

# PCR protocol

**RBRC** 02240      **Strain name** STOCK Grid2<tm1Mim> Tg(Pcp2-Grid2\*R514K)1Yuza

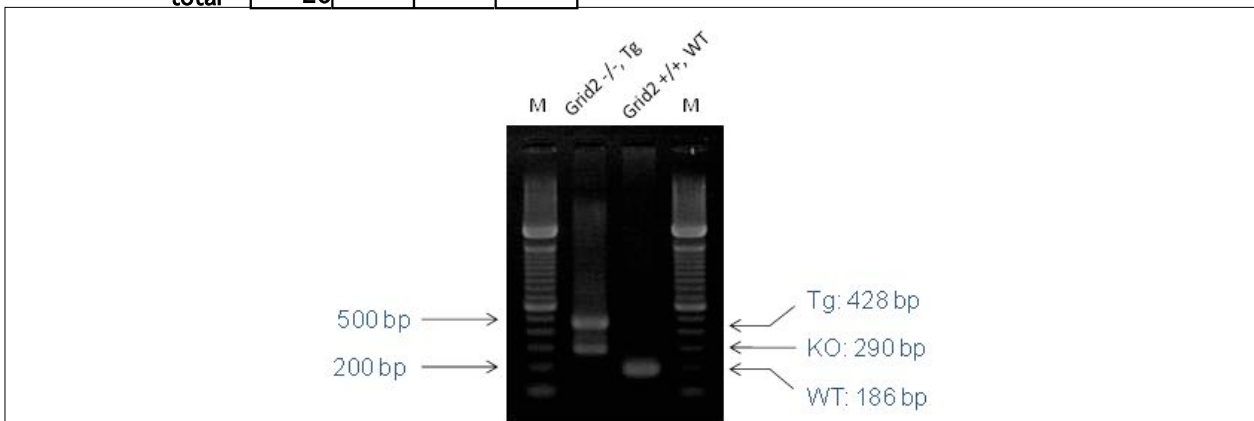
Primer	primer_name	primer_seq	length
1		CATCCCATACAAAAGCATAG	20
2		GATTGCAAATGGGATCGATG	20
3	D2P1	GGAAGTCCCATACACA AACTCTGGCAACCCG	30
4	D2P2	CATGCCCAGCATTGTCAGGTTTGGTGACA	30
5	Neo-P1	GCCTGCTTGCCGAATATCATGGTGAAAAAT	30

**Reaction Components**
**Enzyme** QIAGEN Multiplex PCR Kit (Cat. No. 206143)

**Reaction Conditions**

	Conc	Run 1	Run 2	Run 3	Run 4
<b>H2O</b>		2.0			
<b>Master Mix</b>	2 X	10.0			
<b>primer 1</b>	10 pmol/μl	2.0			
<b>primer 2</b>	10 pmol/μl	2.0			
<b>primer 3</b>	10 pmol/μl	1.0			
<b>primer 4</b>	10 pmol/μl	1.0			
<b>primer 5</b>	10 pmol/μl	1.0			
<b>DNA</b>		1.0			
<b>total</b>		20			

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles
5	72	600	


**Marker**

100 bp ladder (Invitrogen, Cat. 15628-050)

**Gel**

2% agarose in 1 X TAE (100 V, 30 min)

**PCR products**

primer_set	product_size	product
primer 1	428 bp	Tg
primer 3	186 bp	wild (Grid 2)
primer 3	290 bp	KO (Grid 2)

**Comment**