

QuantiGene Technical Note

Preparation of Plant Tissue Homogenates for use in QuantiGene and QuantiGene Plex Assays

About this Technical Note

Instructions are provided for the preparation of plant tissue homogenates for use in QuantiGene 2.0 or QuantiGene Plex 2.0 assays. For more information about these assays, please refer to the appropriate QuantiGene Reagent System User Manual.

Required Materials

Item	Source
Homogenizing Solution	Panomics P/N QS0518, QS0516 or QG0517
Proteinase K (50 µg/µL)	Panomics P/N QS0510, QS0511, QS0512
RNase Zap	Ambion P/N 9780
Liquid nitrogen-cooled mortar (mortar, bowl, and housing)	Fisher P/N 12-947-1
Mortar (extra, convenient when preparing many samples)	Fisher P/N 12-947-2
96-well polypropylene plate (collection plate)	Fisher P/N 07-201-156 (Corning 3371)
0.45 µm cellulose nitrate filter plate	Whatman P/N 7700-3307
Adhesive plate seal	Major laboratory supplier (MLS)
Pestles	MLS
Spatulas	MLS
Liquid nitrogen (500 mL/sample)	MLS
Dry ice	MLS
2-mL tubes and/or 15 mL centrifuge tubes (to hold prepared solution)	MLS

Before You Start

Treat all surfaces with RNase Zap according to manufacturer's instructions.

! WARNING ! Wear safety glasses at all times during this procedure.

About this Procedure

This procedure is for the preparation of plant tissues.

**Preparing Plant
Tissue
Homogenates**

To prepare plant homogenates:

Step	Action
1	Pre-chill tubes, spatula, mortar and pestle on dry ice.
2	<p>Prepare an appropriate volume of Working Homogenization Solution by combining the following, per 2 punches or 5 mg of plant material:</p> <ul style="list-style-type: none"> ◆ 300 μL of Homogenizing Solution ◆ 3 μL of 50 μg/μL Proteinase K solution <p>Vortex briefly to mix.</p> <p>IMPORTANT If you want to prepare more concentrated samples, for example, 4 punches/300 μL of Working Homogenizing Solution, we strongly recommend that you validate the preparation as outlined in "Determining Complete Tissue Homogenization" on page 3.</p>
3	Add a small amount of liquid nitrogen (LN2) to a clean mortar while it is sitting on dry ice.
4	Add the pre-weighed, cut tissue sample to the mortar containing the LN2.
5	<p>Place one hand over the top of the mortar to prevent the tissue from ejecting, and pulverize the tissue with the pestle. Add small amounts of LN2 as it evaporates during the pulverization.</p> <p>IMPORTANT Never grind the tissue without LN2.</p>
6	When the tissue becomes a fine powder, allow the LN2 to evaporate, then, before it thaws, transfer the powder to an appropriate sized, pre-chilled tube.
7	Add 300 μ L of Working Homogenizing Solution per 2 punches or 5 mg plant sample.
8	Incubate the homogenized sample at 65 °C for 30 minutes. Vortex at maximal speed for 1 minute every 10 minutes during this incubation.
9	<p>For QuantiGene assays:</p> <p>Centrifuge the sample at 16,000 x g for 15 minutes to pellet the remaining cellular debris, then transfer the supernatant to a new tube. Repeat this step once more. Use the homogenate immediately in a QuantiGene assay, or store at -80 °C for later use.</p> <p>For QuantiGene Plex assays:</p> <ol style="list-style-type: none"> a. Determine the number of wells to use on the cellulose nitrate filter plate, based on the number of samples and volume prepared for each sample. b. Seal the wells that will not be used with an adhesive seal. <p>IMPORTANT Do not add more than 300 μL to each well.</p> <ol style="list-style-type: none"> c. Add the samples to the 0.45 μm cellulose nitrate filter plate. d. Place the cellulose nitrate plate (with samples) on top of the collection plate. e. Spin the cellulose nitrate plate/collection plate assembly in the microplate centrifuge at 1,444 x g for 2-5 minutes at room temperature. If the sample has not filtered through, spin an additional 2-3 minutes. f. Use the homogenate immediately in a QuantiGene Plex assay, or seal the plate with an adhesive seal and store at -80 °C for later use.

Determining Complete Tissue Homogenization

We strongly recommend that you validate your tissue homogenization process by doing the following:

- ◆ Examine the homogenate. It should be clear of any debris and small fibers.
- ◆ Perform a serial dilution of the homogenate and run a QuantiGene 2.0 or QuantiGene Plex 2.0 assay on the dilution series.
- ◆ Verify the expected fold change matches the observed fold change. For example, a 3-fold dilution should generate a 3-fold change ($\pm 20\%$) in the signal (background subtracted) of the target genes.

Running the QuantiGene 2.0 or QuantiGene Plex 2.0 Assay

Refer to the QuantiGene 2.0 or the QuantiGene Plex 2.0 User Manual as appropriate. Follow the “Capturing Target RNA from Fresh, Frozen, or FFPE Animal Tissues” section for setup of the overnight hybridization reaction and the section “Signal Amplification and Detection of RNA Targets” for the day 2 procedure.

Recommended Sample Input

The amount of homogenate to use in either the QuantiGene 2.0 or the QuantiGene Plex 2.0 assay varies depending on factors such as plant type and the target gene expression level. The amount of homogenate should be titrated to determine the optimal sample input. Typically, between 5 μL and 20 μL of homogenate is recommended.

Troubleshooting

Please refer to the QuantiGene 2.0 or QuantiGene Plex 2.0 User Manual for troubleshooting.

Contacting Panomics

For technical support, contact the appropriate resource provided below based on your geographical location. For an updated list of FAQs and product support literature, visit our website at www.panomics.com.

Location	Contact Information
North America	1.877.726.6642 option 3 techsupport@panomics.com
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