

## C57BL/6-Tg(Ins2-OVA)307Wehi/WehiJ

Stock No: 005432

Protocol 29366: Standard PCR Assay - Tg(Ins2-OVA)

Version 4.2

### Notes

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

### Expected Results

Tg/0 Area = 2

Tg/0 Tm = 81.2 +/- 1

Tg/02 Area = 10

Tg/02 Tm = 89.7 +/- 1

Tg/Tg Area = 2

Tg/Tg Tm = 100 +/- 0.6

internal control Area = 10

internal control Tm = 89.7 +/- 1

### Evagreen

Tg/0 Area = 2

Tg/0 Tm = 82.5 +/- 1

Tg/02 Area = 10

Tg/02 Tm = 89.7 +/- 1

internal control Area = 10

internal control Tm = 89.7 +/- 1

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR6021		CAA GCA CAT CGC AAC CA			A	
oIMR6022		GCA ATT GCC TTG TCA GCA T			A	
oIMR6023		CGA GCT CGA GCC TGC CTA TCT TTC AGG TC			A	
oIMR6024		CGG GAT CCT AGT TGC AGT AGT TCT CCA G			A	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR6021	0.50 uM
oIMR6022	0.50 uM
oIMR6023	0.50 uM
oIMR6024	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

