xcelris genomics
An Abellon Company

XcelGen | DNA Gel/PCR Purification Mini Kit

User Guide

Cat No: XG3511-01/3514

XcelGen

Ver.: 0.2/13

Revised Protocol

Table of Contents

Introduction

This fast and reliable kit is designed to recover DNA from agarose gels and purify DNA fragments from PCR, RFLP, phosphorylation, labeling, ligation, hybridization and other enzymatic reactions. DNA fragments from 100 bp to 20 kb can be purified using the mini column with over 80-90 % recovery.

Overview

If using the DNA Gel/PCR Purification Mini Kit for the first time, please read this booklet to become familiar with the procedures. Samples are homogenized and lysed in a high salt buffer. The DNA is bound to the column while proteins and other impurities are removed by wash buffer. The purified DNA or PCR products is suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

Storage and Stability

All components can be stored at room temperature. All kit components are stable up to 12 months.

Kit Contents

Product	XG3511-00 XG3514-00	XG3511-01 XG3514-01
Preps	4	50
Buffer BL	2 ml	22 ml
Buffer GC	3 ml	40 ml
DNA Wash Buffer*	2 ml	15 ml
Elution Buffer	1 ml	10 ml
Mini Columns	4	50
User Manual	1	1

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important

- Add 8ml (XG3511/XG3514-00) or 60ml (XG3511/XG3514-01)100% ethanol to **DNA Wash Buffer** before use.
- **A gel slice of 100 mg equals to a volume of 100 µl.**
- **Buffer GC** may form precipitates under cool ambient condition. Warm up the buffer at 37°C to dissolve before use.
- **Buffer BL** precipitates below room temperature. It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.
- Keep the cap tightly closed for **Buffer BL** after use.
- Pre-warm aliquots of **Elution Buffer or ddH_2O** at 55-60°C waterbath.

Safety Information

Buffer GC contains acidic acid and chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

Perform all steps including centrifugation at room temperature!

DNA Gel/PCR Purification Spin Protocol

Note: *Fresh* ***TAE buffer*** *as running buffer is recommended. Reusing running buffer will result the increase of the pH and then reduce yield.*

1. Add **400µl of Buffer BL** into the spin column, incubate at room temperature for 2 minute, centrifuge for 2 minute at 12,000 rpm and discard the flow-through. The column is ready and will work well for binding DNA.
2. ***For cycle-pure (PCR reaction):*** Add ***3 volumes*** of ***Buffer GC*** to ***1 volume*** of the PCR reaction and mix completely by vortexing. Briefly spin the tube to collect any drops from the inside wall and tube lid.

   For agarose gel: Excise the DNA fragment from the agarose gel and weigh it in a 1.5 ml microfuge tube. Add **3 volume** of **Buffer GC** to **1 volume** of gel to the 1.5 ml microfuge tube and incubate the mixture at 55-60°C for 8 minute. Mix the tube by tapping the bottom every 2 minute till the gel has melted completely. Cool the tube to room temperature. Add 1 volume of isopropanol.
3. Transfer up to **700µl DNA/Buffer GC** mixture to a spin column with a collection tube. Centrifuge at 13,000g for 1 minute at room temperature. Discard the flow-through and put the column back to the collection tube. Repeat this step to process the remaining solution.
4. Add **500µl** of **Buffer GC** into the DNA Mini Column. Centrifuge at 13,000g for 1 minute at room temperature to wash the DNA Mini Column.
5. Add **650µl DNA Wash Buffer** to the column and centrifuge at 13,000g for 1 minute at room temperature. Discard the flow through and insert the column, with the lid open, back to the collection tube. Repeat step "5".

   Note: *Ensure that ethanol has been added to DNA Wash Buffer as instructed .*
6. Centrifuge the empty DNA column, **with the lid open**, at 13,000g for 2 minute to dry the ethanol residue in the matrix.

   Note: *The residual ethanol will be removed more efficiently with the column lid open during centrifugation.*
7. Place the column into a clean 1.5 ml microfuge tube and add **30-50 µl** pre-warmed (60°C) **Elution Buffer or ddH_2O** to the center of the column. Incubate at room temperature for 1 minute. Centrifuge at 13,000g for 1 minute to elute the DNA. Reload the eluted DNA solution to the column for a second elution.

Note: *Pre-warm elution buffer or ddH_20 at 60°C and incubate the column at 60°C for 5* minute *after adding Elution Buffer or ddH_20 will increase the DNA yield.*

Note: *For fragment larger than 8 kb, incubate the column at 60°C for 15* minute *after adding Elution Buffer or ddH_20 will increase the DNA yield.*

Note: *The first elution normally yields 60-70% of the DNA bound. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA that makes the total yield up to 90%.*

DNA Gel/PCR Purification Vaccum/Spin Protocol

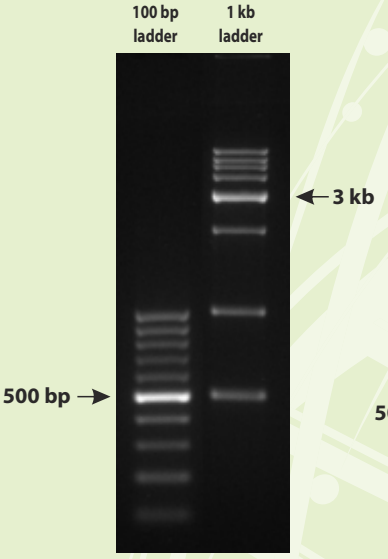
1. Follow the instruction described on step 1 & 2 on page 4. Briefly spin the tube to collect any drops from the inside wall and tube lid.
2. Prepare the vacuum manifold according to manufacturer's instructions. Attach the spin column to the manifold.
3. Load the **Gel or PCR reaction/ Buffer GC solution** to a spin column that is attached to the manifold. Turn on the vacuum to let the solution pass through the column.
4. Wash the column by adding **650µl DNA Wash Buffer.** Repeat **step 4**. Vacuum the column for 1 minute.
5. Put the column, **with the lid open**, in a collection tube, and spin at top speed for 3 minute.

