

PCR Strategy

Primer pair		Template	Product	Product length	Usage	PCR annealing temperature	PCR extending time	Enzyme	Person in charge	Comment
pSB4A5-J6702	J06702-attB9	J06702 plasmid	J06702-OK	900	SET/RESET	58	1min20s	PrimerSTAR HS	XCY	
J23110-attB1	J23110-attP1	J23110 plasmid	J23110-BP1	75		58	20s	PrimerSTAR HS	XCY	
J23110-attB2	J23110-attP2	3 <sup>pc</sup> 产物 (J23110-BP1)	J23110-BP2	115		60	25s	PrimerSTAR HS	LHQ	
J23110-attB3	J23110-attP3	4 <sup>pc</sup> 产物 (J23110-BP2)	J23110-BP-OK	155	SET	58	30s	PrimerSTAR HS	XQ	
AttP9-K137058	K137058-pSB4A5	K137058 plasmid	K137058-SET-OK	900	SET	52	1min20s	PrimerSTAR HS	CF	
J23110-attL1	J23110-attR1	J23110-plasmid	J23110-LR1	75		58	20s	PrimerSTAR HS	XCY	
J23110-attL2	J23110-attR2	7 <sup>pc</sup> 产物 (J23110-LR1)	J23110-LR2	115		60	25s	PrimerSTAR HS	LHQ	
J23110-attB3	J23110-attP3	8 <sup>pc</sup> 产物 (J23110-LR2)	J23110-LR-OK	155	RESET	58	30s	PrimerSTAR HS	XQ	
AttP9-K137058	K137058-I0500-9	K137058 plasmid	K137058-RESET-OK	900	RESET	52	1min20s	PrimerSTAR HS	CF	
K137058-9-I0500	I0500-Xis9	I0500 plasmid	I0500-OK	1230	RESET	55	1min45s	PrimerSTAR HS	XCY	
Xis9-B0015	K137058-pSB4A5	K137058 plasmid	B0015-OK	160	RESET	51	30s	PrimerSTAR HS	XQ	
pSB4A5-J06702	attP-red9	2,5试剂盒连接产物 (J06702~J23110-BP)	J06702~J23110-BP-OK	900	SUPPORT DEVICE	60	1min20s	PrimerSTAR HS	LHQ	
attP9-red	red-GFP9	pKD46	RED-OK	1900	SUPPORT DEVICE	54	2min40s	PrimerSTAR HS	CF	
red9-GFP	K137058-pSB4A5	K137058 plasmid	K137058-SD-OK	1237	SUPPORT DEVICE	52	2min50s	PrimerSTAR HS	CF	
HL-J23110-1	J23110-RBS9bp	J23110 plasmid	J23110-HL-1	65		55	20s	PrimerSTAR HS	CF	
HL-J23110-2	J23110-RBS9bp	16 <sup>pc</sup> 产物 (J23110-HL-1)	J23110-HL-OK	85	INT	55	20s	PrimerSTAR HS	LHQ	
J231109bp-B0031-1	HR	27,28试剂盒连接产物 (Int-B0015)	B0031-Int-B0015-1	1521		60	2min	PrimerSTAR HS	LHQ	
J231109bp-B0031-2	HR	18 <sup>pc</sup> 产物 (B0031-Int-B0015-1)	B0031-Int-B0015-OK	1535	INT	58	2min	PrimerSTAR HS	LHQ	
J231109bp-B0033-1	HR	27,28试剂盒连接产物 (Int-B0015)	B0033-Int-B0015-1	1516		60	2min	PrimerSTAR HS	LHQ	
J231109bp-B0033-2	HR	20 <sup>pc</sup> 产物 (B0033-Int-B0015-1)	B0033-Int-B0015-OK	1532	INT	58	2min	PrimerSTAR HS	LHQ	
J231109bp-B0034-1	HR	27,28试剂盒连接产物 (Int-B0015)	B0034-Int-B0015-1	1520		60	2min	PrimerSTAR HS	LHQ	
J231109bp-B0034-2	HR	22 <sup>pc</sup> 产物 (B0034-Int-B0015-1)	B0034-Int-B0015-OK	1533	INT	58	2min	PrimerSTAR HS	LHQ	
J231109bp-6 N-1	HR	27,28试剂盒连接产物 (Int-B0015)	6N-Int-B0015-1	1513		60	2min	PrimerSTAR HS	LHQ	
J231109bp-6 N-2	HR	24 <sup>pc</sup> 产物 (6N-Int-B0015-1)	6N-Int-B0015-2	1529		58	2min	PrimerSTAR HS	LHQ	
J231109bp-6 N-3	HR	25 <sup>pc</sup> 产物 (6N-Int-B0015-2)	6N-Int-B0015-OK	1546	INT	58	2min	PrimerSTAR HS	LHQ	
J231109bp-B0031-1	INT-B00159bp	K907000 plasmid	Int-OK	1520		55	2min	PrimerSTAR HS	CF	
INT9bp-B0015	HR	B-RBS-HR	B0015-HR-OK	183	INT	55	30s	PrimerSTAR HS	LHQ	
HL-J23110-2	J23110-C9bp	16 <sup>pc</sup> 产物 (J23110-HL-1)	J23110-REINT-HL-OK	87	REINT	55	20s	PrimerSTAR HS	LHQ	
B9bp-B0031	HR	19 <sup>pc</sup> 产物 (B0031-Int-B0015-OK)	B0031-Int-B0015-BOK	1709	REINT	58	2min15s	PrimerSTAR HS	LHQ	
