TetO-EGFR Genotyping

Primer name	Sequence (5’ to 3’)
EGFR3421	ACTGTCCAGCCCACCTGTGT
mp-1R	GCCTGCGACGGCGGCATCTGC

PCR Amplification Protocol

1. **Denaturation**
    - Heat the reaction mixture to **95°C** for **5 minutes** to denature the DNA.
2. **Cycling Phase** (Repeat for **35 cycles**):
    - **Denaturation:** Heat to **95°C** for **30 seconds**.
    - **Annealing:** Lower the temperature to **58°C** for **30 seconds** to allow primers to bind.
    - **Extension:** Increase the temperature to **72°C** for **30 seconds** to allow DNA polymerase to synthesize new strands.
3. **Final Extension**
    - Maintain the reaction at **72°C** for **5 minutes** to complete strand synthesis.

Transgenic Confirmation by Southern Blotting

1. Extract **tail DNA** from transgenic founder specimens.
2. Digest the DNA using **XhoI-SalI** restriction enzymes.
3. Perform **Southern blotting** using a **~500 bp XhoI-SalI probe** targeting the **5' end of the construct** to confirm transgene integration.