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

Recommended protocol for digestion			Recommended protocol for PCR products		
Nuclease free water	16	uL	PCR reaction mixture (0.5 ug of DNA)	10	uL
10X Buffer B	2	uL	nuclease-free water	18	uL
DNA (0.5 -1 ug/uL)	1	uL	10X Buffer B	2	uL
ApaI (0.5-2 uL)	1	uL	ApaI (1 -2 uL)	1	uL
Total:	**20**	**uL**	**Total:**	**31**	**uL**
Mix gently and spin down for a few seconds			Mix gently and spin down for a few seconds		
Incubate at 37 C for 1 - 16 hours			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			for Plasmid		
Nuclease free water	23.5	uL	pCM184	43.5	uL
10X Buffer B	5	uL	nuclease-free water	0	uL
DNA (0.5 -1 ug/uL)	20	uL	10X Buffer B	5	uL
SacI	1	uL	SacI	1	uL
ApaI	0.5	uL	ApaI	0.5	uL
Total:	**50**	**uL**	**Total:**	**50**	**uL**

	DNA 1		DNA2		
c	7.3	ug/mL	c	72.1	ug/mL
length	1085	bp	length	6760	bp
Avg Mw (ss)	329593.5	g/mol	Avg Mw (ss	2053091	g/mol
Avg Mw (ds)	659187	g/mol	Avg Mw (ds	4106182	g/mol
n (ss)	2.21485E-11	mol/mL	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
ApaI, 10U/uL	1	uL	ApaI, 10U/uL	4	uL
Total:	**200**	**uL**	**Total:**	**181**	**uL**

ApaI	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 ApaI Enzyme	0.1	uL	5	uL
min SacI Enzyme: 2x ApaI	0.2	uL	10	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:	**56.75**	**uL**	**Total:**	**56.75**	**uL**

Double Digestion with ApaI, SacI
We recommend:
B buffer
ApaI
2-fold excess of SacI
Incubate at 37°C

29/09/2015 29/09/2015 10:32

EcoRI

DD ApaI&SacI	pcm184 150 ng/uL		DD ApaI&SacI	1st_SC 55 ng/uL	
Reaction vol	**100 uL**		**Reaction vol**	**200 uL**	
nuclease-free water	8	uL	nuclease-free water	8	uL
10X Buffer EcoRI	2	uL	10X Buffer EcoRI	2	uL
Plasmid purified DNA, buff elut	9	uL	PCR purified DNA H2O eluted	9	uL
EcoRI	1	uL	EcoRI	1	uL
Total:	**20**	**uL**	**Total:**	**20**	**uL**

Mass pcm184 1.35 ug Mass 1st_SC 0.495 ug

EcoRV

DD ApaI&SacI	pcm184 150 ng/uL		DD ApaI&SacI	1st_SC 55 ng/uL	
Reaction vol	**100 uL**		**Reaction vol**	**200 uL**	
nuclease-free water	8	uL	nuclease-free water	8	uL
10X Buffer R	2	uL	10X Buffer R	2	uL
Plasmid purified DNA, buff elut	9	uL	PCR purified DNA H2O eluted	9	uL
EcoRV	1	uL	EcoRV	1	uL
Total:	**20**	**uL**	**Total:**	**20**	**uL**

Mass pcm184 1.35 ug Mass 1st_SC 0.495 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

! Do heat inactivation before the ligation start!!!

80 C 20 min