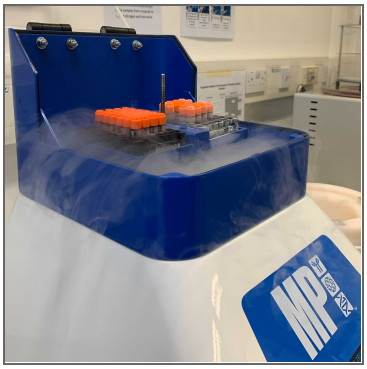

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We use this protocol and it's working

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Sanger Tree of Life Sample Homogenisation: Cryogenic Bead Beating of Plants with FastPrep-96

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ABSTRACT

Disruption under cryogenic conditions is vital to interrupt the often tough, flexible and rigid structure of plant tissues whilst maintaining nucleic acid integrity. Cryogenic bead beating on the FastPrep-96, using 3 × 3 mm stainless steel beads in 1.9 mL Tri-coded FluidX tube, allows for the rapid disruption of up to 96 plant samples simultaneously. Further, the minimal handling time per sample and closed environment of the tube during disruption results in minimal risk of ambient temperature exposure, sample loss or cross contamination.

We recommend an input mass of 10–100 mg flash-frozen plant tissue, dissected into <1 cm^2 pieces. Input requirements will vary depending on the downstream protocol to be performed, the plant species used, tissue type and sample quality. High-quality, young leaf material should be preferentially selected for optimal outcomes with ToL's downstream applications; alternative tissue types can be selected (e.g. herbaceous stem, petiole or flower), but outcomes may vary. Non-pliable, rigid or highly fibrous tissues (e.g. woody or rigid, fibrous stem) should be avoided for optimal disruption outcomes, however if a necessity, these structures should be preemptively interrupted and dissected into pieces smaller than 5 mm^2. Modifications to bead type and number of disruptions can be made to accommodate alternative sample types.

Following disruption, prepped tissues can be used immediately for appropriate downstream procedures, or can be stored long term at –70 °C with few detrimental effects. Approved downstream procedures of beat beaten plant tissue includes: HMW gDNA extraction for long-read PacBio HiFi sequencing, RNA extraction for RNA-seq and Hi-C genomic analysis.

Acronym

HMW: high molecular weight
gDNA: genomic DNA
HiFi: high fidelity

GUIDELINES

- For optimal disruption and downstream outcomes, select high-quality, young leaf material, which generally has less secondary metabolite accumulation and is most pliable. Alternative pliable tissue types (e.g. petiole, soft stem or root) can be selected and should disrupt with few problems, but downstream outcomes may vary.
  Recalcitrant sample types (e.g. tough, rigid, thick or fibrous tissues) can be selected, but should be dissected to pieces smaller than 5 mm^2, and structures should be manually interrupted by crushing prior to disruption. Disruption and downstream outcomes may vary. If frozen whole tissue remains in the sample, additional disruption cycles or alternative bead types (e.g. 2 × 3 mm tungsten carbide) will be required.
- High quality plant tissues with no observable damage, disease or other stressors present should be preferentially
  selected for optimal downstream outcomes. Lower quality plant tissues with signs of stress can be selected, but downstream outcomes may vary.
- Plant tissues should be preserved by flash-freezing and stored under constant cryogenic conditions thereafter. Alternative preservation techniques (e.g. air drying, silica gel) can be performed, but disruption and downstream outcomes may vary. Whole-frozen or disrupted plant tissue can be stored long term at –70 °C with few detrimental effects. Avoid freeze/thaw cycles at all stages prior to the addition of lysis buffer (or equivalent steps); prolonged exposure can degrade nucleic acids.
- All dissection, weighing or general handling of plant tissues should be performed on sterilised surfaces over dry
  ice to avoid contamination and freeze/thaw cycles.
- Following cryogenic disruption, ensure prepped plant tissues are completely disrupted into a fine powder. Additional
  disruption cycles or alternative bead types (e.g. 2 × 3 mm tungsten carbide) becomes essential if whole frozen tissue persists. Complete disruption is crucial to ensure optimal extraction yields and nucleic acid integrity; poorly disrupted tissue drastically decreases downstream procedure efficiencies and can impact all quantifiable outcomes.
- The Tree of Life project operates using Tri-coded FluidX tubes for sample tracking purposes. For this procedure, 2 mL reinforced tubes (SPEX PN 2310) can be used in place of 1.9 mL Tri-coded FluidX tubes during disruption.

