**IL-7 genotyping PCR protocol (cytokine)**

**Primers**
- $\text{mIL7geno1: GGA AGC TGC TTT TCT AAA TC}$
- $\text{mIL7geno2: GTC CAC TCT CAC CTT ACT TG}$

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount (multiply by number of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddH2O</td>
<td>$14 \mu L$</td>
</tr>
<tr>
<td>$25 \text{mM MgCl}_2$</td>
<td>$1.5 \mu L$</td>
</tr>
<tr>
<td>$\text{Fermentas 10x Taq buffer (+ (NH}_4\text{)}_2\text{SO}_4}$</td>
<td>$2.5 \mu L$</td>
</tr>
<tr>
<td>$2.5 \text{ mM dNTP}$</td>
<td>$2.5 \mu L$</td>
</tr>
<tr>
<td>$10 \mu M (each) primer mix$</td>
<td>$1 \mu L$</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>$0.5 \mu L$</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

Add $3 \mu L$ of DNA to each tube to bring total volume to $25 \mu L$.

<table>
<thead>
<tr>
<th>PCR parameters</th>
<th>Time</th>
<th>Temperature</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denature</td>
<td>4 min</td>
<td>95°C</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>30 sec</td>
<td>95°C</td>
<td>35</td>
</tr>
<tr>
<td>Anneal</td>
<td>30 sec</td>
<td>58°C</td>
<td></td>
</tr>
<tr>
<td>Extend</td>
<td>2 min</td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>7 min</td>
<td>72°C</td>
<td>1</td>
</tr>
<tr>
<td>Cool</td>
<td>Indefinitely</td>
<td>4°C</td>
<td>1</td>
</tr>
</tbody>
</table>

**Products**
- WT Band: 145bp
- KO Band: ~600bp