Extracting high-purity DNA from stool samples

----D3141 HiPure Stool DNA Kit

Introduction

With the development of molecular biology, stool, a new non-invasive sample, has been widely used in the research of animal molecular genetics, population ecology, behavioral ecology and some intestinal disease diagnosis. Stool samples includes gut microbial DNA, food residue sample DNA, and alimentary tract exfoliated cell DNA. The primary problem encountered when using stool sample for molecular biology research is the low content of exfoliated cells in the digestive tract and a certain degree of degradation of genetic material in stool. Another issue in molecular scatology research based on PCR is the presence of a large number of inhibitors in stool that can affect Taq enzyme activity, leading to downstream detection inactivation. These inhibitors include polysaccharides, plant polysaccharides, bile acids, bile salts, bile pigments, digestive juices, mucus, etc. Therefore, selecting appropriate extraction methods to obtain high-quality DNA is the key to successful downstream detection of stool DNA. At present, the pretreatment methods used in the laboratory, such as phenol/chloroform extraction, cetyltrimethyl bromide (CTAB) lysis, and guanidine isothiocyanate lysis, lack universality in different species, and the success rate of extracting DNA for PCR amplification is also very low. The HiPure Stool DNA Kit provided by Magen Company has opened up a new approach for DNA extraction from stool samples with good universality, high cost-effectiveness, high yield and purification. The reagent kit adopts a unique solution system and inhibitory factor adsorbent (HTR Reagent), which can efficiently remove various impurities in stool samples. The purified DNA can be directly used for PCR, quantitative PCR and other applications.

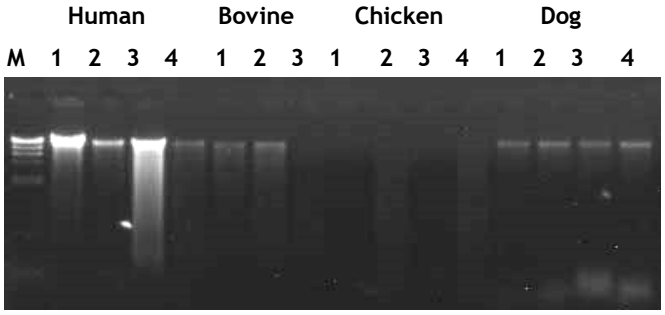
1. The yield and purity of DNA

Extract DNA from healthy human stools (4 samples), bovine stools (3 samples), chicken stools (4 samples), and dog stools (4 samples) using HiPure Stool DNA Kit. Measure OD260, OD280, and OD230 using Nanodrop 2000, results are as listed below. It can be seen that OD260/OD280 is between 1.7~1.9, OD260/OD230 is between 1.8~2 and the OD320 is very low, which indicates high purity of the purified DNA.

Sample	OD260	OD280	OD230	Purity	Yield(μg)
Human1	1.7970	0.9800	1.4150	1.83	8.98
Human 2	0.5650	0.3540	0.9741	1.6	2.83
Human 3	2.9330	1.5860	1.6571	1.85	14.66
Human 4	0.4130	0.2520	0.6164	1.64	2.07
Bovine1	0.3420	0.2320	0.6980	1.47	1.71
Bovine2	0.5920	0.3660	1.1170	1.62	2.96
Bovine3	0.1800	0.0990	0.3913	1.82	0.9
Chicken 1	0.1420	0.0830	0.1919	1.7	0.71
Chicken2	0.7500	0.4010	0.4870	1.87	3.75
Chicken3	0.2470	0.1370	0.2714	1.8	1.24
Chicken4	0.8320	0.4280	0.5073	1.95	4.16
Dog1	1.1810	0.5790	0.6906	2.04	5.9
Dog2	1.1510	0.5610	0.7061	2.05	5.76
Dog3	1.4500	0.7020	0.8101	2.07	7.25
Dog4	1.3400	0.6460	0.7053	2.08	6.7

2. Electrophoretic analysis of genomic DNA

Take 10μl purified DNA and analyze by 0.8% agarose gel electrophoresis analysis. From the figure, it can be seen that relatively complete genomic DNA can be obtained using this kit. The low yield of chicken stool may be due to the fact that the samples have been stored for a long time during sampling.



3. PCR detection

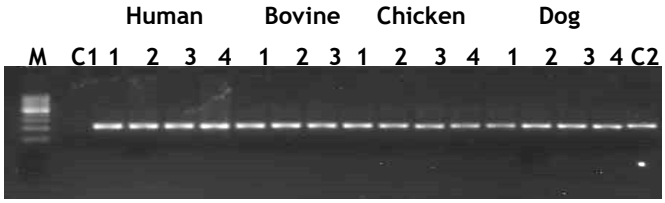
3.1 Bacterial detection

Using 5μl of purified DNA as PCR template, a 200bp fragment was amplified using a universal primer for bacterial 16S (Wu et al., 2002)

U1:5'-TGAAATTGTTGAAAGGGAA-3'

U2:5'- GACTCTTGGTCCGTT-3', and 35 cycles.