MATERIALS

- Cotton glove liners (recommended)
- Dry ice
- Cryogenic gloves
- Safety spectacles
- Liquid nitrogen
- 1.9 mL Tri-coded FluidX tubes (or equivalent)

Equipment

- FastPrep-96 (Cat. no. SKU:116010500)
- Corning® CoolRack CF45 (Cat. no. 432051) or equivalent
- Dewar flask
- Tongs or equivalent
- Ice bucket/s
- Sample tube: FluidX tube (or equivalent)
- FluidX 48-rack (or equivalent)
- 3 mm sterile stainless steel beads (Qiagen Cat. no. 69997)
- Alternative bead type (if required): 3 mm tungsten carbide beads

Protocol PDF:

Sanger Tree of Life Sample Homogenisation_ Cryogenic Bead Beating of Plants with FastPrep-96.pdf

SAFETY WARNINGS

- Users should wear powder-free nitrile gloves and a lab coat at all times when performing this procedure.
- Glove liners are strongly recommended when handling cryogenic substances.
- In addition to all PPE listed above, users should wear eye protection and an oxygen depletion alarm at all times when handling or operating near liquid nitrogen.
- In addition to all PPE listed above, users should wear a face shield and cryogenic gloves when decanting liquid nitrogen
- Users should have training appropriate for the handling of all hazardous equipment used in this procedure (e.g. sharps, cryogenics).
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar) and disposed of in accordance with local regulations.

Laboratory protocol

1 Prepare all necessary equipment prior to starting and place any applicable items (e.g. cold blocks, tools) onto dry ice.

2 Add 3 x 3 mm sterile stainless steel beads (Qiagen PN 69997) to the required number of 1.9 mL Tri-coded FluidX tubes and place into a cold block on dry ice. Alternatively, beads can be added after the sample as long as they have been pre-chilled.

3 Weigh 10–100 mg of the selected whole-frozen tissue on dry ice, dissecting into <1 cm^2 pieces if required, and transfer into a prechilled 1.9 mL FluidX tube containing beads.

- Whole-frozen tissue can be dissected to size with scissors or a scalpel, or fragmented by crushing with an appropriate tool.
- Ensure tissues remain under cryogenic conditions at all times to avoid freeze/thaw cycles.
- Recalcitrant tissue types (see 'guidelines'), should be dissected into pieces smaller than 5 mm^2, and structures should be manually interrupted by crushing.

4 Repeat step 3 for each sample, and transfer all to a FluidX 48-rack on dry ice.

- Up to 96 samples can be processed simultaneously over 2 FluidX 48-racks.
- If only one FluidX 48-rack is used, a second rack containing an equal number of empty 1.9 ml Tri-coded FluidX tubes should be prepared to balance the FastPrep-96 during operation.

5 Submerge the FluidX 48-rack/s containing samples in liquid nitrogen (LN2) until temperature equilibrates (bubbling stops, ~15 seconds).

- Ensure the appropriate PPE is worn when handling, operating near or decanting LN2, see 'Health & Safety warnings'.

6 Remove the FluidX 48-rack/s containing samples from the LN2 with tongs, allow any remaining LN2 to drain, and place onto the FastPrep-96.

- Samples should be handled efficiently once removed from the LN2 to limit exposure to ambient temperature.

7 Homogenise the frozen samples on the FastPrep-96 for 30 seconds at 1,600 rpm.

8 Remove the 48-rack/s containing samples from the fastPrep-96 and resubmerge in LN2 until temperature equilibrates (bubbling stop, ~15 seconds).

9 Repeat steps 6 to 8 twice, for a total of 3 disruptions, until tissues are completely disrupted into a fine powder.

- LN2 submersion is not necessary following the final disruption, but may be performed to avoid samples thawing.
- Manual inspection of samples is recommended for novel specimens; if any whole frozen tissue remains, steps 6 to 8 can be repeated up to a total of 6 times. After 6 disruptions, using an alternative specimen and/or tissue type or alternative bead type (e.g. 2 x 3 mm tungsten carbide beads) is recommended.

10 Place the FluidX 48-rack/s containing samples onto dry ice for >3 minutes to ensure all disrupted tissue remains frozen.

11 Proceed to the required downstream procedure, alternatively, disrupted plant tissue can now be stored long term at −70 °C with little detrimental effects observed.

- Disrupted plant tissues in cryogenic storage should not be thawed at any time prior to its immediate use in downstream applications.