$gk^{A456V.-Neo}$ & gk^{wt} PCR Genotyping Protocol
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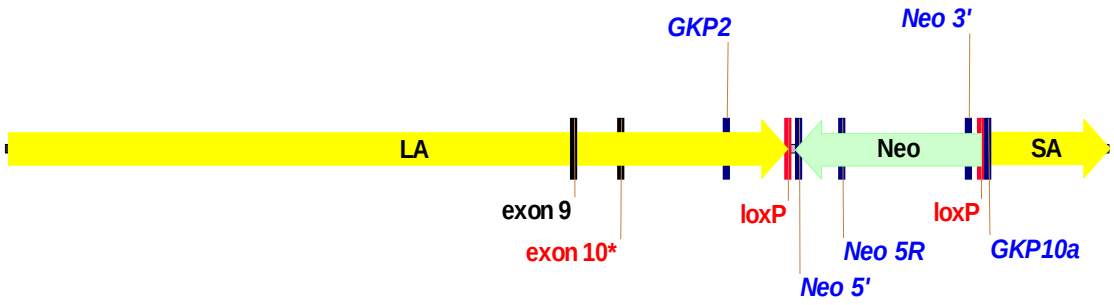
The following conditions are used to screen for the presence or absence of the Neo cassette in the loci targeted by BOB2.A456V. Reaction GKP2 + GKP8 is used to screen for both the *wild-type* and targeted mutation -*Neo allele* (gk^{wt} & $gk^{A456V.-Neo}$).



BOB2.A456V genotyping map

**Primer GKP10a has been replaced by GKP8 shown in the figure above. GKP8 lies 31 bp downstream of primer GKP10a.*

PCR reagents:

1. oligo primers at 20 µM
   GK primers (GKP2 + GKP8 amplify 636 bp wt &/or a 741 bp A456V-Neo fragments)
   GKP2 5'-TGT CTC AAT TTG CTG TGT CCT CCA-3' (top)
   GKP8 5'-ATG TGT GAG TGT GCC AAT ATG AGT-3' (bottom)
2. Perkin Elmer PCR buffer with $MgCl_2$
3. dNTP premix (I make my own dNTP premix using 100 mM NEB dNTP's. The premix contains 250 µl of each dNTP - A, C, G, &T - and 19 ml sterile water and is stable at -20°C. I freeze this in 1 ml aliquots and thaw and refreeze as needed.)
4. Perkin Elmer Amplitaq Gold
5. genomic DNA samples diluted to 50 ng/µl with sterile water

PCR reaction mixture:

15.5 µl sterile water
2.5 µl 10X PCR buffer
4 µl dNTP premix
0.75 µl primer GKP2
0.75 µl primer GKP8
1 µl dil. DNA template
0.2 µl Amplitaq Gold
25 µl total volume

Cycling conditions:

1 cycle - 94°C x 6 min.
40 cycles - 94°C x 1 min., 60°C x 30 sec., 72°Cx 30 sec.
1 cycle - 72°C x 7 min.
hold at 4°C.

Analysis of PCR products:

Load 10 µl aliquots of reactions + 2 µl gel loading buffer in a 1% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down.

Sample gel illustrating amplicons for $gk^{A456V.-Neo}$ & gk^{wt} alleles. .

GKP2 + GKP8 sample gel