DNA EXTRACTION

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Intended Use

- The Large Fragment DNA Extraction Kit provides a streamlined method for the rapid purification and concentration of high-quality large-sized DNA from agarose gels. Simply add the specially formulated Agarose Dissolving Buffer (ADB) to the gel slice containing a DNA sample, let dissolve, and then transfer to the supplied DNA Binding Column. There is no need for organic denaturants or chloroform. Instead, the product utilizes unique spin column technology to yield high-quality, purified DNA in just minutes. DNA purified using the Large Fragment DNA Extraction Kit is ideal for PCR, sequencing, endonuclease digestion, ligation, etc. The entire procedure typically takes about 15 minutes.

Specifications

- DNA Purity: High-quality, purified DNA is especially well suited for sequencing and ligation reactions.
- DNA Size Limits: From ~50 bp to >200 kb.
- DNA Recovery: Typically, up to 10 µg total DNA per column can be eluted into ≥ 10 µL of low salt DNA Elution Buffer or water. Recovery of DNA ranges from 70-95%.
- Sample Sources: DNA in excised agarose gel slices.
- Product Detergent Tolerance: ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS

Materials Supplied

- List of component

Component	25 Reactions	100 Reactions
Agarose Dissolving Buffer (ADB)	50 mL	100 mL
DNA Wash Buffer*	6 mL	24 mL
DNA Elution Buffer	1 mL	4 mL
DNA Binding Columns	25 tubes	100 tubes
Collection Tubes	50 tubes	200 plates

* Ethanol must be added prior to use as indicated on the DNA Wash Buffer label.

Storage Instruction

Component	Storage Information
Agarose Dissolving Buffer (ADB)	Room temperature
DNA Wash Buffer	Room temperature
DNA Elution Buffer	Room temperature
DNA Binding Columns	Room temperature
Collection Tubes	Room temperature

Materials Required but Not Supplied

100% ethanol or 95% ethanol

Assay Protocol

- **Reagent Preparation**

Before starting:

 Add 24 mL 100% ethanol (26 mL 95% ethanol) to the 6 mL DNA Wash Buffer concentrate.

 Add 96 mL 100% ethanol (104 mL 95% ethanol) to the 24 mL DNA Wash Buffer concentrate

Assay Procedure

- All centrifugation steps should be performed between

11,000 - 16,000 x g.

1. Excise the DNA fragment from the agarose gel using a razor blade or scalpel and transfer it to a 1.5 mL microcentrifuge tube. Note: The amount of agarose excised from the gel should be as small as possible.
2. Add 3 volumes of ADB to each volume of agarose excised from the gel (e.g. for 100 µL (mg) of agarose gel slice add 300 µL of ADB).
3. Incubate at 37-55°C for 5-10 minutes until the gel slice is completely dissolved. Note: Do not incubate above 60°C. It is important that the gel slice dissolves completely. This can be facilitated by gentle mixing during the incubation.
4. Transfer the melted agarose solutions to a DNA Binding Column in a Collection Tube. 5.

5. Centrifuge for 1 minute. Discard the flow-through. Note: Remove the flow-through by aspiration. Avoid contamination of the Collection Tube rim.
6. Add 200 µL of DNA Wash Buffer to the column and centrifuge for 30 seconds. Discard the flow-through. Repeat the wash step.
7. Add ≥10 µL DNA Elution Buffer or water directly to the column matrix and wait for 1 minute. Place column into a 1.5 mL tube and centrifuge for 30 seconds to elute DNA. Ultra-pure DNA is now ready for use. Note 1: DNA Elution Buffer: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. Note 2: Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. The total yield may be improved by eluting the DNA with 60-70°C DNA Elution Buffer

Resources

- **Troubleshooting**

 Low Recovery

 Ensure Agarose is Fully Dissolved There may be small globules of undissolved agarose in the sample, containing DNA inaccessible for recovery. Undissolved agarose can also inhibit DNA recovery by clogging the column and leeching salts into the eluate.

 Gel Dissolved at Temperatures Above 60 °C If dissolved at a higher temperature, DNA may be denatured affecting recovery. For optimal results, dissolve the gel slice between 37-55 °C.

 Improperly Prepared/Stored DNA Wash Buffer Make sure ethanol has been added to the DNA Wash Buffer concentrate. Cap the bottle tightly to prevent evaporation over time.

 Addition of DNA Elution Buffer Add elution buffer directly to the column matrix, not to the walls of the column. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10kb.

