

## **PCR Protocol for Genotyping SV40 (Immorto):**

### **A. Primers:**

*WT-F*: 5'-GATCTGCCTGAGGTGTTACTTG-3'

*WT-R*: 5'-GGATGGCATCACTAGTCATGAC-3'

*tsA-F*: 5'-AGTCCTCACAGTCTGTTTCATGATC-3'

### **B. Digestion of mouse tail: (Sigma-Aldrich Extract-N-Amp™ Tissue PCR Kits)**

1. Add 50ul of Extraction solution
2. Add 12.5ul of Tissue Preparation Solution
3. Incubate at room temperature for 10min.
4. Incubate at 95 °C for 3min.
5. Add 50ul of Neutralization Solution B
6. Vortex for 20 sec.
7. Store at -20 °C (Digest is now ready for PCR)

### **C. PCR Reactants:**

Biomix	10.0ul
ddH <sub>2</sub> O	6.2ul
MgCl <sub>2</sub>	0.2ul
Primer 1	0.3ul
Primer 2	0.3ul
DNA template	3.0ul
Total Volume	(20ul)/reaction

\*DNA is added last

### **D. PCR Conditions:**

<b>Heated Lid:</b>	105°C	
<b>Initial Denaturation:</b>	95°C	2 min
<b>Cycles:</b>	35x	
	94°C	20 sec
	58°C	40 sec
	72°C	40 sec
<b>Final Extension:</b>	72°C	5 min
<b>Final Hold:</b>	10°C	

### **E. Product:**

Mutant: 500bp

SV40: 300bp

### **F. Notes:**

1. Tails can be store at -20°C until digested
2. When genotyping embryo tails, do not add dye in the PCR reactants and reduce the DNA amount to 1.0ul