhibition can be tested with a control where one of your extracts is spiked with a known quantity of DNA. Alternatively, if you are using qPCR, the slope and end-point of the plot gives a good indication of inhibition.
  2. Add the *Enhancer* to the PCR mastermix. Adding 2.5 µl of the Enhancer in a 25 µl PCR may improve results from plants with high polyphenolic content.
  3. Add BSA to the PCR mastermix. 1 µl of a 10 mg/ml solution of BSA in a 25 µl reaction is sufficient for reducing the effects of common PCR inhibitors.
  4. Try adding less sample to your PCR. Usually 1 µl or less of the extract is enough for a successful PCR. It is good to optimise the amount of extract added for every new sample type you test.