NGS Multiplex PCR Master MixIII, 2X

NGS Multiplex PCR Master MixIII is a PCR Master Mix designed for unbiased targeted amplification in next-generation sequencing (NGS). By optimizing enzymes and buffers, this product significantly improves the success rate and consistency of amplification efficiency for GC/AT-rich amplicons. Primers with different GC contents can use a uniform annealing temperature, enabling unbiased PCR amplification, up to 10,000X multiplex PCR, and can tolerate PCR inhibitors such as SDS, guanidine salts, and heparin. The product contains green, blue, and yellow dye reagents, allowing direct electrophoresis after PCR reaction without the need for additional dye addition. It has a fidelity more than 100 times higher than Taq and can be used for targeted NGS sequencing of viral, microbial, tumor/heritable DNA.

Cat. No.	Contents	Storage Conditions
NM2001	NGS Multiplex PCR Master MixIII, 2X, 40 rxns	Store unopened at −15°C to −25°C until the expiration date on the label. After opening, the master mix may be stored at −15°C to −25°C until the expiration date on the label, or at 4°C for up to 30 days.
	• NGS Multiplex PCR Master MixIII, 2X (1 × 1 mL)	
NM2002	NGS Multiplex PCR Master MixIII, 2X, 400 rxns	
	• NGS Multiplex PCR Master MixIII, 2X (1 × 10 mL)	
NM2003	NGS Multiplex PCR Master MixIII, 2X, 2000 rxns	
	• NGS Multiplex PCR Master MixIII, 2X (5 × 10 mL)	

Protocol

Note: Before setting up the PCR reactions, prepare a Primer Mix with 0.5 μM of each primer.

Prepare the PCR Reaction Mix

1. Allow all reagents to thaw completely. Mix gently by inverting the tube. Spin briefly. Put all reagents on ice.
2. Using a 96-well optical reaction plate, add the following to one well per sample:

Component	Volume or Mass	Final Concentration
NGS Multiplex PCR Master MixIII, 2X	25 μL	1X
Primer Mix (0.5 μM each)	5 μL	50 nM each primer[1]
Template DNA	0.1–0.2 μg	2–4 ng/μL
Nuclease-free water	Adjust to 50 μL	n/a

[1] The final concentration of each primer in a typical PCR is between 0.05-0.4 μM. In most cases, a final concentration of 0.15 μM gives satisfactory results. Increasing the primer concentration up to 0.4 μM may increase the yield.

3. Seal the reaction plate with Clear Adhesive Film.

Amplify DNA for Analysis

Choose an amplification protocol based on your analysis method

Amplify for Multiplex PCR

1. Configure the run method as outlined in your instrument's user manual. Use the following parameters:

Step	Time	Temperature (°C)
Hold	2 min	95
35 Cycles	20 sec	95
	90 sec	60 [1]
	90 sec	72
Hold	5 min	72
Hold	∞	4

[1] Primers with different GC contents can use a uniform annealing temperature, and a lower annealing temperature can achieve high

sensitivity.

2. Mix well and briefly spin the reaction plate.
3. Load the reaction plate into the instrument, and start the run. See your instrument's user manual for detailed instructions on how to load and run the plate.
4. Analyze the data according to the instructions in the user manual for your gel electrophoresis instrument.