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genomics
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XcelGen | Bacterial gDNA Mini Kit

User Guide

Cat No: XG2411-01

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Introduction

XcelGen Bacterial gDNA kit provides a rapid and reliable method for purification of high quality genomic DNA from a wide varieties of bacterial species. Bacterial cells are grown to log-phase, harvested and process to DNA purification. The bacterial cell wall is initially digested with appropriate enzyme to loosen cell wall. Following initial lysis, bacteria is lysed with chaotropic salt solution to promote complete lysis and DNA release from the cells. Our proprietary DNA binding system that allows the high efficient binding of DNA to our binding matrix while proteins and other contaminates are removed under certain optimal conditions. The DNA bound to the DNA mini silica column and purified DNA is easily eluted with sterile water or elution buffer.

Purified DNA is suitable for PCR, restriction digestion hybridization techniques and other applications.

Highlights

- Spin column technology
- Quick and simple procedure for complete recovery of gDNA
- 10^9 bacterial cells can be processes per column
- Common protocol for gram-positive and gram-negative bacteria
- Organic solvent (Phenol, Chloroform) free system
- Recovery of 15-30µg of bacterial gDNA, depend on the type of strains

Storage and Stability

- Shelf life for provided kit is 12 months from the date of purchase.
- Once reconstituted in water, Proteinase K and lysozyme must be stored at -20°C.
- Store RNase A at 4°C.
- Other components can be stored at 22°C-25°C.
- BL/TL buffers may forms precipitate upon storage. If necessary redissolve by warming to 37°C, and then store at room temperature

Kit Contents (Kit is supplied at prescribe temperature)

Product	XG2411-01	Storage
Preps	50	RT
DNA Mini Columns	50	RT
2 ml Collection Tubes	100	RT
Buffer TL	15 ml	RT
Buffer BL	15 ml	RT
Buffer KB	28 ml	RT
DNA Wash Buffer	15 ml	RT
Glass Beads	1.5 g	RT
Elution Buffer	15 ml	-20°C
Lysozyme	50 mg	-20°C
Proteinase K	30 mg	-20°C
RNase A (20 mg/ml)	270 µl	-20°C
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Buffer BL contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

Before Starting

- Read the entire procedure prior to starting the experiment.
- Work area, equipments and hand should be completely free from dust
- Carry out all experimental procedure/centrifugation at room temperature
- Use pre-warm water/elution buffer at 65°C for efficient elution of gDNA
- **DNA Wash Buffer:** Add 60 ml absolute (96%-100%) ethanol and store at RT

Preparation of stocks:

ProteinaseK Preparations

Prepare a stock solution of Proteinase K (provided) as follows and aliquots into sterile eppondroff tube and store. Bring Proteinase K solution to room temperature before use.

XG2411-01	Dissolve with 1.3 ml of Elution Buffer

Lysozyme Preparations

Prepare a lysozyme stock solution at 50 mg/ml and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use.

XG2411-01	Dissolve with 1 ml of Elution Buffer

Materials to be provided by User

- Table top microcentrifuge
- Water bath set to 30°C
- Shaking water bath set to 55°C
- Dry bath or water bath set to 65°C
- Absolute ethanol (96%-100%)
- Nuclease-free 1.5 ml tubes, tips, pipette

Bacterial gDNA Purification Spin Protocol

This method is designed for isolation of gDNA from 1-3 ml log phase grown Bacteria culture.

1. Pellet 1-3 ml culture by centrifugation at 12,000 x g for 2 min at room temperature.
2. Discard medium completely and resuspend the pellet in 180 µl TE Buffer or Elution Buffer. Add 18 µl of 50 mg / ml lysozyme solution and 5 µl RNase A, incubate at 30°C for 15-30 min.

   Note: Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield more genomic DNA.
3. Centrifuge the cells at 5,000 x g for 5 min at room temperature. Discard supernatant and leave10 µl residual liquid in the tube. Resuspend the cell pellet by vortexing.
4. Add 25 mg glass beads and 200 µl Buffer TL. Vortex at maxi speed for 5 min. Allow the beads to settle down to the bottom of the tube and transfer supernatant to a new 1.5 ml centrifuge tube.
5. Add 25 µl Proteinase K and vortex for 10 seconds. Spin down briefly.
6. Incubate the mixture at 55°C in a shaking water bath for 30 min. If no shaking waterbath is available, briefly vortex the samples every 2-3 min.

