

# Forever Size Markers

Forever Multi Ladder Premix Personalizer

Forever 100 bp Ladder Premix Personalizer

User Manual

Catalog No. M0150, M0100

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# 1 Notices to Customers

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## 1.1 Product Warranty and Liability

Seegene guarantees the performance of all products as described when they are used according to the instructions in this manual. Any problem that occurs for reasons other than misuse should be reported to Seegene immediately. This warranty limits our liability for product replacement.

## 1.2 Safety Warning and Precautions

This product is limited to research use only. It is not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

## 1.3 Notice to Customers

The PCR process is covered by patents owned by Hoffman-La Roche Inc. No license or immunity under any other patent is either expressed or implied by the sale of any Seegene product.

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# 2 Introduction

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## 2.1 Introduction

Our unique permanent ladder system (patent pending) is clearly different from existing commercial 50 bp/ 100 bp DNA ladders. Our system supplies templates (plasmids) that encode 12 genes whose amplification with the supplied primers generate fragments that range in size from 100 bp to 1550 bp. When the plasmids are used together, they form a ladder with an increment of 50 bp/ 100 bp. Since the 12 genes are cloned into plasmids, this system has the unique feature of endless usage. Another key feature is that the user can select specific size of the templates to generate a personalized size marker ladder. In addition, the 12 genes can be used as control probes for other applications.

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## 2.2 Key Features

- **Lifetime usage:**

Sufficient template and primers are supplied in order that a typical laboratory will have adequate size markers for years. In addition, the plasmids (Amp<sup>R</sup>) can be transformed to yield an unlimited supply of templates if necessary. Moreover, the primer sequences are supplied such that the original primers can be replaced by the user.

- **PCR-Ready Format:**

12 different plasmids are supplied in ready-to-PCR format conditions. The PCR conditions and the concentration of each template (plasmid) are designed to amplify all 24 fragments in the same reaction to generate a ladder of bands of approximately equal intensity. It is also possible to generate a ladder with higher intensity to indicate the orientation. 2~3 sets of PCR products amplified from templates of a specific size can be combined to increase the band intensity. This method has the advantage of quickly generating a ladder without the need to quantify each PCR product.

- **Create your own personalized ladder:**

Create a personalized ladder by simply choosing the desired plasmid template(s) with a correct size inserts and amplifying them with the provided primers.

- **Using differently sized fragments as control probes:**

The 12 different plasmids bear different genes, namely, those encoding p53, GAPDH, *c-myc*, *c-fos*, TNF, NOS, CD4, IFN, GM-CSF, IL-1 $\alpha$ , SCI/MIP-1a, and Beta 2-microglobulin. These genes can be used as control probes in other experiments, such as hybridization-related experiments. In addition, this ladder can be radiolabeled by using T4 DNA polymerase.

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# 3 Components

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## 3.1 List of Components

Cat. No. M0100 (Forever 100 bp Ladder Premix Personalizer)

- **12 DNA ladder plasmids**, each 100 ul (10 ng/ul)
- **Primer 1 for 100 bp**, 500 ul (10 µM)  
5'-GTCTACCAGGCATTCGCTTCAT-3'
- **Primer 2 for 100 bp**, 500 ul (10 µM)  
5'-CTGTGAATGCTGCGACTACGAT-3'
- **6X Loading dye**, 1 ml
- **2X SeeAmp™ ACP™ Master Mix**, 1 ml
- **User Manual**

Cat. No. M0150 (Forever Multi Ladder Premix Personalizer)  
M0100 plus

- **Primer 3 for 50 bp**, 250 ul (10 µM)  
5'-TAAAACGACGGCCAGTGAATTC-3'
- **Primer 4 for 50 bp**, 250 ul (10 µM)  
5'-CTCTAGAGGATCCCCGGGTACC-3'

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### **3.2 Storage Conditions**

Store all reagents below  $-20^{\circ}\text{C}$  except the SeeAmp™ ACP™ Master Mix. In case of Master Mix,  $4^{\circ}\text{C}$  for short-term storage and  $-20^{\circ}\text{C}$  for long-term storage are recommended. Avoid repetitive thawing as it may decrease the activity of the Master Mix. The Master Mix is proven to be stable for at least 3 months at  $4^{\circ}\text{C}$ .

