

Primers for 454 genotyping

First-round PCR primer, forward: 5' GAGCCAATTCCCATACATTATTGT 3'

First-round PCR primer, reverse: 5' GCCCATAGTGCTTCTCTGCTGCTCCCAAGAACC 3'

Second-round PCR primer, forward: 5' AATGCCAAAACCATAATAGTACA 3'

Second-round PCR primer, reverse: 5' GAAAAATTCCTTCCACAATTAAA 3'

Fusion primer: 5' GCCTCCCTCGCGCCATCAG 3'

Deep sequencing barcode tags:

- (A) ACGAGTGCGT
- (B) ACGCTCGACA
- (C) AGACGCACTC
- (D) AGCACTGTAG
- (E) ATCAGACACG
- (F) CGTGTCTCTA
- (G) CTCGCGTGTC
- (H) TAGTATCAGC
- (I) TCTCTATGCG
- (J) TGATACGTCT
- (K) TACTGAGCTA
- (L) ATATCGCGAG

Example of complete deep sequencing forward primer, tag A:

(fusion primer, then *barcode A*, then **PCR primer**)

5' GCCTCCCTCGCGCCATCAGACGAGTGCGTAATGCCAAAACCATAATAGTACA 3'

Thermal cycler protocols

RT-PCR

30' 52°C

2' 94°C

15'' 94°C

30'' 55°C

1'30'' 68°C

5' 68°C

40x

Second-round PCR

2' 94°C

15'' 94°C

30'' 55°C

50'' 72°C

5' 72°C

35x