Rapid Extraction of High-purity DNA from Tissue Samples

Introduction

Tissue samples contain abundant DNA, which are important sources for molecular biology research, forensic detecting and genetic disease detection. The extraction of DNA from tissue samples involves two most important processes: lysis and purification. The lysis process refers to the process of adding SDS/Tris/EDTA lysate and proteinase K to digest tissue samples after grinding or homogenization (optional), so that DNA can be completely dissociated from chromatin/nucleosome complex to form free DNA. The purification process refers to the removal of impurities such as proteins, cell fragments, carbohydrates, lipids, etc. from the sample digestion solution, in order to obtain highly purified DNA for downstream applications.

At present, there are many purification methods, including phenol chloroform extraction, silica gel column purification, silica magnetic particle purification (magnetic purification), ion exchange layer column, and salt precipitation method. Each of these methods has its own advantages and disadvantages. For example, phenol chloroform extraction can be simply prepared in the laboratory to save funds, but there are adverse factors such as the use of hazardous chemicals and the low purity of DNA obtained.

Magen's HiPure Tissue DNA Kits are designed specifically for biological samples such as animal tissues. This series is based on a silica gel column purification method, which can quickly extract highly purified total DNA from various amounts of animal tissue samples. The entire process does not require contact with phenol chloroform or alcohol precipitation. The DNA band pattern is single and has good integrity, with a fragment length of approximately 20-60kb. DNA has high purity and can be directly used for PCR, enzyme digestion, Southern hybridization, etc.

Experiment Data

1. HiPure Tissue DNA Mini Kit

1.1 The yield and purity of DNA

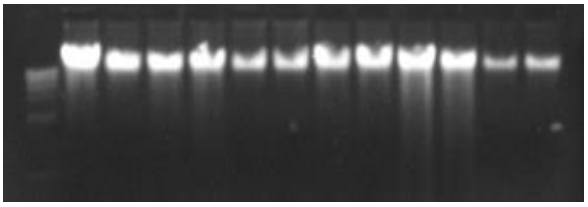
Take different animal tissues (repeat 3 extractions for each type of tissue), extract by HiPure Tissue DNA Kit, and measure their OD values using Nanodrop 2000 after extraction. The results are as follows. From the data, it can be seen that the obtained DNA has an OD260/280 of approximately 1.70-1.90, indicating high DNA purity. From the yield, it can be seen that for three repeated extractions, the yield fluctuates by 10%, which is stable.

Tissue(10mg)	Amount	A260/280	Yield (μg)
Mouse ear	25mg	1.88-1.89	34.4-36.4
Mouse tail	20mg	1.86-1.89	21.9-33.8
Chicken kidney	10mg	1.79-1.81	12.91-14.49
Chicken brain	10mg	1.82-1.86	5.8-6.4
Frog spleen	5mg	1.83-1.90	31.7-35.6
Frog skin	20mg	1.80-1.90	13.96-20.98
Frog lung	10mg	1.81-1.89	37.6-40.7
Frog toe	10mg	1.80-1.81	13.73-16.60
Fish liver	5mg	1.82-1.85	13.5-17.4
Fish heart	10mg	1.80-1.85	7.36-9.36
Fish meat	20mg	1.84-1.90	5.66-7.98

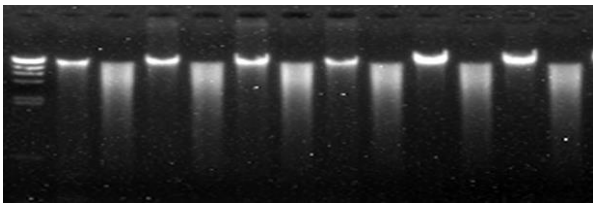
1.2 DNA integrity and enzymatic cleavage effect:

Take the genomic DNA (1μg) purified above and cut it with Hind III enzyme for 16 hours. Add the digestion product and the original genomic DNA on 0.8% agarose gel for electrophoretic analysis. The results are as follows. As shown in the figure, the genomic DNA has a single band and good integrity. After HindIII digestion for 16 hours, the results were good. (M：lambda-HindIII DNA Marker)

M | Liver | Kidney | Heart | Lung | Spleen | Muscle |

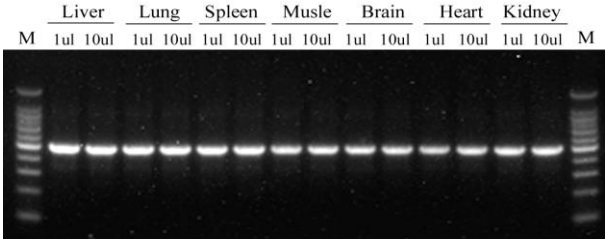


M | Liver | Kidney | Heart | Lung | Spleen | Muscle |



1.3 PCR amplification efficiency

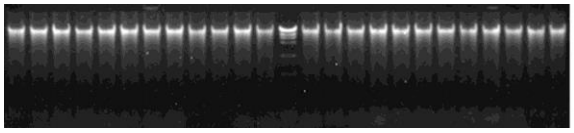
Take 20mg different tissue samples from chickens, extract by the kit and wash out DNA with 400μl Elution Buffer. Take 1μl and 10μl of purified DNA and amplify 1000bp B-actin gene fragment using PCR for 35 cycles. The obtained PCR products were analyzed on 1.5% agarose gel electrophoresis. As shown in the figure, the obtained DNA can be directly used for PCR amplification without inhibiting PCR.



