

GENOTYPING by Real-Time PCR PROTOCOL

CR885 Gabpa 1bp insertion (InDel)

Protocol:

Reagent/Constituent	Volume (µL)
Cat No./ID 204541	
QuantiTect Multiplex PCR Master Mix	5.0
Water	3.6
Probe mix	0.4
-21 µM Forward primer	
-21 µM Reverse primer	
-7 µM Mutant probe	
-7 µM Wildtype probe	
Sample	1.0
TOTAL VOLUME OF REACTION:	
	10.0 µL

Comments on protocol:

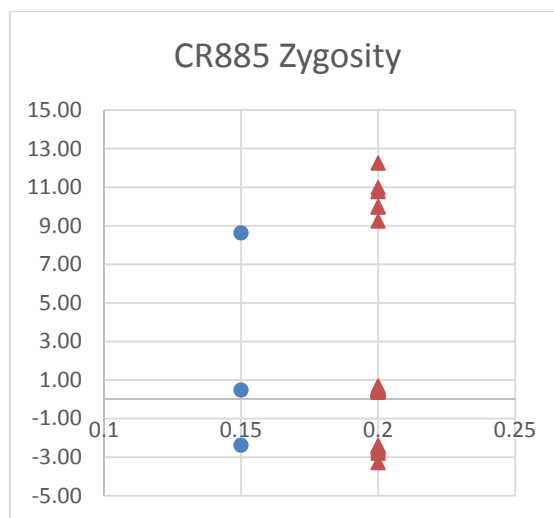
- Protocol may work with other DNA extraction methods or reagent mixes.
- Real-Time probes were ordered with 6FAM and VIC dyes. Both probes use Life Technologies minor groove binder non-fluorescent quencher (MGB-NFQ)

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	15:00	1
2. Denaturation	95	0:30	40x
3. Annealing/Elongation	60	1:00	40x
4. To step 2 for 40 cycles			

Primers:

Name	Nucleotide Sequence (5' - 3')
1. TM_CR885-F	CCATGACTAAGAGAGAAGCAGAAGAGC
2. TM_CR885-R	CCTTTCTTCTGTGCACTCTGCTTTCTC
3. TM_CR885-MGBF (Fam, mutant)	tgagattcgacggga
4. TM_CR885-MGBV (VIC, wildtype)	tgagatcgacgggac



most positive dCT cluster=WT
 most negative dCT cluster=HOM
 cluster between WT and HOM=HET