Genotyping protocol

General information:

Strain name	Q

Primers:

Name	Sequence	Primer type
E2	tggaggtctactacgaaggc	please select one
R1	gaggtgggcacagactaatc	please select one
F1	atcgggaattcagactgctg	please select one
		please select one

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
e.g. wt	E2	R1
e.g. mut	F1	R1

Reaction mix:

Enzyme	10	µl
Forward primer (10uM)	1	µl
Reverse primer (10uM)	1	µl
Water	6	µl
DNA	2	µl
		µl
		µl
		µl
Final volume		µl

PCR program:

95	°C	5	min	
95	°C	30	sec	X30
62	°C	30	sec	
72	°C	30	sec	
72	°C	2	min	

Expected fragment size:

wt	460	bp
mutant	410	bp

Comments/Additonal information:

wt and null alleles are detected in different PCR reactions. It's important to note that the PCR reaction for the detection of KO allele is also able to amplify wt and conditional alleles although due to the large molecular weight expected (around 1 kb) PCR can fail and is not advisable for the genotyping of these two alleles.
Check Martin et al., 2015 (EMBO J)