

User Guide



YOUSEQ

Next Generation Sequencing Multiplex DNA MasterMix

For high multiplex library preparation protocols

Version 1.3

Regulatory status

This product has been developed for Research Use Only and is not intended for human diagnostic use. It should not be used for diagnosis of human disease unless specifically approved by the regulatory authorities in the country of use.

Product description

Introduction

YouSeq NGS MasterMix is a convenient high fidelity 2x mix designed for PCR based NGS library preparation protocols with large numbers of primer pairs (50-5000) where greater sequence accuracy is required.

YouSeq NGS MasterMix contains an engineered and highly processive Polymerase, developed for fast and versatile high-fidelity PCR. The enzyme is derived from Pfu DNA polymerase for its 3'-5' exonuclease (proofreading) activity. Several proprietary mutations significantly improve DNA binding and processivity, resulting in faster extension (10-30s/kb), higher yields and the ability to amplify longer and more difficult targets.

The high accuracy and enhanced 3'-5' exonuclease activity of our Polymerase result in fidelity that is approximately 100 times higher than Taq DNA polymerase. The enzyme is ideally suited to applications where greater accuracy is required, such as sequencing. PCR products generated with this enzyme are blunt ended.

YouSeq NGS MasterMix uses an advanced buffer system including dNTPs, Mg and enhancers, enabling high fidelity PCR of a wide range of targets and fragment sizes with minimal or no optimisation required.

YouSeq Recommendations

Designing Primers

We recommend using our expertly designed primers, however, the mastermix is compatible with home brew designs.

Primers Tm

Predicted melting temperature of approx. 60°C

Primer Concentration

Around 150 pM but optimization required for any panel, dependent on number of primer pairs.

Denaturation

Should be 95°C, however, presence of high GC template, increase it to 98-100°C to improve results

Annealing

Recommend performing temperature gradient to determine optimal annealing temperature. We'd recommend 62°C annealing temperature then increase in 2°C increment to remove non-specific product

Template Concentration

We recommend using 5-100 ng genomic DNA

Reaction Set-Up

Component	Volume
YouSeq NGS 2x MasterMix	12.5 µL
Primer Pool (e.g. 5 µM)	2 µL
Template DNA	5-100ng
DNase/RNase free water	x µL
FINAL VOLUME	25 µL

Cycling Conditions

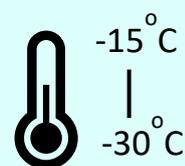
	Temp	Time
	95°C	1-3 mins
20-30* cycles	95°C	10-30 secs
	60°C-70°C	1-8 mins**

*Choose lower number of cycles for larger multiplexes

**Choose longer annealing time for larger multiplexes

Storage Conditions

On arrival the kit should be stored between -30°C and -15°C.



- Avoid prolonged light exposure
- If stored correctly, retain full activity for at least 12 months

Freeze Thaws (F.T) ❄️

- The kit can go through **10** freeze/thaw cycles with no loss of activity.
- If plan >10 F/T, aliquot the MasterMix into smaller volumes