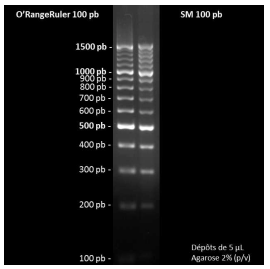
Aug 01, 2024

Home made SM 100bp DNA ladder for agarose gel



DOI
dx.doi.org/10.17504/protocols.io.dm6gpz535lzp/v1

Stéphane Mauger[1]

[1]LIttoral ENvironement et Sociétés - UMR 7266 - CNRS - La Rochelle Université

Stéphane Mauger

LIttoral ENvironement et Sociétés - UMR 7266 - CNRS - La Roc...

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Protocol Citation: Stéphane Mauger 2024. Home made SM 100bp DNA ladder for agarose gel. **protocols.io https://dx.doi.org/10.17504/protocols.io.dm6gpz535lzp/v1**

Protocol status: Working
We use this protocol and it's working

Created: August 01, 2024

Last Modified: August 01, 2024

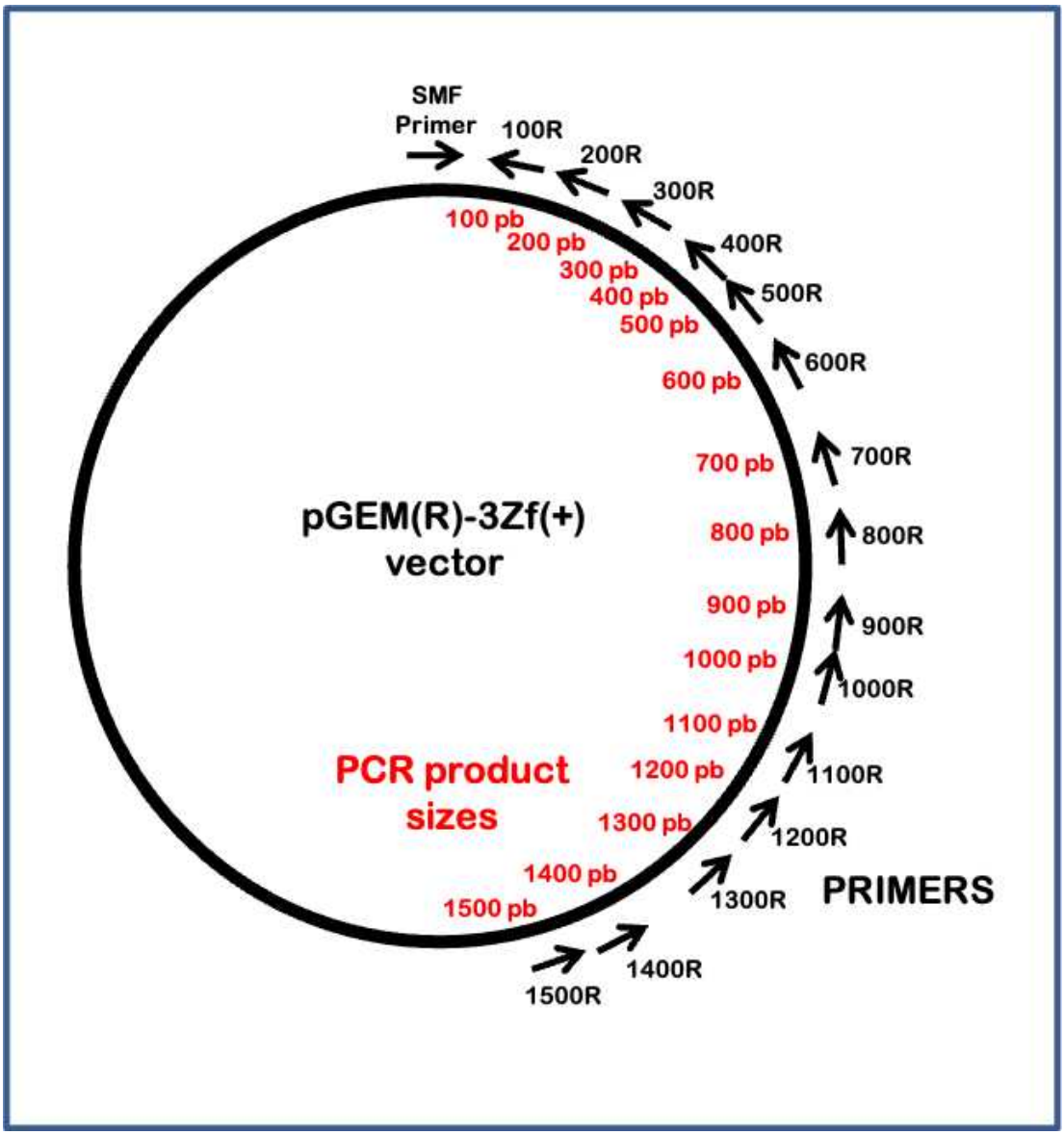
Protocol Integer ID: 104413

Keywords: double-stranded DNA sizing, agarose gel, 100pb ladder

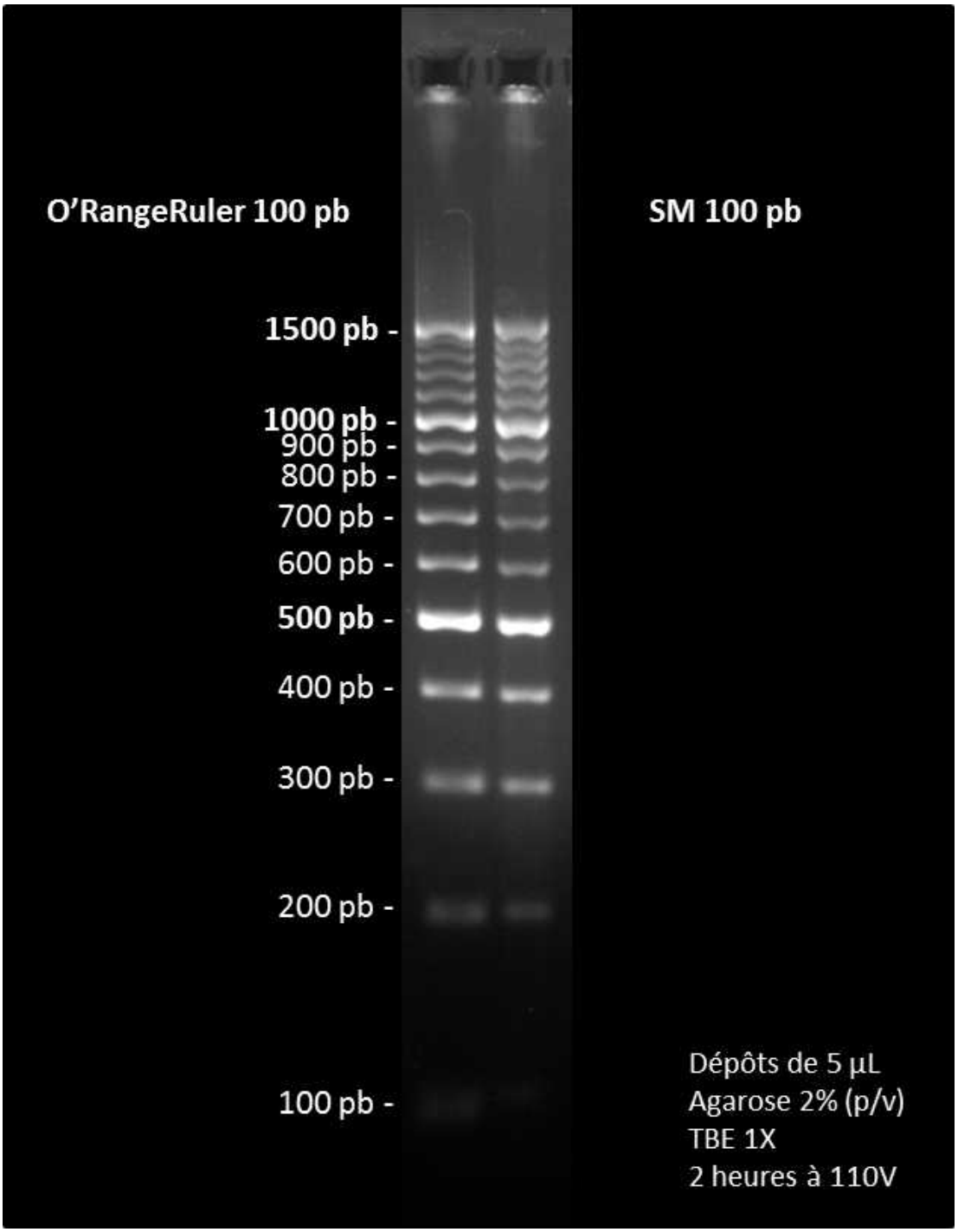
Abstract

The SM 100pb DNA Ladder is a standard size marker equivalent to the Fisher's O'RangeRuler® 100 pb DNA Ladder (#SM0623). The SM 100pb Ladder allows to determine the size of double-stranded DNA fragments between 100 bp and 1500 bp and it is composed of 15 double-stranded DNA fragments of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 and 1500 bp. Like the O'RangeRuler 100 bp, the SM 100 bp gives more intense bands at 500, 1000 and 1500 bp.