Take 3μl of PCR product, analyze by electrophoresis results. The results indicate that the purified DNA using this kit can be directly used for the detection of bacterial DNA.



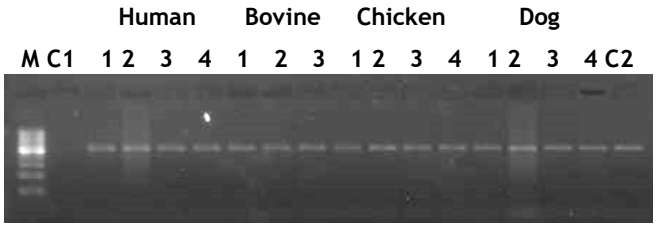
M：100bp DNA Marker

C1: Negative control

C2: Positive control

3.2 Additional human genome (20ng) detection

Take 5μl purified DNA, add 20ng of human genome DNA as the PCR template, and use human primers to amplify 200bp fragments for 35 cycles. Take 3μl PCR product, analyze by electrophoresis. The results showed that in the case of very little human genome DNA (20ng), clear PCR amplification bands could be obtained in the first round of PCR, indicating that the removal of inhibitors was ideal.



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C1: Negative control

C2: Positive control

F & Q

1. Do all the DNA extracted from stool samples come from animals themselves?

Stool samples only contain a small portion of digestive tract exfoliated cells, and the genomic DNA of digestive tract exfoliated cells accounts for approximately 0.01% of the total DNA. Most of the DNA is bacterial DNA and food source DNA.

2. How to preserve stool samples to minimize the degradation of genetic material?

Currently, the best preservation method is to mix stool with dried silica beans or store them in DMSO/EDTA/Tris salt solution, which can effectively slow down DNA degradation.

3. What is the yield of DNA from stool samples?

Answer: Most of the DNA in stool samples comes from bacteria and food. The content of bacteria in stool varies greatly. Therefore, the content of stool DNA varies greatly, generally ranging from 200ng to 20μg.

4. What are the components of PCR inhibitors present in stool samples?

Stool samples contain various microorganisms, of which the polysaccharides, plant polysaccharides, cholic acid, bile salts, bile pigments, digestive juices, mucus, etc. can affect the activity of Taq enzymes and further affect the downstream detection of stool DNA.

5. How to reduce contamination of stool samples?

Due to varying degrees of exposure to the environment, stools are prone to contamination by exogenous genetic materials. Pollution has become an important factor affecting research results in the extraction of stool DNA and PCR amplification. Therefore, it is necessary to conduct blank control experiments in all steps of DNA extraction and PCR amplification. In addition, strict disinfection measures can effectively reduce the chances of contamination during the collection, storage, and extraction process of fecal samples.

6. Are all specific fragments that cannot be amplified by downstream PCR detection affected by inhibitors?

Due to the large amount of PCR inhibitory factors in stool samples, if they are not removed during the purification process, it will affect the amplification of specific fragments. However, the amount of template is also a key issue. Although PCR detection is very sensitive to the template, it also has its normal detection domain, which is generally more than 100ng. Stool samples contain very little nuclear DNA. If the output is not high in the purification process, the specific fragments cannot be amplified because the amount of template addition is outside its normal detection domain.

7. What is the application of this kit?

This kit has good universality and can be used for extracting DNA from animal stools of different feeding habits. This kit has been tested for human stools, pig stools, dog stools, chicken stools, bovine stools, horse stools, etc.

8. Why is the extraction process different when used for bacterial DNA testing and host DNA testing?

Due to the presence of cell walls in bacteria, it is difficult to lyse. It is beneficial to release bacterial DNA by 70°C water bath for 10 minutes. When detecting certain Gram positive bacteria, the water bath temperature needs to be raised to 95°C. Because vast majority of stool samples are bacterial DNA, reducing bacterial DNA contamination is beneficial for improving detection signals if the extracted DNA is used for host DNA testing. Removing the heating process during lysis is beneficial for reducing bacterial DNA contamination.

9. What if the PCR test results are all negative?

Due to the abundant inhibitory factors in stool samples, some inhibitory factors may not be completely removed thus inhibit PCR. It is recommended to add a final concentration of 0.1μg/μl BSA to the PCR reaction solution to improve the sensitivity of PCR.