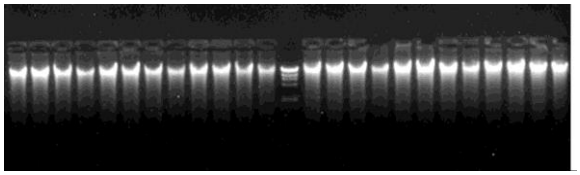
2. Tissue DNA 96 Kit

Take the tails and ears of 24 mice and extract DNA by Tissue DNA 96 Kit. Take 10% DNA and analyze it on 0.8% agarose gel electrophoresis. The results are as follows.

Mouse tail

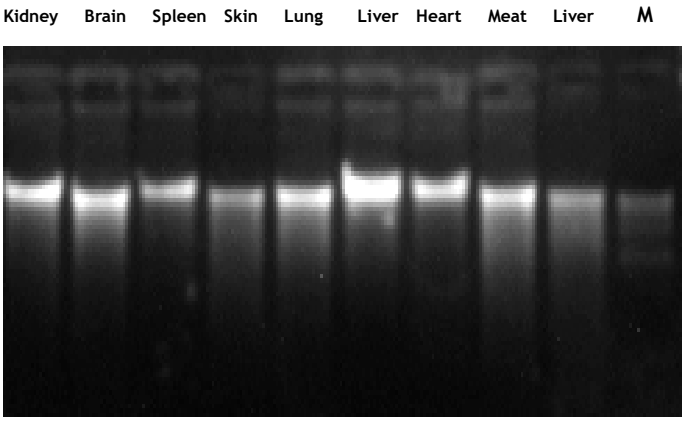


Mouse ear



3. Tissue DNA Midi Kit

Tissue	Sample	A260/280	A260/230	Yield (μg)
Chicken kidney	200mg	1.86	2.2	250
Chicken brain	200mg	1.88	2.19	112
Chicken spleen	50mg	1.8	1.92	366
Chicken skin	200mg	1.81	1.85	208
Chicken lung	100mg	1.86	2.14	370
Chicken liver	100mg	1.78	1.76	274
Chicken heart	200mg	1.87	2.29	150
Chicken meat	200mg	1.79	2.08	70
Fat	200mg	1.79	2.08	99



F&Q

1. Is it necessary to grind the sample with liquid nitrogen when extracting genomic DNA?

Generally, liquid nitrogen grinding is not required. Although liquid nitrogen grinding can break up the sample and accelerate its decomposition and digestion, it is very time-consuming and dangerous. Other methods can also achieve the same effect, such as mechanical homogenization, glass homogenization, or cutting tissue into small fragments as much as possible. These methods are simple and convenient, and the advantages are more obvious when processing multiple samples.

2. How large is the DNA fragment obtained by the kit?

The genomic DNA obtained by this method is generally about 20-60Kb. When extracting high molecular weight genomic DNA (50-150KB), it is recommended to use SolPure Tissue DNA Kit

3. The elution volume and frequency of DNA

Due to the large size of genomic DNA fragments, it is difficult to elute them from the filter membrane of the silica gel column. Research has shown that preheating the elution buffer to 65°C and transferring it to the filter membrane for 3-5 minutes before elution can significantly improve the elution efficiency. When the yield is high, 2-3 washings are recommended. If a higher concentration is required, it is recommended to have a minimum elution volume of 50μl and elute 2-3 times. The DNA concentration obtained by this method is generally between 100-600ng/μl.

4. How to increase DNA yield?

Extracting from 10mg samples with rich genomic DNA content, such as liver, kidney, spleen, lungs can obtain high-yield DNA (20-90μg). Extracting from 10mg samples with low genomic DNA content, such as muscles, skin and bones can generally only obtain 2-10μg. At this time, the sample amount can be increased, and the volume of Buffer ATL, Proteinase K, RNase A, Buffer DL, and ethanol can be proportionally increased. Then, a column can be used to filter and adsorb DNA multiple times. The volume of Buffer GW1 and Buffer GW2 are no need to increase. Research has shown that when treating fish muscles, the sample amount can reach up to 60mg without blocking the column.

5. How to detect the integrity of genomic DNA?

The integrity of genomic DNA can be detected by electrophoresis. When detecting the integrity of genomic DNA, 0.8% agarose gel electrophoresis analysis is generally used, and Lambda DNA/HiBind III DNA Marker is added as the control. The complete genomic DNA

electrophoresis shows a single band, located near the largest band (23kb) in the DNA Marker. If the bands show dispersion during electrophoresis and there is no obvious main band, it indicates DNA degradation. Since PCR only needs to detect a small portion of genes (100-2kb), this degradation does not affect PCR. Research has shown that genomic DNA can still be used for PCR detection even if degraded to below 1kb.

6. Samples suitable to the kit

This kit is suitable for various animal tissue samples, cultured cells, bacteria, saliva, swabs, mouthwash, cotton swabs, paraffin embedded tissues, etc.

7. Why is the OD320 reading relatively high? How to calculate the ratio and yield?

When processing polysaccharide samples, such as liver samples from amphibians and fish, the OD320 readings are generally relatively high, mainly because the samples are rich in polysaccharide impurities.
When calculating the ratio, it should use
(OD260-OD320)/(OD280-OD320),or
(OD260-OD320)/(OD230-OD320), to obtain the correct ratio.
The calculation of DNA concentration should be:
C= (OD260 OD320) x 50 x dilution ratio

8. How would it be more convenient to homogenize tissue samples when using 96 well plates?

When processing 96 samples, if each sample needs to undergo liquid nitrogen grinding or homogenization, it will take a lot of work, which is not recommended. The simplest operation is to use scissors or blades to cut the samples into small pieces (3 to 10 times for each sample), and then transfer to a 96 well plate containing lysis buffer. Process the second sample, wipe off the tissue block with clean absorbent paper, wash the scissors/blades and tweezers with sterile water once, wash with 70% ethanol once, bake on an alcohol lamp for 10 to 30 seconds, and then wash with sterile water once before proceeding with the operation.