

## LOA genotype protocol (EMMA No. 1847)

### Primers:

LOA-L: 5' AA<sub>g</sub>gAA<sub>g</sub>TC<sub>g</sub>AT<sub>g</sub>gTCT<sub>g</sub>gA 3'

LOA-R: 5' T<sub>g</sub>gACCTT<sub>g</sub>AACTT<sub>g</sub>TC<sub>g</sub>T<sub>g</sub> 3'

### PCR conditions:

MgCl<sub>2</sub>: 1.5mM

Primers: 0.5μM (0.5μl)

dNTPs: 0.2mM (2μl)

Taq: 1U/reaction (0.2μl)

### Cycles (35)

3 minutes: 94 degrees

1 minute: 94 degrees

1 minute: 55 degrees

1 minute: 72 degrees

10 minutes: 72 degrees

### Cutting:

Final volume: 10μl

BSA: 0.1μl/reaction

BSGR-1: 0.15μl/reaction (Cut only when LOA mutation is present)

Temperature: 37 degrees, at least 4 hours

PCR: 3μl

```
ccgagggtgctccttgatgctgccgacgccaacaccattgaggaggtgaacc
tggcctacgagaatgtcaaggaagtcgatggtctggatgtttccaaagaag
ggacggaggcctgaggaggccgcatgaagagatacgatgagaggatcgacc
gtgtggagaccgcacaccgcccgcctccgagatcagctcggcacggcca
agaatgccaatgagatgtcaggatcttctccagggttcaatgcaactgttcg
tccgcccacacatccgaggggccattcgtgaataccagaccagctgatcc
aacgtgtgaaagatgacatcgaatctctgcacgacaagttcaagggtccagt
acc
```

gagatgtacaggatctt at LOA