B9bp-B0033	HR	21 <sup>pc</sup> 产物 (B0033-Int-B0015-OK)	B0033-Int-B0015-BOK	1706	REINT	58	2min15s	PrimerSTAR HS	LHQ	
B9bp-B0034	HR	23 <sup>pc</sup> 产物 (B0034-Int-B0015-OK)	B0034-Int-B0015-BOK	1707	REINT	58	2min15s	PrimerSTAR HS	LHQ	
B9bp-6N	HR	26 <sup>pc</sup> 产物 (6N-Int-B0015-OK)	6N-Int-B0015-BOK	1720	REINT	58	2min15s	PrimerSTAR HS	LHQ	
HL-J23110-2	J23110-A9bp	16 <sup>pc</sup> 产物 (J23110-HL-1)	J23110-A-OK	88	SOCKET	58	20s	PrimerSTAR HS	XQ	
J231109bp-A	B-RBS9bp	sequence1	ATTB-OK	369	SOCKET	58	45s	PrimerSTAR HS	XQ	
A-KAN	KAN-B	pSB1K3	Kan-AB-1	856		58	1min20s	PrimerSTAR HS	CF	
A	B	35 <sup>pc</sup> 产物 (Kan-AB-1)	Kan-AB-OK	912	GOI	66	1min20s	PrimerSTAR HS	CF	
C-KAN	KAN-D	pSB1K3	Kan-CD-1	859		58	1min20s	PrimerSTAR HS	CF	
C	D	38 <sup>pc</sup> 产物 (Kan-CD-1)	Kan-CD-OK	900	GOI	66	1min20s	PrimerSTAR HS	CF	
A	KAN-C	35 <sup>pc</sup> 产物 (Kan-AB-1)	Kan-A-OK	874	GOI	55	1min20s	PrimerSTAR HS	CF	
XYWS1F	XYWS1R	sequence1-plasmid	sequence1	444	SINGLE	52	50s	PrimerSTAR HS	LHQ	
XYWS2F	XYWS2R	sequence2-plasmid	sequence2	358	DOUBLE	52	50s	PrimerSTAR HS	LHQ	
K404113-500 F1	K404113-500 R1	K404113 plasmid	500-1	540		58	1min	PrimerSTAR HS	XQ	
K404113-500 F2	K404113-500 R2	43 <sup>pc</sup> 产物 (500-1)	500-OK	580		58	1min	PrimerSTAR HS	XQ	
K404113-100 OF1	K404113-100 OR1	K404113 plasmid	1000-1	1040		58	1min30s	PrimerSTAR HS	XQ	
K404113-100 OF2	K404113-100 OR2	45 <sup>pc</sup> 产物 (1000-1)	1000-OK	1080		58	1min30s	PrimerSTAR HS	LHQ	
PSB4C5-HL	J23110-RBS9bp	J23110-HL-OK	J23110-HL-PSB-OK	105		55	20s	PrimerSTAR HS	LHQ	
J231109bp-B0031-2	HR-PSB4C5	B0031-Int-B0015-OK	B0031-Int-B0015-PSBOK	1700		58	2min10s	PrimerSTAR HS	LHQ	
J231109bp-B0033-2	HR-PSB4C5	B0033-Int-B0015-OK	B0033-Int-B0015-PSBOK	1700		58	2min10s	PrimerSTAR HS	LHQ	
J231109bp-B0034-2	HR-PSB4C5	B0034-Int-B0015-OK	B0034-Int-B0015-PSBOK	1700		58	2min10s	PrimerSTAR HS	LHQ	
J231109bp-6 N-3	HR-PSB4C5	6N-Int-B0015-OK	6N-Int-B0015-PSBOK	1700		58	2min10s	PrimerSTAR HS	LHQ	
PSB4C5-HL	J23110-C9bp	J23110-REINT-HL-OK	J23110-REINT-HL-PSBOK	105		55	20s	PrimerSTAR HS	LHQ	
B9bp-B0031	HR-PSB4C5	B0031-Int-B0015-BOK	B0031-Int-B0015-BOKPSB	1729		58	2min10s	PrimerSTAR HS	LHQ	
B9bp-B0033	HR-PSB4C5	B0033-Int-B0015-BOK	B0033-Int-B0015-BOKPSB	1726		58	2min10s	PrimerSTAR HS	LHQ	
B9bp-B0034	HR-PSB4C5	B0034-Int-B0015-BOK	B0034-Int-B0015-BOKPSB	1737		58	2min10s	PrimerSTAR HS	LHQ	
B9bp-6N	HR-PSB4C5	6N-Int-B0015-BOK	6N-Int-B0015-BOKPSB	1740		58				
PSB4C5-HL	J23110-A9bp	J23110-A-OK	J23110-A-PSBOK	105		58				
PSB4C5-F	PSB4C5-R	PSB4C5 Plasmid	PSB4C5	3221		55	3min40s	PrimerSTAR HS	LHQ	
PSB4C5-F	PSB4C5-R	PSB4A5 Plasmid	PSB4A5	3395		55	3min40s	PrimerSTAR HS	LHQ	
pbsk1500F1	pbsk1500R1	gpt2	1500-1	1540		58				
K404113-100 OF2	K404113-100 OR2	1500-1	1500-OK	1580		58				
pbsk2000/300F1	pbsk2000R1	gpt2	2000--1	2040		58				
K404113-100 OF2	K404113-100 OR2	2000--1	2000-OK	2080		58				
pbsk2000/300F1	pbsk3000R1	gpt2	3000--1	3040		58				
K404113-100 OF2	K404113-100 OR2	3000--1	3000-OK	3080		58				