xcelris
genomics
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XcelGen | Soil gDNA mini Kit

User Guide

Cat No: XG2413-01

XcelGen

Ver.0.0/11

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Introduction

The Soil gDNA Kit is designed for a rapid and reliable purification of high-quality genomic DNA from various soil samples. Up to 1 gram of soil samples can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of matrix with a propriety buffer system to eliminate PCR inhibiting compounds such as humic acid from soil samples. Purified DNA is suitable for PCR, restriction digestion and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

In this procedure, soil sample is homogenized and then treated in a specially formulated buffer that contains detergent. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-frozen step. DNA is further purified with a DNA spin-column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in water or low ionic strength Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the Soil gDNA Kit should be stored at 22°C-25°C. Under these conditions, DNA has successfully been purified and used for PCR after 12 months of storage. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by incubation of solution at 65°C.

Kit Contents

Catalog #	XG2413-00	XG2413-01	XG2413-02
Preps	4	50	250
DNA columns	4	50	250
2 mL collection tubes	8	100	500
Glass beads	lg	12g	55g
DH reagent	1.2 ml	12 ml	60 ml
Buffer LX	5 ml	40 ml	200 ml
Buffer P21.	5 ml	15 ml	60 ml
Buffer BL	3ml	30 ml	150 ml
Elution Buffer	1.5 ml	20 ml	100 ml
DNA Wash Buffer	2ml	15ml	3x24ml
RNaseA	15 µl	160 µl	800 µl
Instruction Manual	1	1	1

*Buffer BL contains chaotropic salts that may form combustive compound with bleach. Use gloves and protective eyeware when handling this solution.

Before Starting

Please read the entire booklet to become familiar with the Soil gDNA Kit protocol.

- Prepare Buffer LX stock solution by adding 10 µl β-mercaptoethanol per 1 ml Buffer LX before use. Each sample will require 600 µl of this solution.
- Preheat Buffer LX and Elution Buffer at 65 °C. Make sure the crystal in Buffer LX is completely dissolved.
- Dilute DNA Wash Buffer with absolute ethanol as follows and store at room temperature. Add 8 ml (XG2413-00) or 60 ml (XG2413-01) or 96 ml (XG2413-02) to each DNA Wash Buffer bottle before use.

Soil gDNA mini Kit Protocol

Materials to be provided by user

- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Water bath or heating block preset to 65°C and 70°C
- β-mercaptoethanol
- Absolute (96%-100%) ethanol
- Isopropanol (100%)

1. Weigh 200 mg of glass beads in a 2 ml centrifuge tube, add 0.2-1 g soil sample. Add 600 µl Buffer LX (add 10 µl β-mercaptoethanol per 1 ml Buffer LX before use). Vortex at maximum speed for 3 minutes or until the sample is thoroughly homogenized.
2. Incubate at 70°C for 10 min, Mix sample twice during incubation by vortexing the tube.

   Optional: for isolation of DNA from gram positive bacteria, do a second incubation at 95 °C for 2 minutes.
3. Add 200 µl Buffer P2, mix thoroughly by vortexing for 30 sec.
4. Incubate the sample on ice for 5minutes.
5. Centrifuge the sample at 13,000 rpm for 5 minutes.
6. Carefully transfer the supernatant to a new 1.5ml microfuge tube, avoid carrying over any debris.
7. Add equal volume of isopropanal and mix thoroughly by invert the tube 5-10 times.
8. Centrifuge at 13,000 rpm for 10 minutes at room temperature.
9. Carefully discard the supernatant and make sure not to dislodge the DNA pellet. Invert the tube on paper towels for 1 minute to drain the liquid. It is not necessary to dry the DNA pellet.
10. Add 200 µl of Elution Buffer to the tube and vortex for 10 sec. *Optional: If RNA-free DNA is required, add 3 µl RNase A (20 mg/ml) in this step. Incubate at 65°C for 10-20 minutes to dissolve the DNA pellet. Centrifuge briefly to collect any liquid drop from the tube cap.*
11. Vigorously mix the bottle of the DH reagent for 30 sec. to ensure the particles are thoroughly resuspended. Add 100 µl of DH reagent and mix thoroughly by vortexing for 10 sec.

Important: DH reagent must be thoroughly suspended before being dispense from bottle. *Tip: Use lml pipettor and cut off the end of lml tip to make it easier for pipetting the DH reagent.*

12. Incubate at room temperature for 2 minutes.
13. Centrifuge at 13,000 rpm for 2 minutes.
14. Transfer cleared supernatant to a new 1.5 ml tube.

    Note: If the supernatant still shows dark color from soil at this point, perform the DH reagent extraction again by repeating step 11-14.
15. Add equal volume of Buffer BL to the cleared soil lysate, mix the sample thoroughly by vortexing. For example: if the sample from step 14 is 500 µl, then add 500 µl Buffer BL.
16. Apply entire sample including any precipitation that may have formed, to a DNA column assembled in a 2 ml collection tube (supplied). Centrifuge at 13,000 rpm for 1 min at room temperature. Discard the collection tube and flow-through liquid.
17. Put the column into a new collection tube; add 300 µl of Buffer BL. Centrifuge at 13,000 rpm for 1 min at room temperature. Discard flow-through liquid and re-use collection tube.
18. Add 650 µl DNA Wash Buffer. Centrifuge at full speed (>13,000 x g) for 30 seconds. Discard the flow-through and re-use collection tube.

