reagent	dosage					
	No.1	No.2	No.3	No.4	No.5	No.6
2 × Mix	10 μl					
Cktcs/cdh-forward	0.5 μl					
Cktcs/cdh-reverse	0.5 μl					
pBlueScript+Cdh	0.5 μl					
$MnCl_2$	0 μl	0.4 μl	0.8 μl	1.2 μl	1.6 μl	2.0 μl
ddH_2O	8.5 μl	8.1 μl	7.7 μl	7.3 μl	6.9 μl	6.5 μl

PCR program

predegeneration	95℃	5min
Cycle one time		
degeneration	95℃	30s
	62℃	60s
	72℃	60s
The annealing temperature is reduced by 1℃ per cycle	**Until 55℃**	
Degeneration	95℃	30s
	55℃	60s
	72℃	60s
Cycle 25 times		
Elongation	72℃	7min
Cycle one time		

Recover PCR products and test for purity and concentration

procedure	reagent	dosage	instrument	time	notice
Agarose gel configuration	agarose	0.6g	electronic scales, weighing paper, medicine spoon	N/A	
	1×TAE	Add to 60ml	measuring cylinder, conical flask	N/A	
Heat till melt	Agarose gel	60ml	Microwave oven	90s	High heat
Solidify	Agarose gel	30ml*2	Gel tray	30min	
SYBR Green working solution configuration	SYBR Green stoste	1μl	centrifuge tube, pipette	N/A	
	ddH20	1ml	centrifuge tube, pipette	N/A	
	6× DNA Loading Buffer	1ml	centrifuge tube, pipette	N/A	
dyeing	PCR sample	6*20μl	centrifuge tube,	5min	

	SYBR Green working solution	6*6μl	centrifuge tube, pipette		
Load sample	PCR production	26μl*6	pipette	N/A	
	5K DNA Marker	20μl*2	pipette	N/A	
Gel electrophoresis	PCR production	2 bar	Electrophoresis apparatus	20min	80V
observe	Gel	N/A	UV transilluminator	N/A	
Cut the Gel	Gel	N/A	UV transilluminator, blade, electronic scales	N/A	Weigh the tube in advance
extraction	Gel	N/A	Agrose Gel DNA Extraction Kit	N/A	
testing	ddH_2O	1μl	Pipette, Spectrophotometer	N/A	
	DNA solution	1μl	Pipette, Spectrophotometer	N/A	

Expression vector construction

Double digestion reaction

procedure	reagent	dosage	instrument	time	notice
Reaction solution （DNA）	DNA（error-prone PCR production*6）	1μg*6 (calculation by concentration)	centrifuge tube. Pipette	N/A	
	10×NEB Buffer	5μl*6			1×
	Apa Ⅰ（ice）	1μl*6			10units
	Sac Ⅱ（ice）	1μl*6			10units
	ddH_2O	Add to 50μl			
Reaction solution （plasmid）	pBlueScript plasmid	1μg*2(calculation by concentration)	centrifuge tube, Pipette	N/A	
	10×NEB Buffer	5μl*2			1×
	Apa Ⅰ（ice）	1μl*2			10units
	Sac Ⅱ（ice）	1μl*2			10units
	ddH_2O	Add to 50μl			
mix	Reaction solution	N/A	Bench centrifuge	N/A	
reaction	Reaction solution*8	N/A	PCR Amplifier	5-15min	37℃
				20min	65℃

Recover PCR products and test for purity and concentration

procedure	reagent	dosage	instrument	time	notice
Agarose gel configuration	agarose	0.9g	electronic scales, weighing paper, medicine spoon	N/A	
	1×TAE	Add to 90ml	measuring cylinder, conical flask	N/A	
Heat till melt	Agarose gel	90ml	Microwave oven	90s	High heat
Solidify	Agarose gel	30ml*3	Gel tray	30min	
SYBR Green working solution configuration	SYBR Green stoste	1μl	centrifuge tube, pipette	N/A	
	ddH20	1ml	centrifuge tube, pipette	N/A	
	6× DNA Loading Buffer	1ml	centrifuge tube, pipette	N/A	
dyeing	PCR sample	8 *50μl	centrifuge tube,	5min	
	SYBR Green working solution	8*15μl	centrifuge tube, pipette		
Load sample	PCR production	30μl*16	pipette	N/A	
	5K DNA Marker	30μl*2	pipette	N/A	
Gel electrophoresis	PCR production	3 bar	Electrophoresis apparatus	20min	80V
observe	Gel	N/A	UV transilluminator	N/A	
Cut the Gel	Gel	N/A	UV transilluminator, blade, electronic scales	N/A	Weigh the tube in advance
extraction	Gel	N/A	Agarose Gel DNA Extraction Kit	N/A	
testing	ddH_2O	1μl	Pipette, Spectrophotometer	N/A	
	DNA solution	1μl	Pipette, Spectrophotometer	N/A	

Ligation reaction

procedure	reagent	dosage	instrument	time	notice

Ligation solution	T4 DNA Ligase Reaction Buffer（10×）	5μl	centrifuge tube, Pipette	N/A	
	Vector DNA	10μl		N/A	
	Insert DNA	30μl*6		N/A	
	ddH_2O	Add to 50μl（2.5μl）		N/A	
	T4 DNA ligase	2.5μl		N/A	
mix	Ligation solution	50μl*6	Bench centrifuge		
reaction	Ligation solution	50μl*6	PCR Amplifier	12h	16℃

Transformation

procedure	reagent	dosage	instrument	time	notice
Heat shock	Ligation solution	50μl*6	PCR Amplifier	10min	65℃
medium	LB medium（solid）	50ml*3	N/A	N/A	
	Ampicillin (AMP)	60μl*3	N/A	N/A	
solidify	medium	6 bar	Culture dish, clean bench	N/A	
mix	Ligation solution	5μl*6	N/A	N/A	
	Competent cells from E. coli, BL21	25μl*6	N/A	N/A	
Ice-bath	Transformation solution	30μl*6	N/A	30min	
Heat shock	Transformation solution	6	Water bath kettle	90s	40℃
Ice-bath	Transformation solution	6	N/A	150s	
culture	Transformation solution	6	shaker	45min	
Spread plate	Transformation solution	6	Culture dish, clean bench	N/A	
culture	Transformation solution	6	Constant temperature incubator	12-16h	37℃