# Animal tissue (mouse tail, mouse ear) direct PCR kit

**Item number:** PC1400

**Specification**: 100T/500T

**Storage**: -20℃ storage, valid for 1 year. DNA Extraction Solution and Stop Solution are stored at 4℃, valid for 3 months.

**Product contents:**

| Kit Composition | 100T (20μL PCR reaction system) | 500T (20μL PCR reaction system) |
|---|---|---|
| DNA Extraction Solution | 9.6mL | 48mL |
| Enzyme Mix | 0.4mL | 2mL |
| Stop Solution | 10mL | 50mL |
| PCR Master Mix (Green, 2×) | 1mL | 5mL |
| Instructions | 1 copy | 1 serving |

**Product description:**

The animal tissue Direct PCR kit allows rapid extraction and PCR amplification of genomic DNA from mouse tail, mouse ear, or other animal tissues such as buccal swabs, hair shafts, and saliva, etc. For genotypic identification, etc. This kit can quickly digest tissue samples to obtain genomic DNA without mechanical fragmentation, organic extraction, column purification and DNA precipitation, and the process of genomic DNA extraction only takes about 20 minutes.

This kit is suitable for genotyping of mice, high-throughput PCR screening of large-scale biological samples, transgene screening, etc. knockout analysis and sequencing. The PCR Master Mix (Green, 2×) provided in this kit contains 2×Taq DNA Polymerase, 2×PCR Buffer, 2×dNTP and 2× loading buffer. PCR amplification can be performed by adding just the right amount of primers, templates and water, greatly simplifying the PCR operation. And the contamination that may be caused during the PCR operation is reduced. After the PCR is completed, the sample can be directly loaded for electrophoresis, without adding the sample buffer.

When using this kit for PCR detection, only a very small number of samples can be amplified and analyzed. The effect of direct PCR of mouse tail genomic DNA extracted by this kit can be seen in Figure 1.

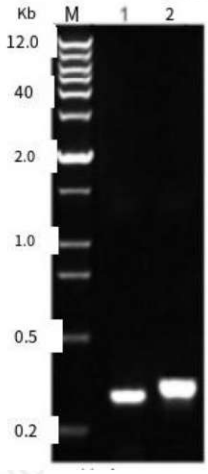


Figure 1. Electrophoretic map of mouse tail genomic DNA extracted by this kit after direct PCR. The primers used for the PCR reaction were designed based on the mouse GAPDH and HSP70 gene sequences, with PCR product sizes of 299bp and 403bp for Lane 1 (GAPDH) and 2 (HSP70), respectively. M, marker.

**Instructions for use:**

**1. Extraction of genomic DNA from mouse tail, animal tissue, hair, or saliva**

1) Preparation of digestive fluid: Preparation of digestive fluid according to the number of samples, the specific preparation method is as follows:

| reagent | 1 sample | 10 samples |
|---|---|---|
| DNA Extraction Solution | 96muL | 960muL |
| Enzyme Mix | 4muL | 40muL |

**Note:** The digestive solution needs to be prepared as is and needs to be used after being thoroughly mixed.

2) Extraction of genomic DNA from different tissue samples.

（a）**Fresh or frozen mouse tails**: Rinse scissors and tweezers with 70% ethanol before the experiment. Cut off 0.2-1cm mouse tail tip and place it in the 100μL digestive solution prepared above, ensuring that the mouse tail is completely immersed in the solution (under normal circumstances, the 1cm mouse tail tip weighing about 15mg can just be immersed in the PCR tube containing 100μL digestive solution, the weight of the mouse tail should not exceed 15mg). For fresh mouse tails, it is recommended to extract genomic DNA within 30 minutes after cutting off the tail, otherwise it should be frozen as soon as possible.

（b）**Hair**: Rinse scissors and tweezers with 70% ethanol before the experiment. To cut off excess hair, just leave the root area of the hair and place it in the 100μL digestive solution prepared above. Only one root hair is needed for each extraction.

（c）**Saliva**: Take 10μL of saliva and add it to the prepared 100μL digestive solution. Swirl or pipette and mix well.

3) Place the sample in a 55ºC water bath or PCR apparatus and incubate for 15 minutes.

4) The sample was placed in a 95ºC water bath or PCR apparatus and incubated for 5 minutes (incomplete digestion of the tissue after incubation is a normal phenomenon, which will not affect the detection effect of the kit).

5) Add 100μL Stop Solution to the above sample and swirl well.

6) Store the above extracted sample at -20ºC or 4ºC or perform PCR testing immediately (for long-term storage of the sample, undigested tissue should be removed or the extract transferred to a new centrifuge tube. In most cases, extracts can be stored for at least 1 month at 4ºC and for at least 1 year at -20ºC).

**2. PCR amplification**

1) Setting of PCR reaction system:

(a) Melt and mix various solutions required for PCR reaction. Place the PCR Master Mix (Green, 2 x) over an ice bath or in an ice box.

(b) Prepare the PCR reaction system on the ice bath by referring to the following table:

| reagent | Final concentration | Volume |
|---|---|---|
| Double steamed water or Milli-Q water | - | 7.4μL |
| Stencils (digestive products) | 2-20ng/mul | 1muL |
| Primer mixture (10μM each) | 0.8muM | 1.6μL |
| PCR Master Mix (Green, 2×) | 1 x | 10muL |
| Total volume | - | 20muL |

(c) Mix lightly with a pipette or lightly Vortex mix and centrifuge at room temperature for a few seconds to allow liquid to accumulate in the bottom of the tube.

