NIPPON GENE

2×M13 Primer Mix

I. Product Description

2x M13 Primer Mix contains M13 forward and reverse primers. The primers can be used in colony PCR for screening for inserts cloned into the M13 vectors and pUC plasmids.

II. Storage

−20°C

It is recommended to store frozen in aliquots to avoid repeated freeze-thaws.

III. Product content

Reagent	Volume	
2x M13 Primer Mix	1.2 ml x 2	for 96 reactions in 50 µl volume

Compositions:

0.8 pmol/µl M13 forward primer (GTTTTCCCAGTCACGACGTT)

0.8 pmol/µl M13 reverse primer (GGAAACAGCTATGACCATGA)

0.1 mmol/l Tris-HCl (pH 7.5)

IV. Remarks

Annealing position of M13 primers in pUC18 DNA:

M13 forward primer →

5' AAGTTGGGTA ACGCCAGGGTTTTCCCAGTCACGACGTTGT AAAACGACGG

3' TTCAACCCAT TGCGGTCCCA AAAGGGTCAG TGCTGCAACA TTTTGCTGCG

5' CCAGTGCCAA GCTTGCATGC CTGCAGGTCG ACTCTAGAGG ATCCCCGGGT

3' GGTCACGGTT CGAACGTACG GACGTCCAGC TGAGATCTCC TAGGGGCCCA

【Multi cloning sites】

5' ACCGAGCTCG AATTCGTAAT CATGGTCATA GCTGTTTCCT GTGTGAAATT

3' TGGCTCGAGC TTAAGCATTAGTACCAGTAT CGACAAAGGA CACACTTTAA

← M13 reverse primer

Sizes of PCR products amplified from the following plasmids:

・ pUC18 or pUC19	121 bp	・ pGEM®-T easy	250 bp
・ pBluescript® Ⅱ	247 bp	・ λ ZAP® Ⅱ	244 bp
・ pT7Blue	172 bp	・ pT7Blue2	388 bp

V. Protocol (example)

Gene RED PCR Mix Plus (Nippon Gene, Japan), which is a 2x premixed PCR reagent containing *Taq* DNA Polymerase, dNTPs, Mg^{2+} and gel-loading buffer, etc., is required in addition.

1. Prepare enough volume of reaction mixture in a 1.5ml microcentrifuge tube for the number of colonies analyzed. For each 50 µl reaction; mix 25 µl of Gene RED PCR Mix Plus with 25 µl of 2x M13 Primer Mix.

Gene RED PCR Mix Plus (2x)	25 µl
2x M13 Primer Mix	25 µl
Total Mix / reaction	**50 µl**

2. Transfer 50µl of the mixture into each PCR tube for equal number of colonies to be tested.

3. Pick a small amount of a colony with a pipette-tip or a toothpick, dip into 50 µl of the mixture and resuspend.

4. Perform PCR:

94°C	3 min.
↓	
94°C	20 sec.
55°C	20 sec.
72°C	10 sec./kbp*

× 25 cycles

*10 sec for less than 1 kbp.

5. Load 5 µl of the reaction mix after PCR in a well of agarose gel for electrophoresis.

For research purposes only. Not for use in diagnostic procedures for clinical purposes.

NIPPON GENE CO., LTD. 1-8-7 Toiya-machi Toyama 930-0834 Japan TEL 076-451-6548
http://www.nippongene.com