SolPure Tissue DNA Kit

Performance Validation Report

High-Molecular-Weight Genomic DNA Extraction from Tissue Samples

1. Introduction

Tissue samples are rich sources of DNA and are widely used in molecular biology, forensic testing and genetic disease research. DNA extraction from tissue samples requires efficient lysis to release DNA from chromatin and nucleoprotein complexes, followed by purification to remove proteins, cellular debris, carbohydrates, lipids and other contaminants.

The SolPure Tissue DNA Kit is based on an improved salting-out method. In this solution-based workflow, proteins and other contaminants are removed by salt precipitation, and genomic DNA is recovered by alcohol precipitation and dissolved in TE buffer. This approach is suitable for scalable tissue and cell DNA extraction and is designed to preserve high-molecular-weight genomic DNA.

To evaluate extraction performance and stability, genomic DNA was purified from multiple animal tissue and cell-related sample types. The purified DNA was analyzed by spectrophotometry, agarose gel electrophoresis, PCR amplification and restriction enzyme digestion.

2. Experimental Design

The following samples were tested. Each sample type was extracted in triplicate unless otherwise stated:

- Mammalian samples: human saliva (100 uL), mouthwash sample (5 mL), and porcine liver tissue (10 mg).
- Avian samples: chicken brain, chicken liver and chicken lung tissues (10 mg each).
- Amphibian samples: frog liver, frog skin and frog lung tissues (10 mg each).
- Fish sample: grass carp liver tissue (10 mg).

For the mouthwash sample, 5 mL of mouthwash solution was collected after rinsing for 1 minute. Cells were collected by centrifugation at 5,000 x g for 5 minutes at room temperature, resuspended in 1 mL Buffer TE, and 100 uL of the suspension was used for DNA extraction.

3. Procedure Summary

- Add 10 mg animal tissue to 300 uL Cell Lysis Buffer and homogenize using a glass homogenizer. For saliva or mouthwash suspension, add 100 uL sample to 200 uL Cell Lysis Buffer and mix by vortexing.
- Add 2 uL Proteinase K (20 mg/mL) and incubate at 60°C for 3 hours.
- Add 2 uL RNase A and incubate at 37°C for 30-60 minutes.
- Place the lysate on ice for 1 minute to bring it back to room temperature.
- Add 100 uL Protein Precipitation Solution and vortex for 30 seconds.
- Centrifuge at 13,000 x g for 3 minutes at room temperature.
- Transfer the supernatant and add 300 uL isopropanol. Mix by inversion 30-50 times.
- Centrifuge at 13,000 x g for 3 minutes at room temperature.
- Carefully discard the supernatant and wash the DNA pellet with 300 uL 70% ethanol.
- Centrifuge at 13,000 x g for 1 minute at room temperature.
- Dry the tube by inverting it on absorbent paper for 5-10 minutes.
- Add 100 uL Elution Buffer, vortex for 10 seconds, and incubate at 65°C for 1 hour.
- Transfer to 4°C overnight to allow complete DNA dissolution.

4. Results

4.1 DNA purity and yield

Purified DNA was diluted 11-fold with Buffer TE and measured using a Beckman DU640 spectrophotometer. OD260, OD280, OD230 and OD320 were recorded, and the average values are summarized below. The results showed A260/A280 values of approximately 1.7-2.0 and A260/A230 values generally within the expected range for purified DNA. Liver tissue produced relatively high DNA yields, while skin and muscle-type samples produced lower yields, consistent with tissue-specific DNA content.

Sample	A260	A280	A230	A260/A280	Yield (ug)
Porcine liver	0.2296	0.1223	0.1145	1.9	25
Frog skin	0.0737	0.0398	0.0469	1.9	8
Frog lung	0.3590	0.1848	0.1509	2.0	39
Chicken lung	0.4895	0.2564	0.1850	1.9	54
Fish liver	0.2257	0.1255	0.2128	1.8	25
Frog liver	0.3604	0.2014	0.0393	1.8	40
Chicken brain	0.0819	0.0449	0.0418	1.9	9
Saliva	0.1080	0.0653	0.0855	1.7	12

Note: OD320 readings were relatively high for some polysaccharide-rich samples such as chicken brain, fish liver and frog liver. OD320 values are not shown in the table.

