



TgN(Thy1-YFP)16Jrs, Version 1

strains ----- 003709 003782

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Notes: *Use 2µl (5-20ng) DNA per reaction. DNA loading dye = 60% sucrose/5mM cresol red. Our genotyping laboratory purchases Cresol Red (Sodium Salt) from Sigma, catalog number C9877.

Products Gel Image

Internal Terd control = ~200 bp

Transgene = ~300 bp

Reaction Components

Reaction Components	Vol/Rxn A	Vol/Rxn B	Final Conc	Total Volume A	Total Volume B
[120	3.140	0.000	QS to 12 µl	3.14	0.00
10X PE Buffer II	1.200	0.000	1 X	1.20	0.00
25 mM MgCl2	0.960	0.000	2 mM	0.96	0.00
2.5 mM dNTP	0.960	0.000	0.2 mM	0.96	0.00
20 µM oIMR 1258	0.600	0.000	1.0 µM	0.60	0.00
20 µM oIMR 1260	0.600	0.000	1.0 µM	0.60	0.00
20 µM oIMR 015	0.400	0.000	0.66 µM	0.40	0.00
20 µM oIMR 016	0.400	0.000	0.66 µM	0.40	0.00
DNA loading dye	1.660	0.000	0.138 µl/µl TV	1.66	0.00
5 U/µl Taq Pol.	0.080	0.000	0.0138 U/µl	0.08	0.00

Cycling Conditions

Cycling Reaction A	Step	Temp	Time	Note
	1	94 °C	1.5 min	
	2	94 °C	30 sec	
	3	62 °C	1 min	
	4	72 °C	1 min	Go to step 2, 35 times
	5	72 °C	2 min	
	6	10 °C		

Separated by gel electrophoresis on a 1.5% agarose gel.

Primers

oIMR0015	5'- CAA ATg TTg CTT gTC Tgg Tg -3' Tm = °C	20-mer A=3, C=3, G=6, T=8	Terd primer: With oIMR016, amplifies a 200 bp DNA fragment from the wild type allele. Tm = 58 C.
oIMR0016	5'- gTC AgT CgA gTg CAC AgT TT -3' Tm = 56.4 °C	20-mer A=4, C=4, G=6, T=6	Terd primer.
oIMR1258	5'- TCT gAg Tgg CAA Agg ACC TTA gg -3'	23-mer A=6, C=4, G=8, T=5	CFP, YFP primer. Thy1F1 from Margo Funk. With oIMR1259, amplifies a 700 bp DNA fragment from the CFP transgene. With oIMR1260, amplifies a 300 bp DNA fragment from

	Tm = 62.0 °C		the YFP transgene.
oIMR1260	5'- CgC TgA ACT TgT ggC CgT TTA Cg -3' Tm = °C	23-mer A=3, C=6, G=7, T=7	YFP primer Thy1F1 from Margo Funk.

This genotyping protocol is used for the following strains:

Stock Number	Strain Name
003709	B6.Cg-Tg(Thy1-YFP)16Jrs/J
003782	B6.Cg-Tg(Thy1-YFP)2Jrs/J

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