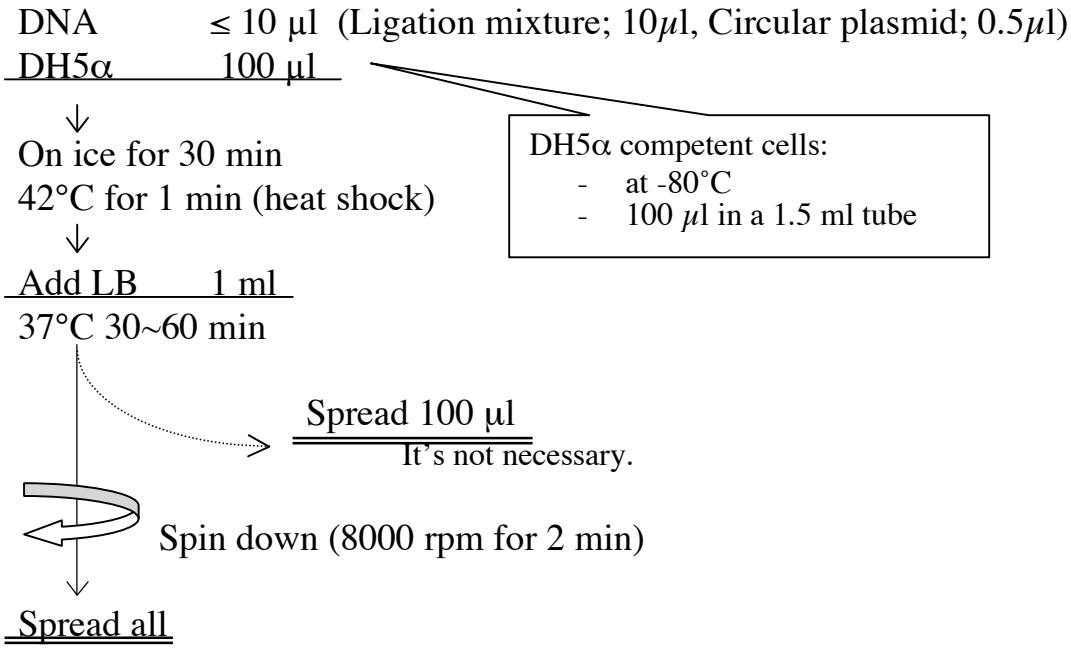
E. coli Transformation

by Chiaki Noguchi



If your plasmid has an ampicillin resistant gene, spread your E. coli cells on to LB + 2 mg/ml ampicillin plate.

We have 50 mg/ml ampicillin in 50% EtOH at -20˚C.

For one LB+amp plate:
Take 50 µl of this stock solution into 50 µl of sterilized milliQ water in a 1.5 ml tube to make a dilution mixture.
(If you have more plates, make more of this mixture.)
And spread 100 µl of it to a LB plate with 6~8 glass beads in.

Making Competent Cells

by Chiaki Noguchi

day 1

17:00 Spread DH5a on LB plate from glycerol stock

Place the plate @37°C over night

day 2

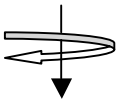
15:00 single colony 1
LB 5ml } x 2 sets in 50ml tubes
Shake @33°C over night
>>>Place 2 sets of 100ml LB in 500ml flasks @33°C room

day 3

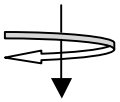
10:00 Put 1 tube of culture cells into warmed 100ml LB in a flask
Shake @33°C 3.5hr

>>>From here, keep it cold!!<<<

13:30 Put 200ml culture cells into 50ml tubes (x4)
on ice 5min

 3500rpm 5min @4°C

Suspend cells with 20ml Tfb1 each tubes

 3500rpm 3min @4°C

Suspend cells of 4 tubes with 8ml Tfb2
Aliquot 100ml in 0.6ml tubes
Put them into liquid nitrogen
Keep it @-80°C

Transformation Buffer 1 & 2 for competent cells

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>>> Tfb1

		stock			
30mM	KoAC	4M	1.88	ml	(4M…19.63g/50ml)
0.1M	RbCl		3.02	g	
10mM	$CaCl_2$	1M	2.50	ml	
50mM	$MnCl_2$		2.47	g	
15%	Glycerol	60%	62.50	ml	
	DW		to 250.00	ml	

↓

0.22mm filtration (40ml/tube x6)

↓

@-20°C

>>> Tbf2

		stock		
10mM	MOPS		0.52	g
10mM	RbCl		0.30	g
75mM	$CaCl_2$		2.08	g
15%	Glycerol	60%	62.50	ml
	DW		to 250.00	ml

↓

pH~4.7 to 6.5 (by KOH)

↓

0.22mm filtration (8ml/tube x6 & 50ml/tube x4)

↓

@-20°C