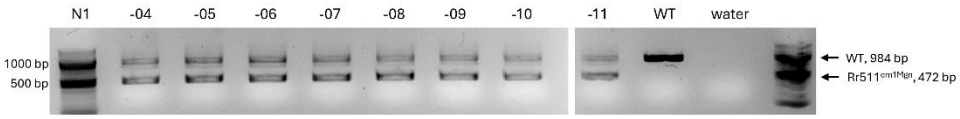
Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: Rr511^{em1Mgn}/Eomes$^{\Delta 1}$

Primer name	Sequence (5' to 3')	Primer
Eom-enh1_5'gtest-FWD	GGTTGCCAGAAGATATACCTGAG	WT and mutant forward
Eom-enh1_3'gtest-REV	GTCCGAGTTTCCCCCAAAGTA	WT and mutant reverse

Expected products:
WT = 984 bp
Rr511^{em1Mgn} = 472 bp



Reaction: Q5 High-Fidelity DNA Polymerase, NEB #M0491L

Component	Final Concentration
5x Q5 reaction buffer	1x
10 mM dNTPs	200 µM
10 µM forward primer	0.5 µM
10 µM reverse primer	0.5 µM
Genomic DNA template	1 µl of tail lysate or purified DNA < 100 ng/µl
Q5 High-Fidelity DNA Polymerase	0.02 U/µl

Thermocycler settings:

Program step	Temperature	Time
1	98°C	1 min
2	98°C	15 sec
3	66°C	15 sec
4	72°C	30 sec
5	72°C	30 sec, go to step 2 for 35 cycles
6	4°C	final hold (16°C for overnight)