   (Optional: Centrifuge at 10,000 x g for 2 min to pellet insoluble debris. Transfer the clear supernatant to a sterile microcentrifuge tube.)
7. Add 220 µl Buffer BL and briefly vortex to mix. Incubate at 65°C for 10 min. A wispy precipitate may form upon addition of Buffer BL (The precipitate does not interfere with DNA recovery).
8. Add 220 µl absolute ethanol and mix thoroughly by vortexing for 20 sec. If any precipitation can be seen at this point, break the precipitation by pipetting up and down 10 times.
9. Transfer the entire sample from Step 8 into a DNA mini column, including any precipitate that may have formed. Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through.
10. Transfer the column into a new 2 ml tube and add 500 µl Buffer KB. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.

    Note: Saturation of column with Buffer KB enhance the binding of DNA to column

11. Place the column into the same collection tube and wash by adding 650 µl DNA Wash Buffer diluted with ethanol. Centrifuge at 10,000 x g for 1 min. Repeat again step "11" to remove contaminatents.

    Note: *Make sure that appropriate volume of ethanol is added in Wash buffer solution (refer to before start)*

12. Discard flow-through and put the column, with the lid open, into a new collection tube and centrifuge at maxi speed (10,000 x g) for 2 min to dry the column..

    Note: *Residue ethanol will be removed more efficiently with the column lid open.*

13. Place the column into a nuclease-free 1.5 ml microfuge tube and add 50µl of pre-warmed (65°C) elution Buffer to DNA Mini column. Allow columns to incubate at 65°C for 2 min.

    Note: Incubating the DNA column at 65°C for 2 min prior to centrifugation will give a modest increase in DNA yield.

14. Centrifuge at 10,000 x g for 1 min to elute the DNA.

15. Repeat the elution with a second 50-100 µl Elution Buffer.

    Note: *Each 50µl elution typically yields 60-70% of the DNA bound to the column. Thus two elutions generally give~90%. However, increasing elution volume reduces the concentration of the final product. Volumes lower than 50 µl greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C upon addition of Elution Buffer.*

Bacterial gDNA Vacuum/Spin Protocol

Note: Please read through previous section of this manual before using this protocol.

1. Prepare samples and column by following step 1 to 9 on page 5.
2. Prepare the vacuum manifold according to manufacturer's instructions and connect the column to the manifold.
3. Transfer the sample/BL/Ethanol mixture to the column. Turn on vacuum to draw the sample through the column.
4. Add 500 µl Buffer KB and allows the buffer to pass through the column by vacuum.
5. Wash the column by adding 650 µl DNA Wash Buffer and draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 650 µl DNA wash buffer.
6. Proceed step 12-15 on page 6.

Trouble Shooting Guide

Problem	Possible Cause	Possible Cause
Column Clogged	Too much bacterial culture	Do not use greater than 3 ml culture at OD_{600} 1.0 or 1 x 10^9 bacterial cells per spin column. For larger volumes, divide sample into multiple tubes
	Incomplete Lysis	Add the correct volume of Buffer TL and incubate at 55oC to obtain complete lysis. It may be necessary to extend incubation time to 30 min.
	Celll not lyse properly	Add more lysozyme or increase the incubation time. It may be necessary to increase incubation to 30 min.
Low DNA Yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume (see note on page 6). Incubation of column at 65°C for 5 min after addition of Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer must be diluted with absolute (96%-100%) ethanol

	Extended centrifugation during elution step	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation - it will not interfere with PCR or restriction digests.
A260 / A280 ratio	incomplete mixing with Buffer BL	Repeat the procedure, this time making sure to vortex the sample with Buffer BL immediately and completely.
	Insufficient incubation.	Increase incubation time with Buffer TL. Ensure that no visible cell clumps remain.
	Trace protein contamination	Following step 11, wash column with 300 µl Buffer KB before proceeding to step 12.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer TL	Mix thoroughly with Buffer BL and incubate at 70°C prior to adding ethanol.
	Incomplete cell wall blasting	Add more lysozyme or extend the incubation time. It may be necessary to increase incubation by 15 min
	Absolute ethanol not added to lysate/Buffer BL mixture	Before applying sample to column, an aliquot of ethanol must be added. See protocol above.
	No ethanol added to Wash Buffer	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.

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 intended for in vitro research use only. Not for use in human. 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.

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Quality Kits made by Xperts

• Plasmid DNA Isolation Kits • Genomic DNA Extraction Kits • RNA Extraction Kits • Polymerase • DNA Ladders • DNA Markers
• Premix Taq • dNTP's • RAPD kits • Agarose • Glycerol • Tms NA Stabilizers & RNA Protectant solutions

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NGS Services

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• Assembly and annotation of prokaryotic and eukaryotic genome • Genome Mapping and SNP discovery
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XcelSeq

Sanger Sequencing Services

• Plasmid /PCR Sequencing Services • r-E. coli Culture Sequencing Services • Primer Walk Sequencing Services
• Microbial Identification Service • 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

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