Screening for the Rhd KO

Screening the KO insertion system is more complicated than screening for replacement homologous recombination, as the Bradley lab 3'hprt insertion vector system used depends on gap repair targeting as part of a strategy to identify correct insertions during ES selection, and the deletion is repaired when the insertion is in the correct gene.
Two types of PCR are used, one detecting the KO insertion, and one detecting the presence of a WT allele.The two are necessary to differentiate KO from heterozygotes.
Multiplex assays to test for both alleles simultaneously could not be set up at the time the line was established (a drawback to correct identification if the DNA quality does not allow long amplification) - so protein expression on RBC was systematically verified.

1. Screening for the **Rhd KO vector insertion**:

One primer is at the extremity of the Rhd sequence
5'-CAG-GTT-CAC-ATC-CAC-AAT-GCA-G-3'
and one primer in 3'hprt,
5'-GCT-TAA-TGC-GCC-GCT-ACA-GG-3'

the PCR cycle is
94°C-2' /
94°C-1';58°C-2'; 72°C-1' for 35cycles/
72°C-7'

the amplified fragment is #200bp.

2. Screening for the **Rhd WT chromosome** :

No polymorphism was found for Rhd. To distinguish homo- and heterozygotes a PCR which amplifies the region homologous to the Rhd insert in the vector on the wt chromosome is used. This yields a # 7kb amplicon

the primers are :
5'-GAT-GCC-TCA-CAA-CTA-TCA-GTA-CAG-TTA-CAG-3'
and
5'-GTC-TTG-TAT-CTG-ACC-TAA-GAC-AGT-GTC-CAT-3'

the PCR cycle is
94°C-1' /
94°C-20s'; 68°C-15' for 14 cycles/
94°C-20s'; 68°C-15'+15s/cycle for 16cycles/
72°C-10'