

## **GeneReleaser® DNA AMPLIFICATION FROM PLANT TISSUE Protocol**

GeneReleaser® is a proprietary reagent which releases DNA from whole blood, cell cultures, bacterial colonies and the like. Lysis is accomplished directly in the amplification tube on a thermocycler. Typically, 1µl of specimen is lysed in a total volume of 20µl and amplification reagents are subsequently added to initiate amplification. GeneReleaser® sequesters cell lysis products which might inhibit polymerases and improves amplification yield and specificity. GeneReleaser® greatly simplifies the amplification of genomic DNA by avoiding the requirement to purify DNA. This avoids lengthy protocols and excessive sample manipulations which may introduce contamination.

### **PLANT 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 H<sub>2</sub>O 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.

### **GeneReleaser® PROCEDURE:**

1. Transfer 1µl of the tissue homogenates obtained above into a 0.5 ml standard amplification tube. NOTE: flick the tube containing the homogenate 3 times before transfer.
2. Resuspend the GeneReleaser® mixture by either vortexing 2-3 seconds or by 10 inversions.
3. Add 20 µl of the GeneReleaser® suspension to the 1µl of homogenate in the amplification tube.
4. Vortex the tube 2-3 seconds.
5. Overlay the mixture with ~50µl of mineral oil.
6. Place tubes in a microwave safe rack.
7. Place the rack in the center of a microwave oven.
8. Microwave on HIGH for 5-7 minutes.
9. Remove rack from microwave.
10. Transfer tubes to thermocycler preheated to 80°C and allow to equilibrate for 5 minutes.
11. Initiate amplification by the addition of 80µl of a 1.25X master mix containing all components for the amplification.
12. Begin your optimized amplification. NOTE: It is very important that the very first denaturing step of the first cycle be at 94°C for 2-5 minutes depending on brand of cycler, reaction volumes etc.
13. Assay the products per your standard method.
14. Select the volume of homogenization buffer which gave the best product yield of the three volumes described above for future homogenizations and amplifications from the same type of source material.

### **NOTES:**

1. If the amplicon is larger than 1KB a magnesium titration should be performed. 2mM Mg is a good starting point for all amplifications with GeneReleaser®.

2. For future amplifications from the same type of source material, select the volume of homogenization buffer which gave the best product yield of the three volumes described above.

If you have further questions, please contact us at [support@bioventures.com](mailto:support@bioventures.com). You may also contact us via fax (1-877-286-0330) or through our toll-free phone number (1-877-852-7846).