Day 01.

Prepare the desire plasmid DNA in a good concentration (Means min conc 0.1 ug/ul) there should not be RNA contamination in preparation.

Prepare the Insert DNA (PCR Product) Clean the product with PCR clean up kit If it is single band product or Gel purify it if it has some nonspecific amplicons.

Now set up reaction as follows

Restriction Digestion. (for single enzyme)

Master mix for Digestion.

Double distilled water	**16 ul**
10X buffer	**2 ul**
Enzyme	**2 ul**
Mix the master mix by vortex and spin down in microfuge.	

Reaction tubes

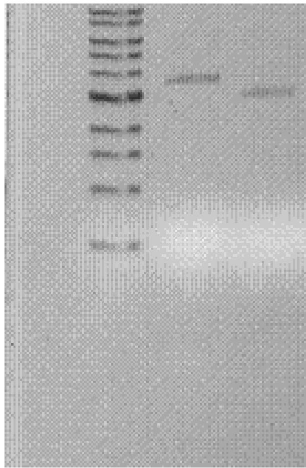
components	**Vector DNA**		**PCR product or Insert DNA**
D D water	**X ul (75- rest volume)**		**Y ul (75- rest volume)**
10X buffer	**7.5ul**		**7.5ul**
DNA	**5 ug (Minimum)**		**All after purification**
Enzyme mix	**10ul**		**10ul**
Total Volume==	**75 ul**		**75 ul**

Incubate at 37 degree for 2 hours and check for digestion with 5ul from Vector DNA tube and 2ul of loading dye on 0.8% Agarose gel.

Lane 1= Marker

Lane 2= Digested DNA (Vector)

Lane 3 = Undigested Vector DNA.



M 1 2

Please see the shift in bands.

Restriction Digestion. (for two enzyme)

Master mix for Digestion.

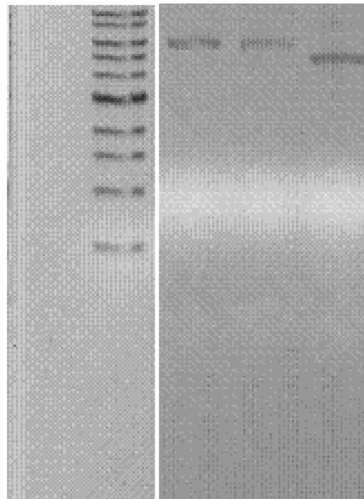
DD Water	**16 ul**	**16 ul**
10X buffer	**2 ul**	**2 ul**
Enzyme 01	**2 ul**	**00 ul**
Enzyme 02	**00 ul**	**2 ul**

Vortex and spin down in a Microfuge.

Reaction set up.

	Vector DNA	**Control for Enzyme 01**	**Control for Enzyme 02**	**PCR product.**
Dd water	**75- Rest**	**20- Rest**	**20- Rest**	**75-Rest**
10X Buffer	**7.5ul**	**2 ul**	**2ul**	**7.5 ul**
DNA	**Min 5 ug**	**1 ug plasmid DNA**	**1 ug Plasmid DNA.**	**All purified product**
Enzyme 01	**10 ul**	**3 ul**	**00 ul**	**7 ul**
Enzyme 02	**10 ul**	**00 ul**	**3 ul**	**7ul**
Total Volume	**75 ul**	**20 ul**	**20 ul**	**75 ul**

Incubate at 37 Degree for 2 hrs, Check on gel from only control tubes for Enzyme 01 and Enzyme 02



M 1 2 3

Lane M= Marker , Lane 1 Control tube for Enzyme 01 ,

Lane 02 = Control tube for Enzyme 02,

Lane 03 = Uncut plasmid.

If u are using two restriction enzyme there is no need of treating your digested vector DNA with Alkaline Phosphatase. After digestion is complete u can gel purify the product or let me know about your kit (I will tell u short cut)

But if u r using only one Restriction enzyme treat it with Alkaline Phosphatase Enzyme. See below protocol

DD Water	100- Rest Volume .
10X Buffer	10 ul
Digested vector DNA	75 ul
Alkaline Phosphatase	2ul
Total volume	100 ul

Incubate as follows

37 Degree= 60 mins ,

70 degree = 10 mins

4 degree = Pause

After treatment is complete u can gel purify the products (Digested and de phosphorylated Vector DNA and Digested Insert DNA) or let me know about your kit (I will tell u short cuts also .)

Now run Purified Double digested dephosphorylated Vector DNA and digested Insert DNA (Use only 1 ul with DNA Loading dye)

Now directly see it on gel with naked eye u shouldsee the intensity of band and approximately analyse how much nano gram DNA is there.

(Usually it will be around40-50ng/ul)

Now set up the digestion reaction as follows.

	1:3	1:5	1:7	--Ve	
DDWater					
10X Buffer	1 ul	1 ul	1 ul	1 ul	
DigestedVector DNA	1.5 ul	1.5 ul	1.5 ul	1.5 ul	
Digested Insert DNA	1.2 ul	1.5 ul	2 ul	00 ul	
T4 DNA Ligase enzyme	1 ul	1 ul	1 ul	1 ul	
Total Volume=	10 ul	10 ul	10 ul	10 ul	

Incubate at 22 degree for 3 hours.

Set Bacterial transformation.