

Catalog No.: D911-Soln

Product Name: **Conquest™ PCR Genotyping Solution Set**

Size: 500 rxns

Description: The **Conquest™ PCR Genotyping Kit** contains the total genomic DNA extraction reagents for PCR and is customized with the **Conquest™ 2X PCR Master Mix**. Total DNA samples can be extracted quickly from many kinds of biological materials including mouse tail, any type of animal tissues, cell cultures, bacteria cultures, blood and other body fluid, and environmental microorganisms. The extracted DNA can be used directly for PCR with the **Conquest™ 2X PCR Master Mix**. The **Conquest™ 2X PCR Master Mixes** are specifically developed for genotyping, genomic cloning, and other various PCR applications, which cover regular PCR and difficult PCR including arbitrary primers, high GC templates, inhibitory raw samples, and other difficult PCR scenarios.

The PCR product can be directly loaded to the wells of a gel for electrophoresis for viewing the PCR results, as there is no need to add DNA loading buffer.

Note: Setup your startup PCR experiment with all four **Master Mixes** in the **Conquest™ Genotyping Starter Kit**, (Cat. No. D911). Once the best PCR result is obtained, one of the four individual **Conquest™ Genotyping PCR Kits** can be chosen for future experiments to consistently get good PCR results.

- Applications:**
- Genotyping
 - Genomic cloning
 - High GC PCR
 - Large fragment PCR
 - Low template PCR
 - Hardship PCR

Kit Contents:

Components	Sizes
Extraction Solution A	45 ml
Extraction Solution B	5 ml

Storage: The whole kit can be stored at 4°C for up to a month. For long-term storage, the **Conquest™ 2X PCR Master Mixes** should be stored at -20°C; do not freeze-and-thaw more than three times.

Note: This Product Is For Research Use Only.

Reorder Information:

Product	Size	Catalog No.
Conquest™ Genotyping Optimizing Kit	4 x 100 rxn	D911
Conquest™ Genotyping PCR Mix 1	5000µl	D911-Mix1
Conquest™ Genotyping PCR Mix 2	5000µl	D911- Mix2
Conquest™ Genotyping PCR Mix 3	5000µl	D911- Mix3
Conquest™ Genotyping PCR Mix 4	5000µl	D911- Mix4

General Protocol

I. DNA sample Preparation:

1. Place the sample into a PCR tube:
 - **For mouse tail tip:** 0.1 - 0.3 cm in length.
 - **Animal Tissues:** 1-2 mg is sufficient.
 - **Cultured cells:** 10 μ l of cell culture.
 - **Other samples:** similar amount or volume as above.
2. Pipette 90 μ l of **Extraction Solution A** into the PCR tube.
3. Place the tube into a PCR machine and heat the tube at 95°C for 15 minutes. Generally, people like to set the PCR machine in a two step PCR mode: 95°C for 15 minutes and 4°C for the time length convenient for you, from a few minutes to a few hours, or even overnight.
4. After step 3 (above), take out the tube and add 10 μ l of **Extraction Solution B** into the tube.
5. Mix well by vortexing or by vigorously shaking the tube a few times.
6. The sample is now ready for PCR. You can store the sample at or below -20°C for future use.

Note:

- The sample may not be digested completely. This is normal and will not interfere with the PCR result. Use the supernatant only for your PCR reaction and avoid any undigested tissues.
- The volume of **Extraction Solution A** and **B** can be proportionally scaled down or up, e.g., use 180 μ l of **Extraction Solution A** and 20 μ l of **Extraction Solution B** for large samples.

II. PCR Amplification:

1. Add the following reagents to a PCR tube or plate, and mix:

<u>2X PCR Master Mix:</u>	<u>10 μl</u>
<u>Primers:</u>	<u>y μl</u>
<u>Sample:</u>	<u>1 μl</u>
<u>Water:</u>	<u>x μl</u>
Total volume:	20 μl

Note:

- Adjust your PCR volume according to your specific case, such as, using 25 μ l or 50 μ l PCR as the final reaction volume; however, for the **2X PCR Master Mix**, always use half of the final volume of your PCR reaction.
 - When multiple samples are processed with the same primers, the **2X PCR Master Mix**, water and primers can be premixed and aliquoted.
2. Perform the thermal cycling. The following table is a typical example of PCR. Use your own favorite PCR profile; or, a touchdown PCR cycle profile can be used for many PCR reactions.

Step	Temperature	Time	Cycles
Initial Denature	95°C	1-3 min	1
Denature	95°C	0.5-1 min	30-35
Annealing	50-65°C	0.5-1 min	
Extension	72°C	1 min/kb	
Final Extension	72°C	5-7 min	1
Hold	4°C	∞	

3. The amplified products can be directly loaded onto an agarose gel for checking PCR results.