### **3.3 Reagents and Equipments to Be Supplied by User**

Thermal cycler  
Micro-centrifuge

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# 4 Protocol

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## 4.1 Protocol for Forever Ladder Premix Personalizer

The Forever Ladder Premix Personalizer consists of 12 different templates (plasmids) in a PCR-ready format. The PCR reaction can be performed on one or all of the templates to generate tailored ladders and/or PCR products from one reaction. Furthermore, the PCR products can be mixed with each other to generate ladders with specific bands of desired intensity.

1. Add the following reagents to a PCR tube.

2 $\mu$ l	Template
1 $\mu$ l	10 $\mu$ M Primer 1 or 3*
1 $\mu$ l	10 $\mu$ M Primer 2 or 4*
21 $\mu$ l	Distilled water
25 $\mu$ l	2X SeeAmp™ ACP™ Master Mix
<hr/>	
50 $\mu$ l	Total volume

\* The intensity of the 100 bp PCR product is likely to be lower than that of the other size markers. To obtain 12 bands of equal intensity, add a separate 100 bp reaction mixture to a reaction mixture containing one or all of the PCR products.

Other commercially available size markers usually contain 500 bp and 1000 bp bands that are more intense than the other bands. This helps the viewer to distinguish among the various bands in the ladder. To create such a ladder, add one or more reaction mixtures for these bands to a reaction mixture for one or all bands.



- The comparison of the primers used differs depending on the desired marker size.

Template (plasmid)	Combination of Primer 1 & 2	Combination of Primer 3 & 4
	Expected size	Expected size
1(100 bp)	100 bp	150 bp
2(200 bp)	200 bp	250 bp
3(300 bp)	300 bp	350 bp
4(400 bp)	400 bp	450 bp
5(500 bp)	500 bp	550 bp
6(600 bp)	600 bp	650 bp
7(700 bp)	700 bp	750 bp
8(800 bp)	800 bp	850 bp
9(900 bp)	900 bp	950 bp
10(1000 bp)	1000 bp	1050 bp
11(1200 bp)	1200 bp	1250 bp
12(1500 bp)	1500 bp	1550 bp

2. Commence the PCR reaction by using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94 °C	5 min
2	35	94 °C	40 sec
		68 °C	40 sec
		72 °C	40 sec
3	1	72 °C	5 min

3. Run 0.5 - 1 µl of each PCR product on a 2% agarose gel containing EtBr.
4. Mix the synthesized PCR products together in the desired combinations.
5. Use appropriate aliquots of the mixture to personalize a DNA size marker.



Usually, each PCR yields sufficient products for 200-300 runs.

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# 5

## Ordering Information

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Product	Cat. No.
Forever Multi Ladder Premix Personalizer	<b>M0150</b>
Forever 100 bp Ladder Premix Personalizer	<b>M0100</b>

