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

ecommended protocol for digestio

Nuclease free water	16	uL
10X Buffer B	2	uL
DNA (0.5 -1 ug/uL)	1	uL
ApaI (0.5-2 uL)	1	uL
Total:	**20**	**uL**

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
ApaI (1 -2 uL)	1	uL
Total:	**31**	**uL**

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
ApaI	0.5	uL
Total:	**50**	**uL**

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
ApaI	0.5	uL
Total:	**50**	**uL**

for Plasmid

pCM184	43.5	uL
nuclease-free water	0	uL
10X Buffer B	5	uL
SacI	1	uL
ApaI	0.5	uL
Total:	**50**	**uL**

	DNA 1		DNA2	
c	7.3	ug/mL	72.1	ug/mL
length	1085	bp	6760	bp
Avg Mw (ss)	329593.5	g/mol	2053091	g/mol
Avg Mw (ds)	659187	g/mol	4106182	g/mol
n (ss)	2.21485E-11	mol/mL	3.51178E-11	mol/mL

Ratios		
length	6.2304	
conc (mass)	9.8767	1.5852
conc (molar)	1.5856	

50 uL

My protocol for double digest

for PCR product			for Plasmid		
Nuclease free water	0	uL	nuclease-free water	153	uL
10X Buffer B	10	uL	10X Buffer B	15	uL
PCR purified DNA	80	uL	Plasmid purified DNA	20	uL
SacI, 10U/uL	2	uL	SacI, 10U/uL	8	uL
ApaI, 10U/uL	1	uL	ApaI, 10U/uL	4	uL
Total:	**93**	**uL**	**Total:**	**200**	**uL**

ApaI	10U/uL	min	5 U	for	1ug	dna
SacI	10U/uL	min	5 U	for	1ug	dna

insert mass		Plasmid mass	
7 ng/uL	560 ng	250 ng/uL	5000 ng

min ApaI Enzyme	0.1	uL
min SacI Enzyme: 2x ApaI	0.2	uL
	25	uL
	50	uL

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
Total:	**50**	**uL**	**Total:**	**50**	**uL**

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
Total:	**56.8**	**uL**	**Total:**	**56.8**	**uL**

Single clone

1 - pcm184, 2 pcm184&pcr1, 3 - hypothetical pcm184&pcr1&pcr2

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EcoRI fast digest

DD BglII&KpnI fast digest	plasmids				
First digest: 2XSacI, 1X Tango Buffer			**Master mix**	**13**	**x**
nuclease-free water	6.5	uL	nuclease-free water	84.5	
Green buffer	1.5	uL	Green buffer	19.5	
Plasmid purified DNA	6	uL	Plasmid purified DNA	78	
EcoRI fast digest	1	uL			
Total:	**15**	**uL**	**Per tube**	**14**	**15**

Clones: 1 -12 Control: pcm184

plasmid concentration 55 ug/ml

Plasmid mass 0.33 ug