Differentiating Research

Product Details
SUPERSHOT™

Genomic DNA preparation for PCR in 10 minutes

Product Overview

The CTAB and phenol–chloroform methods for genomic DNA extraction have been irreplaceable for the past four decades. The age-old protocols have stood the test of time largely because of the difficulties associated with genomic DNA isolation. Rigid cell walls, inhibitors released during lysis, low DNA yields, and the propensity for DNA degradation have made it extremely hard to protect DNA in crude lysates from which target amplicons can be generated reproducibly. For the first time in history, we have designed a single-buffer system SUPERSHOT™ that isolates and protects DNA in crude lysates of plants, animals, and microbes with utmost consistency and efficiency. The buffer is also compatible with the widely used phenol-chloroform and silica methods for DNA purification, making it an indispensable component of the DNA extraction field today.

Catalog Details

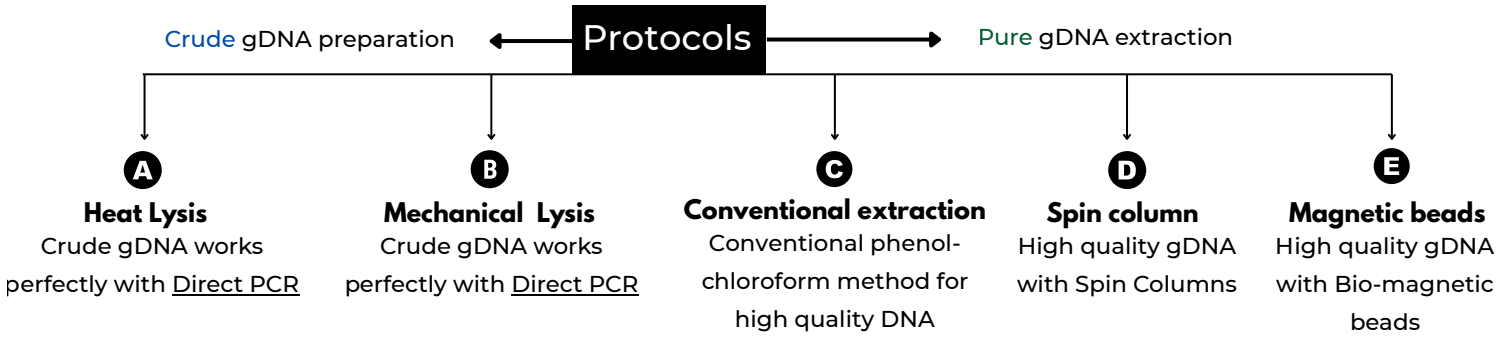
RC50 100 ml Pack

Storage details

Storage: Supershot buffers can be stored in room temperature for a few weeks. For prolonged storage beyond a month, store the buffers in 4 °C.

Critical Note Before using Supershot

SuperShot can be used in 5 different ways depending on your need. Choose ***ONE PROTOCOL ONLY.***



A Preparing PCR-ready genomic DNA through heat lysis

1. **Prepare.** Take 200 µL of supershot buffer and add 2.5% Beta-mercaptoethanol (5µl).
2. **Mix.** Add the following amounts of sample into the buffer:
   a. Plants - 5-10 mg of plant leaf torn into pieces allowing it to completely immerse in 200 µL Supershot buffer. For seeds, crack open the seed coat with a mortar and pestle. Make sure to add one entire cracked seed into the buffer. If the seed is large, increase the buffer volume to allow the sample to be dipped entirely in the buffer.
   b. Microbes: Pick an entire isolated colony of the microorganism from the plate and suspend the same in the buffer.
   c. Animals: 10^4 counted cells from any of the cell lines or 1 cm of the scraped or entire cut tissue suspended into the buffer.
3. **Treat.** Vortex briefly and transfer the vial into a heat block/water bath at 90 °C and incubate for 10 minutes.

4. **Process:** Dilute 1 µL of the lysate to 60 µL with nuclease free water and use 2 µL of the diluted DNA for 20 µL PCR mix.

Note: *From extensive studies in a large reportoire of samples, 1:60 dilution lysate provided the best results uniformly. Kindly do not deviate.*

B Preparing PCR-ready genomic DNA through mechanical lysis

1. **Prepare and Mix** samples using the same methodology mentioned in the previous protocol (A1-A2).
2. **Treat.** Lyse the samples using a beadmill homogenizer or a mortar and pestle to the maximum extent possible.
3. **Process.** Dilute 1 µL of the lysate to 60 µL with nuclease free water and use 2 µL of the diluted DNA for 20 µL PCR mix.