At the end, the SM 100 pb DNA Ladder is 25 times cheaper than the O'RangeRuler 100 pb.



Map of the universal cloning vector pGEM®-3Z(+) showing the position of the 16 primers



Comparaison between O'rangeRuler 100pb (Thermofisher) and SM100bp ladder on 2% agarose gel

Protocol materials

GoTaq® Flexi DNA Polymerase **Promega Catalog #**M8291	Step 3
pGEM®-3Zf( ) Vectors **Promega Catalog #**P2271	Step 2

Primers and pGEM®-3Zf(+) Vectors preparation

1 **Oligo primers dilution**

in 16 1.5 mL microtubes, dilute 1:10 each oligo primer at 100µM to final concentration 10µM, **see the SM100pb_primers.xlsx file below.**

50 µL oligo primer (100µM)

450 µL nuclease free water

Homogenize and store at 4 °C (or at -20 °C for a long-term storage).

SM100pb oligo primers sequences (salt free purification):

SM100pb_primers.xlsx 12KB

2 **pGEM®-3Zf(+) Vectors dilution**

In 1.5 mL microtube, dilute 1:100 pGEM®-3Zf(+) vector at 1µg/µL to final concentration 10ng/µL.

5 µL pGEM®-3Zf(+) (1µg/µL)

495 µL nuclease free water

Homogenize and store at 4 °C (or at -20 °C for a long-term storage).

pGEM®-3Zf(+) Vectors **Promega Catalog #**P2271

PCR amplification to generate 100pb-1500pb double-stranded DNA fragments

10s

3 **PCR mix preparation**

10s

SM100pb is produced using **28 PCRs amplifications** in a total of 50 µL reaction volume:

1 x 50 µL for 600pb, 700pb, 800pb, 900pb, 1100pb, 1200pb, 1300pb and 1400pb fragments

2 x 50 µL for 200pb, 300pb and 400pb fragments

3 x 50 µL for 1000pb and 1500pb fragments

4 x 50 µL for 100pb and 500pb fragments

Defreeze and vortex all reagents, except enzymes (stored at -20 °C), for approximately 00:00:05

Spin down all reagents for approximately 00:00:05 and place On ice.

In 1.5 µL microtube, prepare the PCR mix according to the following table :

	A	B	C	D	E
		Initial concentration	Final concentration	n=1	n=28
	SMF forward primer	10µM	400mM	2µL	56µL
	Green GoTaq buffer	5X	1X	10µL	280µL
	MgCl2	25mM	1mM	2µL	56µL
	pGEM®-3Zf(+) vecto	10ng/µL	20ng	2µL	56µL
	dNTP mix	2.5mM	150µM each	3µL	84µL
	GoTaq polymerase	5 u/µL	1.75U	0.35µL	9.8µL
	nuclease free water			28.65µL	802.2µL
	TOTAL			48µL	1344µL

PCR mix composition

GoTaq® Flexi DNA Polymerase **Promega Catalog #**M8291

4 **Reverse primers and mix combinaison**

Defreeze and vortex all the 15 reverse oligo primers at 10µM

In a 96-well plate PCR, transfer 2 µL of each reverse oligo primers (10µM) according to the following map :

	A	B	C	D	E	F	G	H
	600R	200R	1000R	500R				
	700R	200R	1500R	500R				
	800R	300R	1500R	500R				
	900R	300R	1500R	500R				
	1100R	400R	100R					
	1200R	400R	100R					
	1300R	1000R	100R					
	1400R	1000R	100R					

Map of the PCR plate

Vortex and spin down the PCR mix tube, transfer 48 µL in each of the 28 wells.
Seal the PCR plate.