- Incomplete Elution

1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) to the column and incubate for several minutes prior to elution.
2. Sequential elusions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

 Low A260/A230 ratio

 Column tip contaminated When removing the column from the collection tube, be careful that the tip of the column does not come into contact with the flow through. Trace amounts of salt from the flow through can contaminate a sample resulting in a low A 260 /A 230 ratio. Ethanol contamination from the flow through can also interfere with DNA elution. The DNA Binding Columns are designed for complete elution with no buffer retention or carryover.

- Following Clean-up, Multiple Bands Appear in an Agarose Gel
- Acidification of DNA Loading Dye :

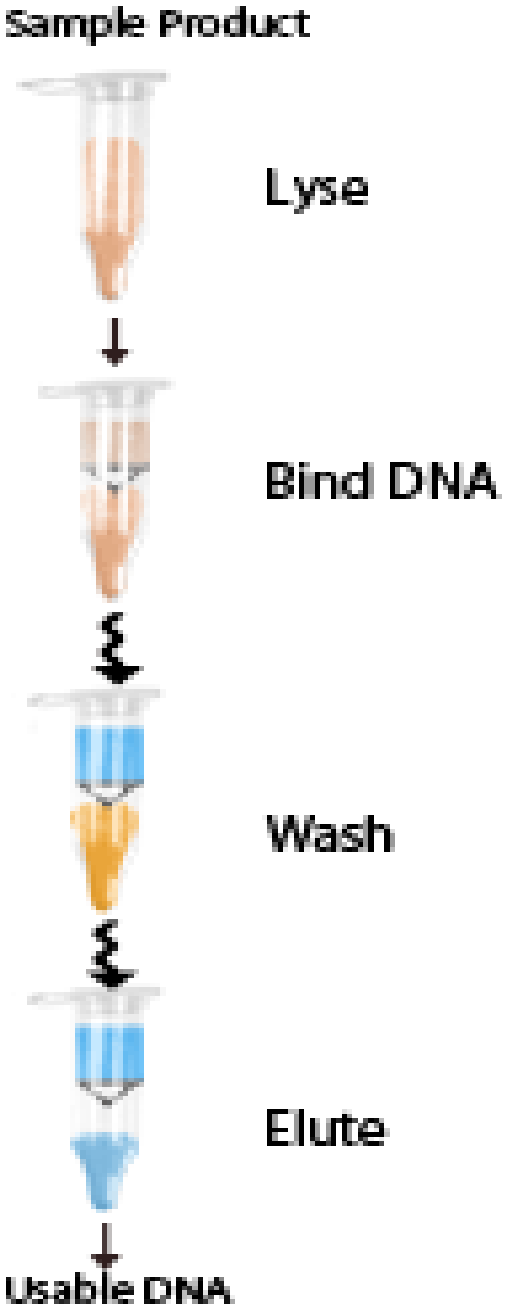
  Most loading dyes do not contain EDTA and will acidify (pH ≤ 4) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended.

Principle

- Genomic DNA Extraction Kit: In the high salt state, DNA purification resin adsorbed DNA specificly; while in a state of low-salt or aqueous solution, DNA was eluted down.


QIAGEN

- The actual process of DNA extraction follows the usual workflow of column-based DNA cleanups. Binding of DNA in high salt conditions to silica membranes, washing with Ethanol based wash buffers and elution in low salt conditions.
- The DNA is extracted in a buffer which contains some Tris, no EDTA and is adjusted to a pH of 8.
- You can choose the elution volume to be **100ul or 200ul.**
- The manual extraction time (excluding Prot K digestion) is about 20min per sample.

