Technical tips for sample management

- 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 creates DNA that can be used for SNiPs, STRs, quantitative, multiplex and routine PCR applications.
- OD_{260} methods for yield estimation are unsuitable for the DNA produced by the PDQeX.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by the PDQeX is approximately 90% single-stranded. If standard fluorescent chelating dyes are to be used for quantification, then this factor should be taken into consideration.
- 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.

ZyGEM

Automated DNA Extraction

PLANT DNA EXTRACTION

(for use with *phyto*GEM- kits)

... simple

Plant DNA Extraction Protocol

This procedure is used in conjunction with the *phyto*GEM crusher tool and storage cards

Sample Types:

- Plant leaves, stems and roots
- Plant pathogens.

Reagent Storage:

- *phyto*GEM A -20°C (Limit freeze/thaw cycles)
- *prep*GEM -20°C
- *prep*GEM B 4°C (do not leave at room temperature)
- GREEN Plus Buffer 4°C

Method:

1. Completely thaw *prep*GEM and *phyto*GEM A, and mix by gently inverting the tubes. Remove GREEN Plus buffer from refrigerator and mix.
2. Prepare a master mix for plant lysis per 100 µl reaction:

10 µl	GREEN Plus buffer
2 µl	*prep*GEM
2 µl	*phyto*GEM A
10 µl	*phyto*GEM B
76 µl	DNA-free water

3. Dispense 100 µl of master mix into each PDQeX tube.
4. Take a sample by crushing the plant onto the *phyto*GEM storage cards using the crusher tool.

   IF USING THE MULTI-USE PUNCHES, RINSE THE PUNCH IN WATER AND ETHANOL, AND BLOT DRY
5. Take 1 - 4 punches from your ZyGEM storage card and add it to the tube. 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 PDQeX cartridge.
7. Load 24-well plate or 8-strip tubes in the collection drawer and put the drawer in place.
8. Insert the PDQeX cartridge into the heating block.
9. Cover the tubes with the hinged flap and close the sliding door.

   MAKE SURE THE PDQeX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL
10. Select the "Plant" program.

Default plant program:

35°C	5 mins.
75° C	5 mins.
115° C	2 mins.

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

PRECAUTIONS

1. Do not load the 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 less than 24 reactions are planned, ensure that the PCR tubes are placed in the holes in the draw corresponding to the channels to be used in the heating block.