### Related Products

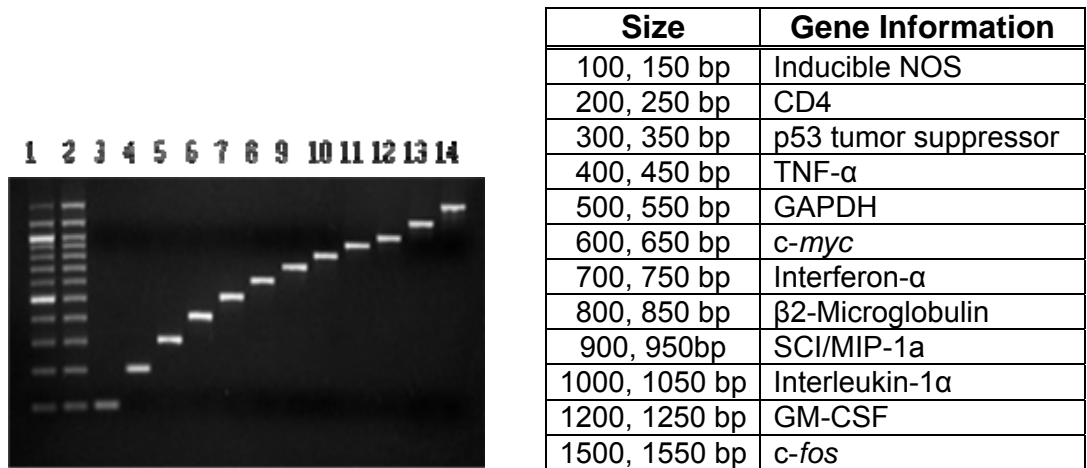
Products	Size	Cat. No.
2X SeeAmp™ ACP™ Master Mix	5 ml	<b>E1010</b>
Primer 1 for 100 bp	150 rxns	<b>P2001</b>
Primer 2 for 100 bp	150 rxns	<b>P2002</b>
Primer 3 for 50 bp	150 rxns	<b>P2003</b>
Primer 4 for 50 bp	150 rxns	<b>P2004</b>

# 6

## Appendix

### 6.1 Information about Each Size Marker

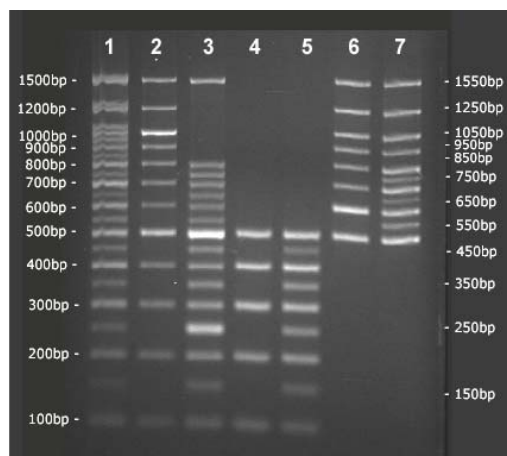
The 12 plasmids are supplied in a ready-to-PCR format. Simply amplify the differently sized fragments from the plasmids and mix them together. The 12 plasmids consist of different genes isolated from mouse (ICR) tissues. These genes can be used as control probes in other research applications, such as hybridization-related experiments.



**Fig. 1. Photograph of DNA ladder with an increment of 100 bp synthesized from the relevant templates by PCR.** Lane 1: a 100 bp DNA ladder whose 500 and 1000 bp bands are markedly more intense. Lane 2: a 100 bp DNA ladder with the bands of equal intensity. Lanes 3 to 14 show each PCR product alone.

## 6.2 Expected Result of Personalized DNA Ladder

To create your own personalized ladder, simply choose the appropriate plasmid templates, amplify them with the two universal primers supplied, and mix them together.



**Fig. 2. Various types of customized DNA ladders created by using the Forever Multi ladder Premix Personalizer.**

Lane 1: A ladder consisting of 100 to 1000 bp fragments in 50 bp increments plus 1200, 1250, 1500, and 1550 bp bands

Lane 2: A ladder consisting of 100 to 1000 bp fragments in 100 bp increments plus 1200 and 1500 bp bands

Lane 3: A ladder consisting of 100 to 800 bp fragments in 50 bp increments plus a 1500 bp band

Lane 4: A ladder consisting of 100 to 500 bp fragments in 100 bp increments

Lane 5: A ladder consisting of 100 to 500 bp fragments in 50 bp increments

Lane 6: A ladder consisting of 500, 600, 700, 800, 900, 1000, 1200, and 1500 bp bands

Lane 7: A ladder consisting of 500 to 800 bp fragments in 50 bp increments plus 900, 1000, 1200 and 1500 bp bands