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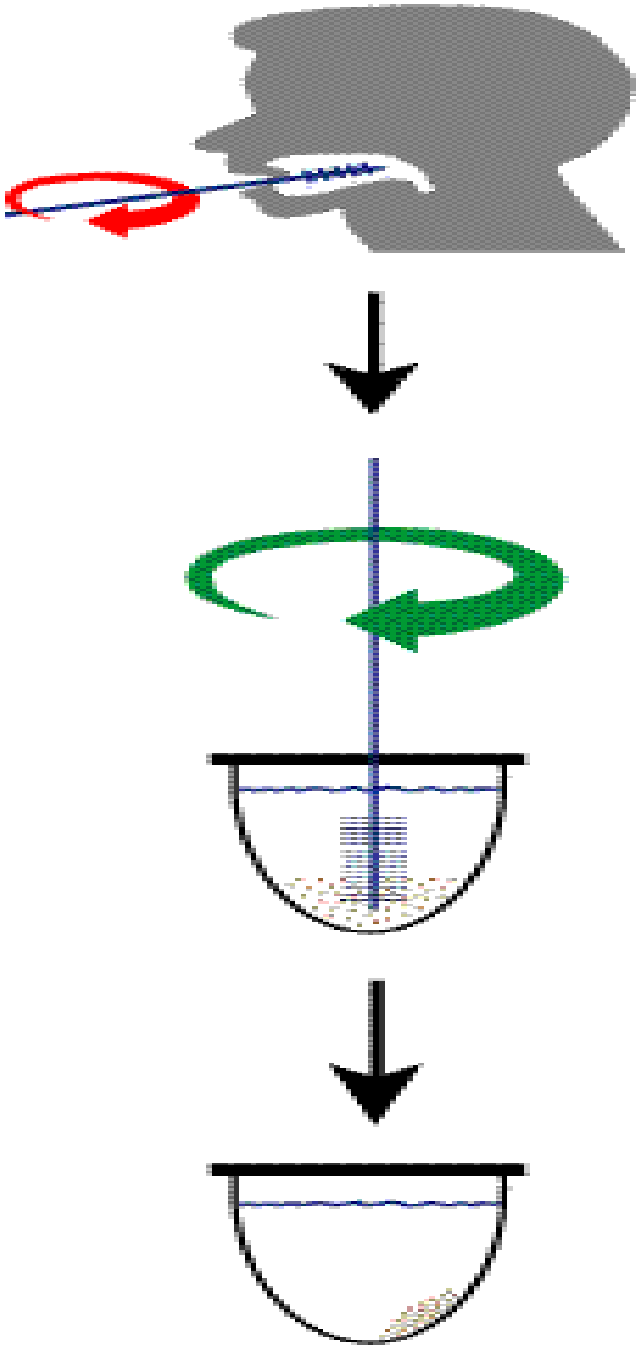

Rotate brush
on the inside
of cheek

Rotate brush
into *MasterAmp*
DNA Extraction Solution

Heat at
65°C for 30 min. and
98°C for 15 min.

Pellet cellular debris.
Supernatant containing
DNA is ready for PCR.

QIAquick Gel Extraction Kit

For gel extraction/cleanup of up to 10 µg DNA (70 bp to 10 kb) from enzymatic reactions

•Up to 95% recovery of ready-to-use DNA
•Fast and convenient procedure
•Cleanup of DNA up to 10 kb in three easy steps
•Gel loading dye for convenient sample analysis

The QIAquick Gel Extraction Kit provides spin columns, buffers, and collection tubes for silica-membrane-based purification of DNA fragments from gels (up to 400 mg slices) or enzymatic reactions. DNA ranging from 70 bp to 10 kb is purified using a simple and fast bind-wash-elute procedure and an elution volume of 30–50 µl. An integrated pH indicator allows easy determination of the optimal pH for DNA binding to the spin column. The procedure can be fully automated on the QIAcube.

In under 4 minutes, from start to finish

DNA Purification Selection Guide

Application	Material	Experimental requirement	Product Choose
Plasmid DNA Extraction	Bacteria	Sample size:1-5ml	High Purity Plasmid Miniprep Kit
		Sample size:1-5ml Safe, Healthy	Eco-Friendly Plasmid Miniprep Kit
		Sample size:100-200ml Simple, Convenient	Plasmid DNA Maxiprep Kit (High Purifity, Silica-membrant Spin Column)
		Sample size:100-200ml	Plasmid DNA Maxiprep Kit (High Purifity, Silica-precipitation)
		Sample size:100-200ml Endotoxin-Free	Plasmid DNA Maxiprep Kit (Endotoxin-Free)
	Yeast	Sample size:1-5ml	Yeast Plasmid DNA Miniprep Kit
DNA Product Purification	Standard Agarose Gel	——	Gel Extraction Kit
		Safe, Healthy	Eco-Friendly Gel Extraction Kit
	PCR Product/ DNA Fragment Solution	——	PCR and DNA Fragment Purification Kit
		Safe, Healthy	**Eco-Friendly PCR and DNA Fragment Purification Kit**
Genomic DNA Purification	Insect/Animal Nematoda	Sample size: less than 10mg tissue	Quick Genomic DNA Extraction Kit
	Blood	Sample size: 0.l-1ml flesh blood/ frozen blood	Blood Genomic DNA Extraction Kit
		Sample size: less than 200ul whole blood	Quick Blood Genomic DNA Extraction Kit
	Tissue/ Cell	Sample size: less than 10mg tissue/ 10^6-10^7 culture cell	Quick Tissue/Culture Cells Genomic DNA Extraction Kit
	Bacteria	Sample size: 0.5-2ml/ less than 10^6-10 bacteria	Quick Bacteria Genomic DNA Extraction Kit
	Yeast	Sample size: less than 1-5x10^7 Yeast	Quick Yeast Genomic DNA Extraction Kit


Thank
you!