## PCR: FasAB_GranzymeA(ASD)

Amplifluor: No

## Primers

Nucleotide sequence (5` --> 3`)

| Primer | Sequence |
|---|---|
| HF-11 | AGGAGCAATATATACCAATGG |
| HF-7AS | AGGTAGGTGAAGGATAGCCAC |
| 01neo2A | CGGAGAACCTGCGTGCAATC |

## Reagents

| Reagents/Constituents | Commercial name | Stock Concentration | Volume (µL) |
|---|---|---|---|
| DNA Sample | | | 1.0 |
| 10x Buffer | | 20.0 mM MgCl2 | 2.5 |
| dNTPs | | 10.0 mM | 1.0 |
| Primer: **HF-11** | | 10.0 µM | |
| Primer: **HF-7AS** | | 10.0 µM | |
| Primer: **01neo2A** | | 10.0 µM | |
| Water | | | 16.45 |
| Taq Polymerase | | | 0.05 |
| Reagent: 25mM MgCl2 | | | 2.0 |

**Comments on protocol:** DNA Sample (ear punch)

## Strategy

| Steps | Temp (°C) | Time (seconds) |
|---|---|---|
| Hot start: **No** | | |
| Initiation/Melting | 94.0 | 180 |
| Denaturation | 94.0 | 30 |
| Annealing | 57.0 | 30 |
| Elongation | 72.0 | 60 |
| Number of cycles (repeat denature, anneal & elgonate): **35** | | |
| Strand completion (i.e. 72°C, 10 min) | 72.0 | 240 |
| Finish (i.e. 4°C, indefinite) | 25.0 | Hold |

## Electrophoresis

| | |
|---|---|
| **Running buffer:** | |
| **% Agarose:** | 2.0 |
| **Volt:** | 360.0 |
| **Estimated running time (min):** | 12.0 |

## Bands

| Number | Band (bp) | Genotype |
|---|---|---|
| 1. | 320 | KO |
| 2. | 460 | WT |