

**Catalog No.:** D911

**Product Name:** Conquest™ Genotyping PCR Optimizing Kit

**Size:** 400 rxns

**Description:** The Conquest™ Genotyping PCR Optimizing Kit contains the total genomic DNA Extraction Solutions and four unique Conquest™ 2X PCR Master Mixes for PCR. Total DNA samples can be prepared quickly from many kinds of biological materials including mouse tail any other type of animal and plant tissues, cell cultures, bacteria cultures, blood and other body fluids, and environmental microorganisms. The extracted DNA can be used directly for PCR with the Conquest™ 2X PCR Master Mixes. The four 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. To set up the startup PCR experiment, all four Master Mixes within the kit should be used, and the best PCR result will be easily obtained. Afterwards, one of the four individual Conquest™ Genotyping Kits can be chosen for future experiments to consistently get good PCR results. 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.

**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
Conquest™ 2X PCR Master Mix 1	1000µl
Conquest™ 2X PCR Master Mix 2	1000µl
Conquest™ 2X PCR Master Mix 3	1000µl
Conquest™ 2X PCR Master Mix 4	1000µl

**Storage:** The whole kit can be stored at 4°C for up to three months. 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™ PCR Genotyping Solution Set	One set	D911-Soln
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:**

- 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  $\mu$ l of cell culture.
  - Other samples:** similar amount or volume as above.
- Pipette 90  $\mu$ l of **Extraction Solution A** into the PCR tube.
- Place the tube into a PCR machine and heat the tube at 95°C for 15 minutes. (**Tech Tip:** People like to set up 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 overnight.)
- After step 3 (above), take out the tube and add 10  $\mu$ l of **Extraction Solution B** into the tube.
- Mix well by vortexing or by vigorously shaking the tube a few times.
- The sample is now ready for PCR. You can store the sample at or below -20°C for future use.

**Optional:** the sample can be centrifuged briefly and use the supernatant for PCR.

**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.

### **II. PCR Amplification:**

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

2X PCR Master Mix:	10 $\mu$ l
Primers:	y $\mu$ l
Sample:	1 $\mu$ l
Water:	x $\mu$ l
<b>Total volume:</b>	<b>20 <math>\mu</math>l</b>

**Note:**

- Adjust your PCR volume according to your specific case, such as, using 25  $\mu$ l or 50  $\mu$ l PCR as the final reaction volume; however, for the **2X PCR Master Mixes**, 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.
- 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	$\infty$	

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