

PCR Protocol for Genotyping for Akt mice

Materials

1. Tail DNA
2. 25 mM MgCl₂ (Roche, Biochemicals)
3. dNTPs (10mM each) (Amersham Pharmacia Biotech, Inc.)
4. Primers. The oligonucleotides used have the following sequence:
Sense 5'-TCTGCACCTTGTCAGTGAGG-3'
Anti-sense 5'CCGGTACACCACGTTCTTCT-3'
5. AmpliTaqGold DNA Polymerase (Roche, Biochemicals)
6. 10X PCR buffer (MgCl₂ free) (Roche, Biochemicals).
7. DNase and RNase free water

Methods

1. For a 25 μ l reaction mixture add the following amounts of the above materials:

<u>Stock</u>	<u>For 1 Reaction</u>	<u>Final Concentration</u>
tail DNA	1.0 μ l	20.0 ng
25 mM MgCl ₂	4.0 μ l	4.0 mM
dNTP mix [10mM each dNTP]	1.0 μ l	0.4 mM
20 μ M Primers	0.4 μ l each	0.32 μ M
5 units/ μ l Taq Polymerase	0.5 μ l	0.1 unit/ μ l
10X PCR Buffer	2.5 μ l	1.0 X
PCR Water	15.2 μ l	
	Total 25.0 μ l	

2. PCR profile: 94°C for 3 min, then 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by one cycle of 72°C for 7 minutes.
3. A 900 bp PCR product (probasin promoter and a part from the 5' end of Human Akt1) can be resolved on a 1.2% agarose gel.

