

PCR Protocol for Genotyping: SV40 (Immorto)

A. Digestion of mouse tail or ear notch, and **embryo tail (in red)**:

1. Add 100µL of Tissue Digestion Buffer and 2µL of Proteinase K per tail (~1-2mm length). For embryos tail add **50µL of Tissue Digestion Buffer and 1µL of Proteinase K**. Make sure tail is immersed in the buffer.
2. In a thermocycler incubate at 55°C for 1 h followed by 95°C for 8 min to inactivate the enzyme and hold at 10°C. For embryos incubate at 55°C for 30 min followed by 95°C for 8 min and hold at 10°C.
3. Vortex and store at 4°C (-20°C for long storage) or use immediately to set up the PCR.

B. PCR Genotyping Protocol: SV40 (Immorto)

Primers			
WT -F	5'-	GAT CTG CCT GAG GTG TTA CTT G	-3'
WT-R	5'-	GGA TGG CAT CAC TAG TCA TGA C	-3'
tsA-F	5'-	AGT CCT CAC AGT CTG TTC ATG ATC	-3'

PCR Reaction		PCR Conditions		
BioMix (Bioline)	10.0 µL		Heated Lid	105°C
Primer F	0.8 µL		Initial Denaturation	95°C 2 min
Primer R	0.8 µL		Number of Cycles	x35
Primer tsA-F	0.8 µL			95°C 20 sec
ddH ₂ O	5.6 µL			58°C 40 sec
DNA template	2.0 µL			72°C 40 sec
Total Volume	20.0 µL		Final Extension	72°C 5 min
			Final Hold	10°C

PCR Product Size (bp)	
Wild type band	500 bp
SV40 band	300 bp

C. Reagents

Reagent	Cat #	Final Concentration	Working Concentration
Tissue Digestion Buffer for ear notch or tail			
Tris pH8.5		50mM	
EDTA		1mM	
Tween20		0.5%	
Proteinase K (Invitrogen)	25530-015	20mg/mL	
BioMix (Bioline)	BIO-25012		
Primers			10mM