GenePurgeDirect® DNA/RNA Releasing Agent

Soil and Plant Tissue

Version: 1.0
Revision date: 31-07-2014

Description

GenePurgeDirect® is composed of proprietary polymeric materials that quickly facilitate the release of nucleic acids from cells in a form suitable for PCR. By segregating inhibitors that are released during lysis as well as preservation agents that may interfere with amplification, GenePurgeDirect® provides amplifiable nucleic acids from minute amounts of material.

Protocols

GenePurgeDirect® Protocol for Soil

1. Prepare a 10% slurry of soil sample in isotonic (0.9%) saline. We recommend 0.25g of sample with 2.25ml of 0.9% saline solution.
2. Gently invert 20-30 times.
3. Centrifuge sample at 500xg for 1 minute.
4. Decant supernatant.
5. For every 0.25g of soil used, add 1 ml of a solution of 0.1% Tween 20 in isotonic saline (990µl 0.9% saline solution + 10µl of 10% Tween 20).
6. Mix gently to resuspend soil slurry.
7. Remove 10µl of the suspended soil slurry and place into a 0.5ml PCR tube.
8. Thoroughly resuspend the contents of the GenePurgeDirect® tube by inverting 10-20 times or vortexing briefly.
9. Add 40µl of the resuspended GenePurgeDirect® to the tube containing the 10µl of soil sample slurry.
10. Place samples onto thermal cycler, with a heated lid, with the following GenePurgeDirect® program:

Step	Temperature	Time
1.	65ºC	30 sec.
2.	8ºC	30 sec.
3.	65ºC	90 sec.
4.	97ºC	180 sec.
5.	8ºC	60 sec.
6.	65ºC	180 sec.
7.	97ºC	60 sec.
8.	65ºC	60 sec.
9.	4ºC	hold

11. Once program is completed, centrifuge tubes at 5,000xg for 5 minute.
12. Remove supernatant to use as template for PCR. Recommend using 1-10ul of supernatant per 20-100µl amplification reaction.
13. Perform amplification reaction according to your optimized protocol.

GeneReleaser® Protocol for Plant Tissue

Part I: Plant Tissue Homogenization

1. Using the 1.5 ml "snap cap" standard conical centrifuge tube, use the cap to punch a round leaf section by inserting the leaf between the base of the open cap and the tube opening and closing the cap.
2. Using a disposable pestle, grind or mince the leaf material with 100µl of sterile H2O or 1X TE in a 1.5ml tube provided. Repeat this with two additional replicates of identical leaf or plant material using 50µl and 25µl of buffer for the homogenization.
3. The homogenized material may be used immediately or stored at -20°C until ready for use.

Part II: GenePurgeDirect® Treatment:

1. Transfer 1µl of the tissue homogenates obtained above into a standard amplification (PCR) tube. NOTE: flick the tube containing the homogenate 3 times before transfer.
2. Resuspend the GenePurgeDirect® mixture by either vortexing 2-3 seconds or by inverting 10-20 times.
3. Add 20 µl of the GenePurgeDirect® suspension to the 1µl of homogenate in the amplification tube.
4. Vortex the tube 2-3 seconds.

5. Place samples onto thermal cycler, with a heated lid, with the following GenePurgeDirect® program:

Step	Temperature	Time
1.	65ºC	30 sec.
2.	8ºC	30 sec.
3.	65ºC	90 sec.
4.	97ºC	180 sec.
5.	8ºC	60 sec.
6.	65ºC	180 sec.
7.	97ºC	60 sec.
8.	65ºC	60 sec.
9.	80ºC	hold

6. Sample is ready to use as PCR template.
7. Add appropriate volume of mastermix (of 80µl of a 1.25X master mix containing all components for the amplification)
8. Perform amplification reaction according to your optimized protocol.

* GenePurgeDirect® lysis can also be achieved using the optional microwave protocol below.

Microwave Lysis Protocol:

We have found that the microwave treatment of specimens affords a rapid sample preparation and facilitates the amplification of the more intractable types of specimens.

A. Evaluation of microwave

Perform the following experiment to determine the optimal conditions for your tubes and microwave.

1. Place 40µl DI water in the same type of tube that you will be using for GenePurgeDirect® treatment.
2. Overlay each tube with mineral oil to prevent evaporation.
3. Close the tubes, place in microwave safe rack (polyethylene or propylene) and heat on high for 5 minutes.
4. If any caps pop or tubes distort in any manner, then place a separate beaker in the microwave with 150ml of room temperature DI water and repeat the above 3 steps, the beaker of water serves as a heat ballast.
5. If tubes open or distort, reduce the power by 10% increments and increase time by 1-minute increments repeating step 4 until tubes no longer open or distort.

Note: Make sure the racks used in this procedure are MICROWAVE SAFE!

B. Microwave Protocol

1. Perform microwave procedure above for time and power conditions
2. Place 1µl of specimen with 20µl of GenePurgeDirect® into either a 0.5ml PCR tube or 1.5ml tube.
3. Vortex the tubes containing specimen and GenePurgeDirect® for ~10 seconds.
4. Overlay with mineral oil to prevent samples from evaporating.
5. Place the closed tubes in a microwave safe polyethylene or propylene rack. Make sure that the lids are loosely closed. If lids are closed too tightly tubes could rupture.
6. Place the rack in a microwave oven and heat at maximum power setting (setting should be based on the microwave evaluation results) for 5-7 minutes. Typically, 5 minutes if wattage is 900 or higher and 7 minutes if wattage is 500.
7. Remove rack from microwave and centrifuge the tubes at 5000xg for 5 minutes. After centrifuging samples, remove supernatant and use as DNA template.
8. Perform the amplification reaction.

References:

Sikorski J, Teschner N, and Wackernagel W. Highly Different Levels of Natural Transformation Are Associated with Genomic Subgroups within a Local Population of Pseudomonas stutzeri from Soil. Applied and Environmental Microbiology. 2002 February; 68(2): 865–873.

Sikorski J,Stackebrandt E, and Wackernagel W.Pseudomonas kilonensis sp. nov., a bacterium isolated from agricultural soil. International Journal of Systematic and Evolutionary Microbiology 2001:1549–1555.