(d) Omit this step if the PCR instrument being used has a hot cap. If the PCR apparatus does not have a hot cap, drop a drop of mineral oil into the tube.

(e) Place the prepared PCR reaction system on the PCR apparatus and start the PCR reaction.

2) The setting of PCR reaction parameters can refer to the following examples:

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initiation of degeneration | 94℃ | 3min | 1 |
| Denaturation | 94℃ | 30sec | 30-35 cycles |
| Anneal | 55℃ | 30sec | |
| Stretch | 72℃ | 1kb/min | |
| Final extension | 72℃ | 10min | 1 |
| Save temporarily | 4℃ | - | - |

**3. Agarose gel electrophoresis**

After PCR reaction, agarose gel electrophoresis was performed by directly loading samples.

**Common problems:**

**1. PCR products have little or no target band**

a. Contaminants in tissue extracts inhibit PCR reactions. To detect inhibitors, a PCR reaction can be performed with an equal-volume mixture of DNA Extraction Solution and Stop Solution, together with a DNA control or a known number of templates (100-500 copies).

b. Tissue digestion is inadequate. The digestion time can be appropriately extended at 55ºC or the amount of Enzyme Mix used can be appropriately increased.

c. Enzyme Mix is not completely inactivated. Appropriately extend the incubation time of digestive products at 95ºC.

d. Poor primer design is the most common problem during PCR. Select the appropriate primer design software for primer design, paying attention to issues with GC content, secondary structure, dimer, annealing temperature, length, specificity, etc. In the primer that is added to the enzyme cut site, it is necessary to pay attention to the GC content, secondary structure, dimer, annealing temperature, length, specificity and other aspects of the whole primer after adding the enzyme cut site. In the case of poor effect of the original primer and the positive control primer can work normally, you can consider replacing the primer.

e. The GC content of the fragment to be amplified is high. When the GC content is high, PCR will become relatively difficult. In this case, a GC-rich buffer suitable for the amplification of DNA fragments with high GC content can be used, and the PCR reaction parameters can be adjusted according to the requirements or instructions of the GC-rich buffer.

f. Amplification of long fragments. Although Taq DNA polymerase can amplify DNA fragments up to 8kb, most of the time it is suitable for amplification of fragments below 2 to 3kb. For longer fragments, other DNA polymerases that are better suited for longer fragment amplification are recommended.

g. The PCR reaction is set up at room temperature and tends to result in non-specific conditions. It is recommended to set the PCR reaction on an ice bath.

h. Due to the presence of a certain secondary structure of the primer or the presence of a certain primer dimer, or the primer is too short, the annealing effect is not good. At this time, methods such as Touch down can be used for annealing, usually using a gradual slow cooling from 65ºC to 55ºC or 50ºC to make the annealing more full.

i. The annealing temperature is not good and needs to be optimized. If you have a temperature gradient PCR machine, you can set the temperature gradient for annealing and feel for the best temperature for annealing.

If you do not have a temperature gradient PCR machine, you can fumble for the best annealing temperature through multiple PCR reactions.

j. Insufficient extension time. The extension time can be set as 1 minute per 1kb fragment, and 1.5-2 minutes per 1kb fragment for more difficult fragments to expand.

k. The GC content of the fragment to be amplified is high or the length is long, and the denaturation is not sufficient. The initial denaturation conditions can be adjusted to 95ºC for 1min or even 95ºC for 2-4min.

l. The PCR reaction is performed on different PCR machines to avoid problems with the PCR machine sometimes.

m. The number of cycles is insufficient, and the number of cycles of PCR should be extended appropriately. Usually, the maximum number of cycles need not exceed 40, and the commonly used cycle number range is 25-35.

n. The template content is too low, appropriately increase the amount of template, or use nested PCR(nested PCR) or secondary PCR. Nested PCR is to design a pair of PCR primers inside the original design of PCR primers, and then dilute the first PCR product and then perform a PCR amplification, so on the one hand can play the amplification effect, but also can amplify specific bands from the first PCR product. Secondary PCR is a relatively simple dilution of the first PCR product with the original primer and then a PCR amplification, which can play a role in amplification, but can not be removed unless the specific band.

o. It is often helpful to be careful to set up appropriate positive and negative controls.

**2. Tissue is not fully digested after incubation**

a. Some tissues are difficult to digest completely. Direct PCR kits produced by Solebao do not require complete digestion of tissue, and the DNA extracted from partial digestion is usually sufficient for PCR testing.

**What to watch for:**

1. When using this kit to digest a mouse tail or other tissue sample, be sure that the tissue sample is fully immersed in the digestive fluid. When preparing the digestive Solution, the DNA Extraction Solution and Enzyme Mix should be used as soon as possible after being mixed; leaving it too long may affect the DNA extraction effect.
2. Because PCR reaction is very sensitive and can amplify the target gene fragment more than 10 million times, please pay attention to avoid contamination of trace DNA to be amplified when using Taq enzyme, and try to consider setting a blank control without template to confirm whether there is contamination of DNA to be amplified.
3. The PCR Master Mix(Green,2X) in this kit still has almost the same PCR amplification effect after 15 times of repeated freeze-thaw and before freeze-thaw, but it is still appropriate to avoid too much repeated freeze-thaw, and before use, it must be completely melted and gently mixed upside down before use, and avoid foaming as far as possible.
4. This product is only used for scientific research by professionals, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, and shall not be stored in ordinary homes.
5. For your safety and health, please wear a lab coat and wear disposable gloves when operating.