    Note: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 3. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.
19. Add another 650 µl of DNA Wash Buffer to the column. Centrifuge at full speed (>13,000 x g) for 1 minute. Discard flow-through liquid and collection tube.
20. Discard liquid and insert the column to a new collection tube, centrifuge the column at 14,000 x g for 2 min at room temperature. This step is critical in removing traces of ethanol that will interfere with downstream applications.
21. Place column into a clean 1.5mL microcentrifuge tube (not supplied). To elute DNA add 50 µl of Elution Buffer directly onto the center of the matrix and incubate at 65 °C for 5 minutes.
22. Centrifuge at 13,000 rpm for 1 minutes to elute DNA.
23. Repeat elution step with a second 30 µl of Elution Buffer.

Trouble Shooting Guide

Problem	Possible Reason	Suggested Improvement
A260/280 ratio is low	Inefficient elimination of inhibitory compounds	Repeat the DNA isolation with a new sample, be sure to mix the sample with DH reagent thoroughly and extract the sample with DH reagent twice.
	Salt contamination	• Repeat the DNA isolation with a new sample. • Make sure the column is dried before elution. • Wash the column with extra DNA Wash Buffer.
	DNA Wash Buffer prepared with lower percentage ethanol	Prepare DNA Wash Buffer with 96-100% ethanol
A260/280 ratio is high	High RNA contamination	Be sure to treat the sample with RNase A. See step 5.
Low DNA yield or no DNA eluted	Sample stored incorrectly	Sample should be stores at -20 °C.
	Poor homogenization of sample.	Repeat the DNA isolation with a new sample, be sure to mix the sample with Buffer LX and glass beads. Use long beads beating time to make sure the sample are fully homogenized and cell are lysed.
	Incorrect Buffer BL was added before loading to the column	Repeat the DNA isolation with a new sample
	DNA washed off.	Dilute DNA Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in down stream applications	Ethanol residue in the elute	Be sure to completely dry the column before elution
Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary.
Sample can not pass through the column	Clogging column	Check the centrifugal force and increase the time of centrifugation

Related Products

1) DNA Gel/PCR Purification Miniprep kit (XG3511-01/XG3514)
2) Agarose, Hipure(LE) (XGA-100)
3) 100 bp DNA Ladder (XGM250)
4) 1 kb DNA Ladder (XGM1k)
5) Premix Taq V2.0 (XG334A)
6) Taq DNA Polymerase (XG00007-1000/ XG00007-10000)
7) Pfu DNA Polymerase (XG00021-100/ XG00021-500)
8) dNTP Mixture, 10 mM each (XG0056)

Limited Use and Warranty

This product is warranted to perform as described in its labeling and in XcelGen's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by XcelGen. XcelGen's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of XcelGen, to replace the products, XcelGen shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technology support or learn more product information, please visit our website at **www.xcelrisgenomics.com**

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Product & Services

Sanger Sequencing Services

PCR Product/Plasmid Sequencing
r-*E.coli* Sequencing/BAC Sequencing
Primer walk sequencing
DNA shotgun Sequencing
Multilocus Sequence Typing

Customised Services

SNP Genotyping by SNaPshot Assay
Microsatellite Genotyping
Golden Gate Assays and Arrays
Gene Expression on Real Time PCR
Gene expression on Agilent / Microarray / Affymetix
Library construction

NGS Services on SOLiD 4.0, GS FLX, HiSeq 2000 Ion Torrent

Whole Transcriptome analysis
Whole Genome Re Sequencing
Denovo genome sequencing
ChIP Seq
Metagenomics
Amplicon Sequencing
Metatranscriptomics
Metagenomics
Small RNA Sequencing
RAD Sequencing
Exome Sequencing

Bioinformatics Research and Data Analysis Services

In silico Primer Design
Microarray Analysis
Physical ,Genetic and QTL mapping
Assembly and annotation of prokaryotic and eukaryotic genome
Genome Mapping and SNP discovery
Transcriptome discovery and analysis
sRNA analysis and discovery
Metagenomics

Custom Oligo Synthesis and Purification Services

10 nmole
25 nmole
50 nmole
100 nmole
200 nmole
1000 nmole

Consumables and Kits

XcelGen Plasmid Isolation Kits
XcelGen High Performance (HP)Plasmid Kits
XcelGen BAC/PAC/ Yeast Plasmid Isolation Kits
XcelGen PCR Purification Kits
XcelGen Genomic DNA/RNA Isolation Kits
Polymerases and Modifying Enzymes

Infrastructure

·SOLiD v4
·GS FLX +
·HiSeq 2000
·Ion Torrent
·ABI 3730XL
·Dr. Oligo 192 (Oligo Synthesizer)
·Advanced Bioinformatics Lab
·Advanced Manufacturing Lab
·BeadXpress
·iScan
·LightCycler® 480

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