GenePurgeDirect® DNA/RNA Releasing Agent

Yeast and Bacteria

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 DNA from Bacterial Colony

1. Place 1 isolated bacterial colony into a standard amplification tube.
2. Thoroughly resuspend the contents of the GenePurgeDirect® tube by inverting 10-20 times or vortexing briefly.
3. Add 20µl of GenePurgeDirect® to the bacterial colony and vortex briefly for 1-2 seconds.
4. Treat samples using either the thermal cycler program (below) or the microwave protocol (page2)
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. Once program is completed, sample is ready to use as PCR template.
7. Add appropriate volume of mastermix (80µl of a 1.25X master mix containing all components for the amplification)
    - Alternatively centrifuge the sample tube at 5,000xg for 1 minute and remove the supernatant to use as a template for PCR. Recommend using 1-10 µl of supernatant per 20-100ul PCR reaction volume.
8. Perform amplification reaction according to your optimized protocol noting the following:
    - The first cycle should have a denaturing time of 2-4 minutes at 94-95ºC.
    - For some primer sets a re-titration of the magnesium concentration may be required.

GenePurgeDirect® Protocol for DNA from Bacterial Culture

1. Place 1µl of cells at 10^3-10^8 cells/ml into a standard amplification tube.
2. Thoroughly resuspend the contents of the GeneReleaser® tube by inverting 10-20 times or vortexing briefly.
3. Add 20µl of GenePurgeDirect® to the bacterial culture sample and vortex briefly for 1-2 seconds.
4. Treat samples using either the thermal cycler program (below) or the microwave protocol (page2)
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. Once program is completed, sample is ready to use as PCR template.
7. Add appropriate volume of mastermix (80µl of a 1.25X master mix containing all components for the amplification)
    - Alternatively centrifuge the sample tube at 5,000xg for 1 minute and remove the supernatant to use as a template for PCR. Recommend using 1-10 µl of supernatant per 20-100ul PCR reaction volume.
8. Perform amplification reaction according to your optimized protocol noting the following:

- The first cycle should have a denaturing time of 2-4 minutes at 94-95ºC.
- For some primer sets a re-titration of the magnesium concentration may be required.

GenePurgeDirect® Protocol for DNA from Yeast Culture

1. Estimate the number of organisms/ml from your culture (typically approx. 10^8/ml).
2. Prepare three (3) dilutions of the culture with isotonic saline so that the concentrations are 10^3, 10^4 10^5 organisms/ml.
3. Thoroughly resuspend the contents of the GenePurgeDirect® tube by inverting 10-20 times or vortexing briefly.
4. Add 20µl of GenePurgeDirect® in each of three individual standard amplification tubes.
5. Add 1µl of each dilution of the yeast culture to one of the tubes containing GenePurgeDirect®, label each tube.
6. Using a pestle, grind the yeast cells in the presence of GeneReleaser® using 10 turns and thrusts of the pestle. Drain the pestle into the tube, repeat for each dilution.
7. Vortex the tubes and transfer the entire contents to a new amplification tube.
8. Treat samples using either the thermal cycler program (below) or the microwave protocol (page2).
9. 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

10. Once program is completed, sample is ready to use as PCR template.
11. Add appropriate volume of mastermix (80µl of a 1.25X master mix containing all components for the amplification)
    - Alternatively centrifuge the sample tube at 5,000xg for 1 minute and remove the supernatant to use as a template for PCR. Recommend using 1-10 µl of supernatant per 20-100ul PCR reaction volume.
12. Perform amplification reaction according to your optimized protocol noting the following:
    - The first cycle should have a denaturing time of 4 minutes at 94-95ºC.
    - For some primer sets a re-titration of the magnesium concentration may be required.

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:

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