

IU 1 ug of lambda DNA in 1 hou at 37 C in 50 uL

#### Recommended protocol for digestion

Nuclease free water	16 uL
10X Buffer B	2 uL
DNA (0.5 -1 ug/uL)	1 uL
Apal (0.5-2 uL)	1 uL
<b>Total:</b>	<b>20 uL</b>

Mix gently and spin down for a few seconds  
Incubate at 37 C for 1 - 16 hours

#### Recommended protocol for PCR products

PCR reaction mixture (0.5 ug of DNA)	10 uL
nuclease-free water	18 uL
10X Buffer B	2 uL
Apal (1 -2 uL)	1 uL
<b>Total:</b>	<b>31 uL</b>

Mix gently and spin down for a few seconds  
Incubate at 37 C for 1 - 16 hours

#### Our protocol (extracted from double digest)

Nuclease free water	24.5 uL
10X Buffer B	5 uL
DNA	20 uL
Apal	0.5 uL
<b>Total:</b>	<b>50 uL</b>

#### Our protocol for double digest

for PCR product	
Nuclease free water	23.5 uL
10X Buffer B	5 uL
DNA (0.5 -1 ug/uL)	20 uL
SacI	1 uL
Apal	0.5 uL
<b>Total:</b>	<b>50 uL</b>

for Plasmid	
pCM184	43.5 uL
nuclease-free water	0 uL
10X Buffer B	5 uL
SacI	1 uL
Apal	0.5 uL
<b>Total:</b>	<b>50 uL</b>

#### DNA 1

c	7.3 ug/mL
length	1085 bp
Avg Mw (ss)	329593.5 g/mol
Avg Mw (ds)	659187 g/mol
n (ss)	2.21485E-11 mol/mL

#### DNA2

c	72.1 ug/mL
length	6760 bp
Avg Mw (ss)	2053091 g/mol
Avg Mw (ds)	4106182 g/mol
n (ss)	3.51178E-11 mol/mL

#### Ratios

length	6.23
conc (mass)	9.877 1.5852
conc (molar)	1.586

50 uL

My protocol for double digest

for PCR product		for Plasmid	
Nuclease free water	77 uL	nuclease-free water	148 uL
10X Buffer B	20 uL	10X Buffer B	20 uL
PCR purified DNA	100 uL	Plasmid purified DNA	1 uL
SacI, 10U/uL	2 uL	SacI, 10U/uL	8 uL
Apal, 10U/uL	1 uL	Apal, 10U/uL	4 uL
<b>Total:</b>	<b>200 uL</b>	<b>Total:</b>	<b>181 uL</b>

Apal	10U/uL	min	5 U	for	1ug	dna
SacI	10U/uL	min	5 U	for	1ug	dna
insert mass				Plasmid mass		
12 ng/uL	1200 ng	1000 ng/uL	1000 ng			

min Apal Enzyme	0.1 uL	5 uL
min SacI Enzyme: 2x Apal	0.2 uL	10 uL

[http://www.methods.info/Methods/RNA\\_DNA/restr\\_analysis.html](http://www.methods.info/Methods/RNA_DNA/restr_analysis.html)

DNA up to 1ug  
Enzyme 1 uL

<https://www.lifetechnologies.com/order/catalog/product/ER1411>

DNA sample up to 30% of the reaction volume

First digest (1 hour 37 C)

for PCR product		for Plasmid	
Nuclease free water	5 uL	pCM184	38 uL
10X Tango	5 uL	nuclease-free water	5 uL
DNA (0.5 -1 ug/uL)	38 uL	10X Tango	5 uL
KpnI	2 uL	KpnI	2 uL
BglII	0 uL	BglII	0 uL
<b>Total:</b>	<b>50 uL</b>	<b>Total:</b>	<b>50 uL</b>

Second digest (1 hour 37 C)

for PCR product		for Plasmid	
First digest	50 uL	First digest	50 uL
10 xTango	6.25 uL	10X Tango	6.25 uL
BglII	0.5 uL	BglII	0.5 uL
	uL		uL
	uL		uL
<b>Total:</b>	<b>56.75 uL</b>	<b>Total:</b>	<b>56.8 uL</b>

### Double Digestion with Apal, SacI

We recommend:

B buffer

Apal

2-fold excess of SacI

Incubate at 37°C

**25/09/2015**

25/09/2015 15:15

DD Apal&Sacl	pcm184	200 ng/uL	DD Apal&Sacl	PCR4	3.5 ng/uL
Reaction vol		100 uL	Reaction vol		200 uL
nuclease-free water	18	uL	nuclease-free water	7	uL
10X Buffer B	10	uL	10X Buffer B	10	uL
Plasmid purified DNA, buff elut	60	uL	PCR purified DNA H2O eluted	80	uL
Apal, 10U/uL	4	uL	Apal, 10U/uL	1	uL
2x Sacl, 10U/uL	8	uL	2x Sacl, 10U/uL	2	uL
<b>Total:</b>	<b>100</b>	<b>uL</b>	<b>Total:</b>	<b>100</b>	<b>uL</b>

Mass	pcm184	12 ug
------	--------	-------

Mass	PCR4	0.28 ug
------	------	---------

Seems to be less than that

Or too long digestion made it degraded

<https://www.thermofisher.com/ca/en/home/brands/thermo-scientific/molecular-biology/thermo-scientific-restriction-modifying-enzymes/restriction-enzymes-thermo-scientific/double-digest-calculator-thermo-scientific.html>

[illegible]

**80 C 20 min**