   Note: *The residual ethanol will be removed more efficiently with the column lid open during centrifugation.*
6. Put the column to a clean 1.5 ml microfuge tube and add **30-50µl Elution Buffer or ddH_2O** to the column. Incubate at room temperature for 2 minute. Centrifuge the tube at 13,000g for 1 minute to elute DNA.

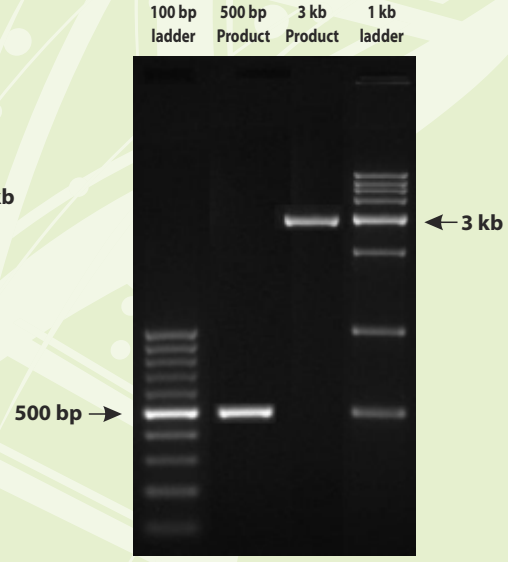
   Note: *Pre-warm Elution Buffer or ddH_20 at 60 °C and incubate the column at 60 °C for 5* minute *after adding elution buffer or ddH_20 will increase the DNA yield.*

   Note: *The first elution normally yields 60-70% of the DNA bound. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA that makes the total yield up to 90%.*

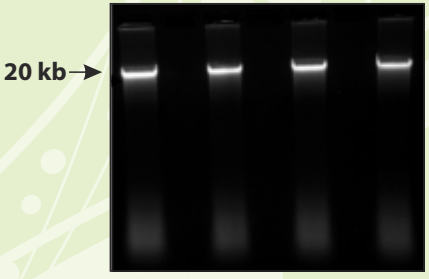
Before Gel purification



After Gel purification

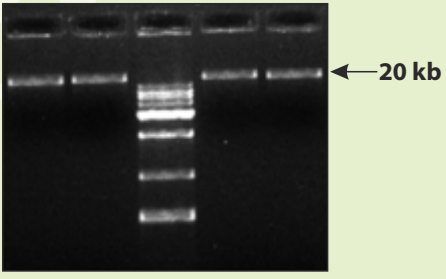


Before Gel purification



Genomic DNA

After Gel purification



Gel Purified genomic DNA

Troubleshooting Guide

Problems	Possible Reasons	Suggestion
Low Yield	1. Not enough Buffer GC	1. Determine the volume of Buffer GC to be used correctly as instructed.
	2. Agarose gel doesn't melt completely	2. Make sure to set the water bath to 55-60°C to allow gel to melt completely. Add more Buffer GC if necessary.
	3. Reused electrophoresis Buffer with increased pH.	3. Use fresh electrophoresis buffer.
	4. Fragment >10 kb	4. Incubate the column (after adding ddH_20 or Elution Buffer) at 60°C for 15 min before elution.
No DNA yield	Forgot to add ethanol to DNA Wash Buffer	Add absolute ethanol to DNA Wash Buffer as instructed before use.
DNA sample floats out of well while loading agarose gel	Ethanol was not completely removed from the column following wash step	After the wash step, centrifuge the empty column with the lid open at top speed for 1-3 min. Repeat once.
Column clogged	Agarose gel doesn't melt completely	Make sure to melt the gel at 55-60°C before loading the sample to DNA column.

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.

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

xcelris genomics
An Abellon Company

Product & Services

XcelGen

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

PrimeX

Oligo Synthesis & Purification Services

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

NXT

NGS Services

• Denovo Genome Sequencing • Whole Genome Resequencing • GBS/RAD Sequencing • Exome Sequencing • Amplicon Sequencing
• Whole Transcriptome Analysis/RNA-Sequencing • Small RNA Sequencing • Metagenomics • Metatranscriptomics
• ChIP Sequencing • Mitochondrial Sequencing • Next Generation Genomic Services on Illumina MiSeq
• Genotyping by Sequencing • Tilling/Ecotilling using NGS • Genome Database development Services

NGS Bioinformatics

• In silico Primer Design • Microarray Analysis • Metagenomics • 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

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

xcelris genomics
An Abellon Company

Xcelris Labs Limited
Old Premchand Nagar Road, Opp. Satyagrah Chhavani, Bodakdev,
Ahmedabad-380015, India. Tel.: +91-79-66197777 / Fax: +91-79-66309341
Website: www.xcelrisgenomics.com
E-mail: bdgenomics@xcelrislabs.com