4.2 Genomic DNA integrity

Two microliters of purified DNA were analyzed on a 0.7% agarose gel at 80 V for 30 minutes, using Lambda DNA/HindIII Marker as the reference. The gel results showed intact genomic DNA with no obvious smearing, and the main DNA fragments were above 23 kb.

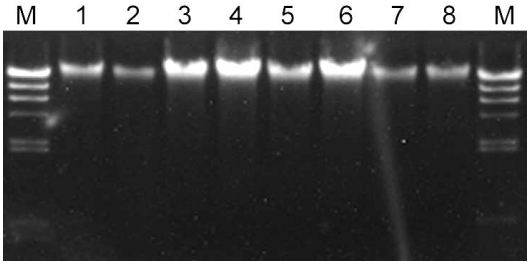

Figure 1. Genomic DNA extracted from different tissue samples. Lanes 1-8: porcine liver, saliva, chicken lung, chicken brain, frog lung, frog skin, frog liver and fish liver. M: Lambda DNA/HindIII Marker.

4.3 Recovery of small DNA fragments

Certain sample types, such as saliva, may contain free DNA or small apoptotic DNA fragments. To evaluate whether the workflow can recover these smaller DNA fragments, 5 uL of purified saliva DNA was analyzed on a 1.0% agarose gel. The result showed a characteristic apoptotic DNA pattern, indicating that small-fragment DNA can be recovered by the SolPure Tissue DNA Kit workflow.

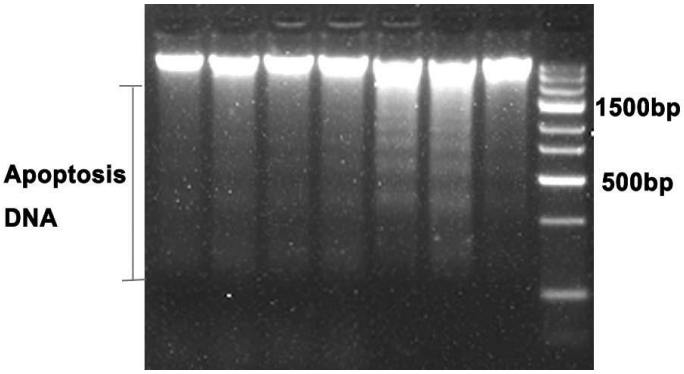

Figure 2. Small-fragment DNA recovery from purified saliva DNA.

4.4 PCR validation

To further evaluate DNA purity and downstream compatibility, DNA from human saliva, chicken liver, chicken brain and chicken lung was serially diluted and used as PCR template for target gene amplification. Clear PCR products were observed after amplification, supporting compatibility with PCR workflows across a wide template input range.

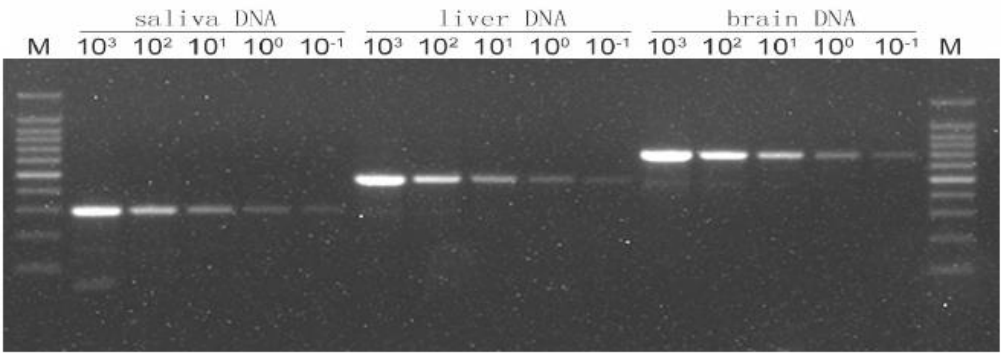

Figure 3. PCR amplification using serially diluted DNA templates. DNA input was diluted from 1 ug to 100 pg as indicated in the original gel image.

4.5 Restriction digestion validation

Purified DNA was also tested by EcoRI restriction digestion. The digestion pattern was normal, indicating that the extracted DNA had sufficient purity for enzymatic downstream applications.

