Kunkel Method

By Chiaki Noguchi
Modified by Jordan Rapp (1/20/07)

= ssDNA prep =

day 1

1) Transform RZ1032 with plasmid DNA on (amp) plate @37°C

day 2

2) Culture 1ml LB(amp) with a single colony @37 °C for about 5.5hr
3) Take 0.2ml RZ-cell to 1.5ml tube, mix 0.1ml helperphage (promega/R408)
   Incubate for 30 min at 37 °C
4) Transfer all to 20ml LB in 50ml flask, culture O/N @37 °C
   w/ 40μl 50mg/ml amp + 10μl 10mg/ml uridine

day 3

5) Transfer culture to polypropylene bottle
   
   12000rpm 15min RT
6) Transfer sup to new bottle
   
   12000rpm 15min RT
7) Transfer sup to new bottle again (glass tube)
   add 5ml 2.5M NaCl / 20% PEG8000, on ice 30min
   
   9000rpm 20min 4 °C
8) Discard sup
   
   9000rpm 20min 4 °C
9) Discard sup completely using pipetman
10) Suspend with 1ml TE, transfer to two 1.5ml tubes
    phe x 2times, chl x 1time
11) Add 1/10vol. 3M NaoAc + EtOH @ -80 °C 30min
    
    Max 10min 4 °C
12) Suspend with 0.2ml TE, merge two to one 1.5ml tube
    phe/chl x 1time, chl x 1time
    EtOH, 70% EtOH wash
    
13) Suspend with 25μl TE
14) Check 1μl on TAE agarose gel, keep -20 °C
    (good for quite long time)

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= Mutagenesis =

Kination

5pmol/μl oligo	15 μl
10x Kination buffer	2 μl
10mM ATP	1 μl
T4-polynucleotide kinase	2 μl
	20 μl

37oC 30min
65oC 20min ===> P-oligo (Keep @ -20oC)

Annealing

UssDNA	1 μl	1 μl
P-oligo	1 μl	-
10x Annealing buffer	1 μl	1 μl
D.W.	7 μl	8 μl
	10 μl	10 μl

70oC 10min
Cool down to RT for more than 3 hours, then put it on ice
(DON'T RUSH! This is important!!)

Synthesis

add 10x Synthesis buffer 1 μl
T4 DNA ligase (3units/reaction)
T4 DNA polymerase (1unit/reaction)

0˚C 5min
25˚C 5min
37˚C 90min

add STOP buffer 90 μl

Chcek 10 μl by agarose gel electrophoresis
Transformation E.coli with 10 μl of product

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= Reagents =

10x Kination buffer (2 μl/reaction)

700mM	Tris-HCl pH7.4
100mM	$MgCl_2$
50mM	DTT

10x Annealing Buffer (1 μl/reaction)

200mM	Tris-HCl pH7.4
20mM	$MgCl_2$
500mM	NaCl

10x Synthesis buffer (1 μl/reaction)

5mM each	dNTP
10mM	ATP
100mM	Tris-HCl pH7.4
50mM	$MgCl_2$
20mM	DTT

T4 DNA ligase (3 units/reaction)

T4 DNA polymerase (1 unit/reaction)

STOP buffer (90 μl/reaction)

10mM	Tris-HCl pH7.4
15mM	EDTA

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