DNA digestion for vector and inserts

1: insert		2: insert		3: insert		4: vector	
DNA:		DNA:		DNA:		DNA:	
H_2O (MilliQ)		H_2O (MilliQ)		H_2O (MilliQ)		H_2O (MilliQ)	
10xbuffer#2		10xbuffer#2		10xbuffer#		10xbuffer#	
Enz:		Enz:		Enz:		Enz:	
Enz:		Enz:		Enz:		Enz:	
Total	40ul	Total	40ul	Total	40ul	Total	40ul

Incubate tubes at 37˚C for 1h.

**If you cut a vector with a single enzyme, then you need to treat the enzyme digested DNA with CIAP.

Vector reaction above (40ul) +MilliQ (4ul) CIAP buffer (5ul) + CIAP (1ul) = total 50 ul

Incubate at 37˚C for another 30 min

Samples may be frozon at -20C at this point

Agarose electrophoresis of digested DNA

Check 4ul of each sample in an agarose gel to examine if digestion is complete

(4ul sample + 8ul of 1x loading dye= 12ul, apply all to agarose gel)

(Rest of the sample can be kept at -20˚C)

DNA fragment purification (vector and inserts)

Add 4ul of 10x loading dye to each sample

Run an agarose gel (two lanes for each sample: about 20ul per lane. Skip one lane between different samples.)

Cut gel piece that contains a desired band

1: _____kb

2: _____kb

3: _____kb

4: _____kb

Purify fragments using the Qiagen kit

Purified samples may be kept at -20˚

Agarose electrophoresis of purified DNA

Check 1ul of each sample (1ul sample + 9ul of 1x loading buffer)

Rest of the sample may be kept at -20˚C

Ligation

Mix the vector and an insert

1: for		2: for		3: for	
Vector:		Vector:		Vector:	
Insert:		Insert:		Insert:	
10xligation buffer	2	10xligation buffer	2	10xligation buffer	2
T4 DNA ligase	1	T4 DNA ligase	1	T4 DNA ligase	1
Total	20	Total	20	Total	20

Incubate samples at 16˚C, 30min~O/N

After the ligation reaction, samples can be kept at -20˚C

E. coli transformation

Transformation of DH5a cells with the legated DNA (5~10ul)

Plate E. coli cells onto LB+amp plates

Seal plates and incubate them at 37˚C, O/N (not more than 18h)

After colonies appear, plates can be kept at 4˚C

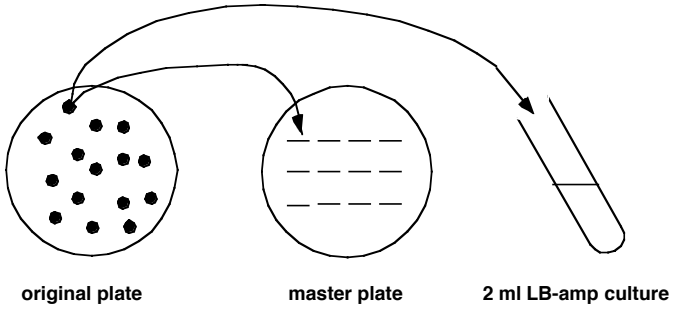
E. coli liquid culture

Inoculate colony in 2ml of LB+amp liquid medium

(we have 500x amp)

At the same time, make a master plate for your colonies

(if you start cultures, you must come to the lab next day to perform plasmid preparation)



Plasmid preparation

Keep the master plate at 4˚C

Prepare plasmid from E. coli culture using Eppendorf Kit

plasmid can be frozen at –20˚C

Plasmid digestion

Cut DNA by _____ (and) _____ to check whether your ligation is successful or not.

DNA	
H2O	
10x buffer #	
Enz:	
Enz:	
total	25ul

Incubate sample at 37˚C, 1h

Samples may be frozon at -20C at this point

Agarose gel electrophoresis of digested plasmid DNAs

add 3ul of 10x loading dye and run a gel

Store the plasmid and update plasmid file correction

If you find a correct plasmid, then you need to store the plasmid in our plasmid collection @-20°C. Give the plasmid a number (ex. pE550), label on the side and lid. Update the plasmid file collection

Glycerol stock of bacteria strains

Grow the bacteria strain corresponding to the plasmid you stored. (O/N @37°C in 2ml LB+amp)

Mix 600 ul of bacterial culture and 200 ul of 60% autoclaved glycerol in a Nalgene ample.

Don't forget to label the sample on the side and lid.

Store @-80°C