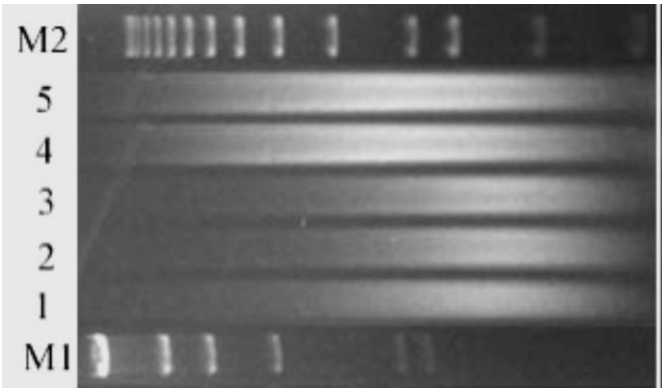

Figure 4. EcoRI restriction digestion of purified genomic DNA.

5. Conclusion

The SolPure Tissue DNA Kit produced high-molecular-weight genomic DNA from multiple animal tissue and cell-related sample types. The purified DNA showed acceptable spectrophotometric purity, intact high-molecular-weight bands by electrophoresis, recovery of small DNA fragments from saliva, and compatibility with PCR and restriction enzyme digestion. These results support the use of the kit for tissue and cell DNA extraction where high molecular weight and downstream enzymatic compatibility are required.

6. Technical Notes and FAQ

1. What factors are most critical for DNA purity?

The salting-out procedure is simple, but it is sensitive to sample input and reagent volume. Sufficient Cell Lysis Buffer and Protein Precipitation Solution are critical. For protein-rich samples, increasing these reagent volumes can improve purity and yield.

2. How should RNase A digestion be handled?

Because isopropanol precipitation does not selectively recover DNA over RNA, RNase digestion time is important. For saliva and muscle-type samples, 15-20 minutes is usually sufficient. For RNA-rich tissues such as liver, kidney and spleen, 1 hour of RNase treatment is recommended.

3. How can yield and consistency be improved for very small samples?

For low-input samples, isopropanol precipitation may recover small DNA amounts less efficiently. Adding glycogen (20 mg/mL) can improve yield and consistency. This is recommended for swabs, dried blood stains or tissues with low DNA content.

4. What DNA fragment size can be obtained?

This method is designed to recover high-molecular-weight genomic DNA. Pulsed-field gel electrophoresis data indicate that the DNA is typically about 30-150 kb.

5. How long is required for DNA dissolution?

High-molecular-weight DNA may dissolve slowly. After adding Elution Buffer or Buffer TE, incubate at 65°C for 1 hour, then place at 4°C overnight for complete dissolution. Low-yield DNA samples may dissolve after 30 minutes at 65°C.

6. How can DNA purity be improved for aquatic animal tissues?

Aquatic animal tissues, especially liver samples from fish or shrimp, may contain polysaccharides and lipids. Increasing reagent volumes and adding an ice incubation step after RNase digestion and after Protein Precipitation Solution addition can improve removal of these contaminants.

6. How can DNA purity be improved for aquatic animal tissues?

Aquatic animal tissues are often rich in polysaccharides and lipids, such as fish oil or DHA-containing components. Liver tissues from fish and shrimp are typical examples, and these substances can be difficult to remove during DNA purification. To improve DNA purity, reagent volumes may be increased when processing these samples. In addition, after RNase digestion, incubate the lysate on ice for 10 minutes, add Protein Precipitation Solution, vortex for 30 seconds, and then incubate on ice for another 20 minutes. Centrifuge at 13,000 × g for 10 minutes at 4°C, collect the supernatant, and continue the extraction according to the kit protocol. This additional precipitation and cooling step helps remove polysaccharides, lipids and other contaminants commonly present in aquatic animal tissues, thereby improving the purity of the recovered DNA.

7. Which sample types are suitable for this kit?

The kit is suitable for animal tissues, cultured cells, bacteria when appropriate pretreatment is used, saliva, swabs, mouthwash samples, cotton swabs and paraffin-embedded tissue samples.

8. Why can OD320 readings be high, and how should ratios be calculated?

For polysaccharide-rich samples, such as amphibian or fish liver tissues, OD320 can be elevated due to sample-derived contaminants. Corrected ratios should be calculated using OD320 correction: (OD260-OD320)/(OD280-OD320) or (OD260-OD320)/(OD230-OD320). DNA concentration should also be calculated as C = (OD260-OD320) x 50 x dilution factor.