Note: *From extensive studies in a large reportoire of samples, 1:60 dilution lysate provided the best results uniformly. Kindly do not deviate.*

C Conventional purification of genomic DNA from samples using Supershot

1. Follow the preparation, mixing, and treatment, just as the previous protocol to get a homogenous lysate of the sample in Supershot buffer (B1-B2).
2. Add 5 µL of RNAse A (10 mg/ml). Invert the vial 4 - 5 times and incubate the lysate at 37 °C for 20 minutes in an incubator.
3. Add 200 µL of chloroform:Isoamyl alcohol mix (24:1) and blend the two solutions to homogeneity by inverting the vial multiple times.
4. Centrifuge at 10,000 rpm for 2 minutes or 3,500 rpm for 15 minutes based on the apparatus used for processing.
5. Collect 150 µL of the aqueous layer from the top gently without disturbing the vial and transfer it to a fresh vial. Add 250 µL of chilled isopropanol. Invert the vial gently and incubate in -20 °C for 5 minutes.
6. Centrifuge at 10,000 rpm for 2 minutes or 3,500 rpm for 15 minutes again.
7. Decant the supernatant and add 400 µL of chilled 70% ethanol. gently tap the vial to allow the pellet to enter into the solution. If no pellet is observed, proceed to the next step irrespective.
8. Centrifuge at 10,000 rpm for 2 minutes or 3,500 rpm for 15 minutes again.
9. Decant the supernatant and air dry the pellet for 10 - 15 minutes.
10. Resuspend the pellet in 100 µL of 1X TE buffer or nuclease free water and load 2 - 5 µL of the purified DNA on a 0.8% agarose gel to ascertain the DNA purity.

D Silica - spin column based purification of genomic DNA from samples using Supershot.

1. Follow steps 1 to 4 from the previous protocol (C1-C4). Collect 150 µL of the aqueous layer from the top gently without disturbing the vial and transfer it to the spin column.
2. Centrifuge at 10,000 rpm for 2 minutes.
3. Discard the flow through and add 400 µL of cold 70% ethanol to the spin column.
4. Centrifuge at 10,000 rpm for 2 minutes and discard the flow through.
5. Repeat step 4 to get rid of residual ethanol and air dry the column for 10 minutes.
6. Elute the DNA from the column by adding 100 µL of 1X TE buffer or nuclease free water and centrifuging again at 10,000 rpm for 2 minutes.
7. Load 2 - 5 µL of the purified DNA on a 0.8% agarose gel to ascertain the DNA purity.

Note: In order to extract RNA from the lysate, treat the lysate with DNAse rather than RNAse and proceed with the same protocol.

E Magnetic beads based purification of genomic DNA from samples using Supershot.

1. Follow steps 1 and 2 from Protocol B (B1-B2) for preparing the lysate ready for biomagnetic bead based gDNA isolation.
2. Centrifuge at 10,000 rpm for 5 minutes or 3,500 rpm for 15 minutes to pellet the cell debris.
3. Carefully transfer the clarified supernatant to a new tube and add 40 µL of the biomagnetic bead slurry.
4. Mix well by inverting and incubate on a vortexer at low rpm in room temparature for 15 minutes to allow the DNA to bind to the beads.
5. Place the tube on a magnetic separation rack and allow the beads to attach to the wall on which side the magnet is.
6. The solution typically clears up in 30 seconds to 1 minute. Aspirate and discard the supernatant containing the cellular contaminants.
7. Remove the tube from the magnet and add 70% ethanol. Mix thoroughly to resuspend the beads completely. Place it back on the rack to allow the beads to pellet and discard the supernatant.
8. Air dry the beads at room temperature for 5-10 minutes allowing the residual ethanol to evaporate.
9. Remove the tube from the rack and add 50 µL of 1X TE buffer to the beads. Mix to resuspend the beads fully and incubate at 60 °C for 10 minutes to facilitate DNA release.

Place the tube back on the rack for 1 to 2 minutes to separate the beads and aspirate the final DNA to a separate tube. Load 2 - 5 µL of the purified DNA on a 0.8% agarose gel to ascertain the DNA purity.

Quality Control

Quality control testing: Supershot buffer was tested for production of a genomic DNA isolate and PCR amplicon from the lysates of cotton leaf. Direct PCR from mechanical lysate resulted in amplicons tested upto 2 kb and 1 kb for heat lysates.
Nuclease tests: No contamination of endo or exonucleases were detected. DNA is safe and stable in lysate.

PCR Tips

Recomended Mastermix for direct PCR: GloryPol Mastermix (2X) , Catalog# R8320

1. Glory-Pol mastermix can amplify targets upto 1 kb from Supershot heat lysates (Protocol A) and 2 kb for mechanically sheared lysates (Protocol B).
2. Use 2 µL of the 1:60 diluted lysate as template for PCR with GloryPol Mastermix (2X) , Catalog# R8320 in a 20 µL reaction mix.
3. Recommended PCR Cycles: 35. If faint or weak band is observed, cycles can be increased upto 40.
4. If non specific ampolicons are observed, set the annealing temperature to 60℃ .

Any Technical Help ?

Please write to us at info@dxbidt.com . Response can be expected within 24Hrs. Our technical team shall be happy to assist you all the time.

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