In thermocycler, run PCR amplification with cycles follows:

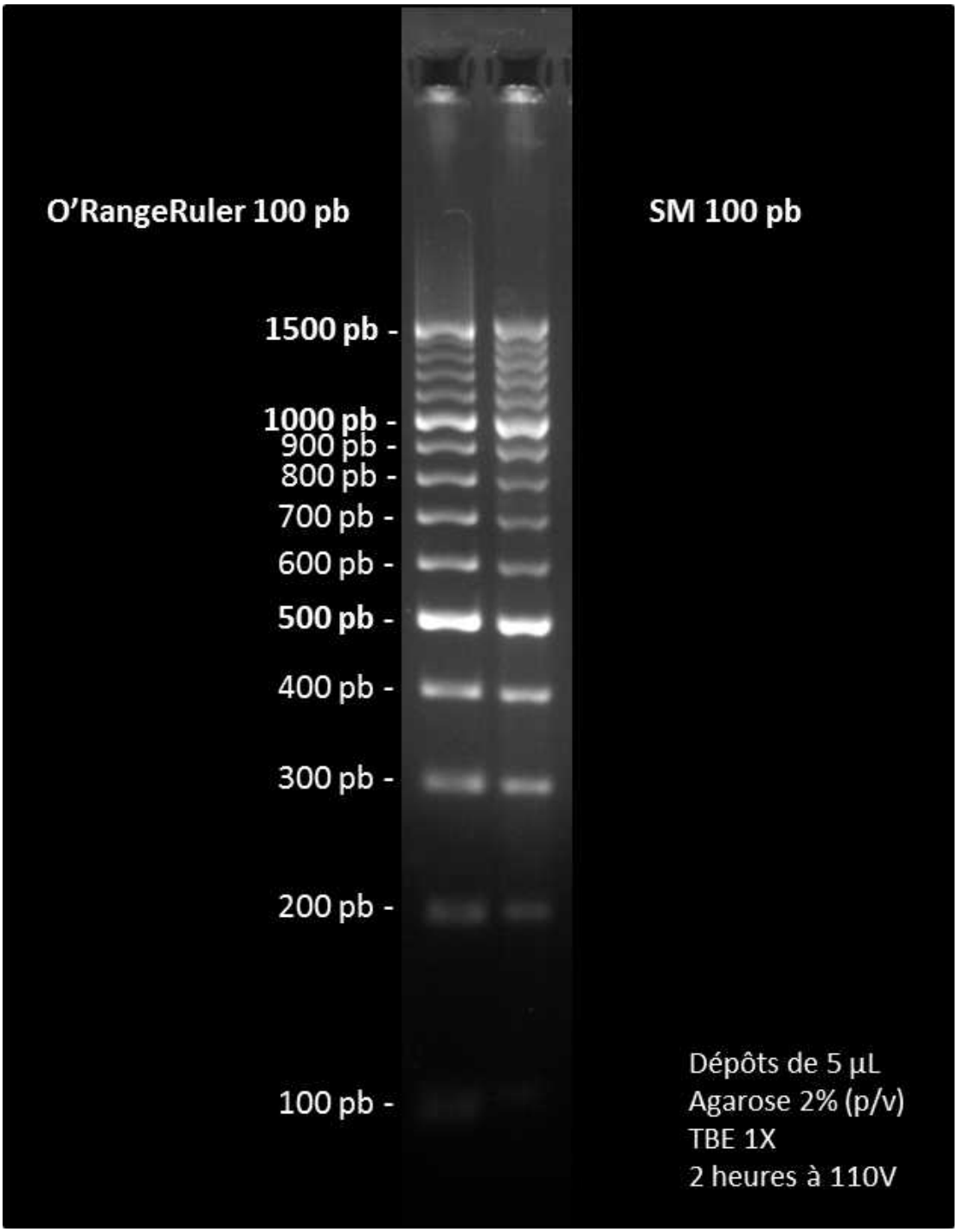
	A	B	C	D
	Cycles step	Temperature	Time	Cycles
	Initial denaturation	94°C	5 min	1
	Denaturation	94°C	30 sec	40
	Annealing	60°C	30 sec	40
	Extension	72°C	30 sec	40
	Final extension	72°C	60 min	1
	Hold	4°C		

PCR program

After PCR, pool and dilute 1:2 the PCR amplification
In a 5 mL tube:
1400 mL of PCR amplification (28 x 50 µL)
420 µL 5X green GoTag buffer
980 µL nuclease free water

Homogenize and store at 4 °C (or at -20 °C for a long-term storage).

Load 5 µL to 10 µL per line in a agarose gel.



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