For research use only Version Number：1.0

Zebra Fish Direct PCR Kit

For performing PCR directly from zebra fish tissue, tail fin or eggs without prior DNA purification

Kit composition (20μl system)		TP-0141T 50T	TP-01411 200T	TP-01412 500T	TP-01413 2000T
Part I	Buffer FP	5 ml	20 ml	50 ml	100 ml × 2
	Foregene Protease	220 μl	880 μl	1.1 ml × 2	8.8 ml
	6× DNA Loading Buffer	1.5 ml	1.5 ml	1.5 ml	1.5 ml × 4
Part II	2× PCR Easy™ Mix	500 μl	1 ml × 2	1.7 ml × 3	1.7 ml × 12
Manual		1	1	1	1

Product introduction

This product uses a unique lysis buffer system to release genomic DNA from zebrafish and other freshwater fish tissues, tail fins or fish eggs samples quickly for PCR reactions. It is particularly suitable for large-scale genetic testing.

The process of releasing genomic DNA from the lysis buffer is completed within 10-30 minutes at 65°C. No other processes such as protein and RNA removal are required, and the released trace DNA can be used as a template for PCR reaction.

D-Taq DNA polymerase is a DNA polymerase specially developed by Foregene for direct PCR reactions. D-Taq DNA polymerase has strong tolerance to a variety of PCR reaction inhibitors, and can efficiently amplify trace amounts of DNA in various complex reaction systems, and the amplification speed can reach 2 Kb/min. It is especially suitable for direct PCR reaction.

Transportation and Storage Conditions

1. Transportation conditions: Low temperature icebox transport throughout the whole process to ensure the kit is in a state of <4°C.
2. Storage conditions: Part I of this kit is stored at 2-8°C; Part II is stored at -20°C.

Features

- Time-consuming and expensive DNA purification are not required.
- The sample demand is small, as little as 1mg fish caudal fin or 10 fish eggs can be tested.
- No special treatments such as grinding and crushing are required, and the operation is simple.
- Optimized PCR system makes PCR have higher specific and stronger tolerance to PCR reaction inhibitors.

Precautions:

- Pay attention to the cleaning of experimental equipment and the experimental operation to avoid cross-contamination between samples.
- Please try to use newly taken zebrafish tissue samples for experiments. If the tissue samples are stored for a long time, avoid repeated freezing and thawing of the samples.
- If the Buffer FP has precipitated out, it can be placed at 37°C until the precipitate disappeared, and the solution should be shaken before use.
- Foregene Protease has a unique formula, please store at 4℃, never at -20℃.
- 2× PCR Easy™ Mix should avoid repeated freezing and thawing, otherwise it will affect the PCR efficiency.
- If the ambient temperature is too high, 2× PCR Easy™ Mix may become turbid. You can put it on ice for 1-2 minutes. When the solution is clear, mix it upside down and mix 3-5 times before use.
- During electrophoresis detection, do not use Loading Buffer containing SDS, otherwise a large group of tailing bright bands will appear in the swimming lane during electrophoresis. It will affect the experimental results.

Prevent cross-contamination between samples

In order to avoid cross-contamination between samples, after each sampling, the cutting edge of the sampling equipment or the part directly in contact with the sample needs to be immersed in 2% sodium hypochlorite solution, washed repeatedly for several times and wiped off the residual liquid with clean paper towel and then it can be used again. Use it again. In order to facilitate the test, multiple sampling equipment can also be prepared and cleaned uniformly after use to ensure that each individual sample uses non-polluting sampling equipment.

Operation guide

A：Sample DNA release

1. Add 100 μl Buffer FP and 4 μl Foregene Protease to the centrifuge tube, vortex gently to mix.
   Note: The mixture of Buffer FP and Foregene Protease should not be stored for a long

time ,please use it as soon as possible .

2. Cut 5-10mg zebrafish tissue (or 1-3mg zebrafish tail fin or take 10 zebrafish eggs) and put it into the above centrifuge tube, vortex gently to mix.

   Note: Cut the tissue or tail fin as small as possible so that the enzymatic hydrolysis reaction can proceed more smoothly.

3. Incubate at 65°C for 10-30 min, then treat at 95°C for 5 min.

   Note: Incubation at 65°C for 10 minutes can meet the requirement of majority PCR . If the amount of DNA required is large or the sample is difficult to digest, the time can be extended to 30 minutes. The tissue mass does not need to be digested completely, and the remaining part can be removed in the subsequent centrifugation step.

4. Centrifuge at 12,000 rpm (~13,400×g) for 5 min.

5. Transfer the supernatant to a new centrifuge tube and place it at 4°C or -20°C for later use or use for PCR amplification directly.

B: PCR reaction identification

1. Add the corresponding 2× PCR Easy™ Mix and specific primers to the PCR tube for later use.

2. Take an appropriate amount of the lysis mixture processed in step A and add it to the PCR system prepared above (see Table 1 for system preparation).

   Note: When used for subsequent PCR detection, the template amount should account for 1-10% of the PCR system, and should not exceed 20%. For example, in a 50 μl PCR system, add 0.5-5 μl lysis buffer, but not more than 10 μl.

3. Carry out the PCR reaction according to the optimized PCR conditions (annealing temperature, etc.) (see the following table 2 for the reaction conditions).

   Note: Try to use the optimized conditions for PCR reaction to get better results.

4. Agarose gel electrophoresis test results.

   Note: It is recommended to use the 6× DNA Loading Buffer delivered with the kit. Do not use the Loading Buffer containing SDS for electrophoresis.

Table 1: PCR reaction system preparation

PCR system additions	Consumption		Final concentration
2× PCR Easy™ Mix	10 μl	25 μl	1×
Forward Primer(10 μM)	0.5 μl	1 μl	0.2-0.25 μM 1*
Reverse Primer(10 μM)	0.5 μl	1 μl	0.2-0.25 μM 1*
Lysis Mix (DNA template) 2*	X μl	X μl	
ddH_2O (Sterilized distilled water)	(9-X) μl	(23-X) μl	
Total Volume	20 μl	50 μl	

1*: Usually, the final concentration of the primer is 0.2-0.25 μM to get better results. When the reaction performance is poor, the primer concentration can be adjusted within the range of 0.1-0.5 μM.

2*: The lysate is used as a PCR template, and the optimal amount of addition is between 1-10% in the PCR system. In actual operation, the template addition amount can be explored to find the optimal template amount.

Note: The preparation of this system is for reference only. The PCR system can be adjusted according to the needs and add a proper ratio of lysis mixture. Prepare the PCR reaction system, put it on a vortex machine, vortex and mix, then centrifuge briefly to collect the reaction solution at the bottom of the tube.

Table 2: Example of reaction conditions

Steps	Temperature	Time	Number of cycles	Content
1	94℃	3 min	1	Predenaturation
2	94℃	10 sec	30-40	Denaturation
3	55-65℃	20 sec		Primer annealing
4	72℃	x min (2kb/min) 2*		Extension
5	72℃	5min	1	Final extension

1*: 2× PCR Easy™ Mix has a good amplification ability for templates with high GC content. During the PCR, we recommend that the annealing temperature of all primers is 2°C higher than the TM value.

2*: For DNA fragments within 1kb, the extension time is recommended to 30 sec.

Note: The PCR conditions in table 2 are for reference only.The PCR conditions varied depending on the structural of the template and primers, etc. In the specific operation, it is necessary to design the optimal reaction conditions, including annealing temperature, extension time, etc. according to the specific conditions of the template type, the size of the target fragment, the base sequence of the amplified fragment and the GC content and length of the primer.