Melt probe design based on I491F Primer Set 6

Reagents we already have…

Primer set 6:

MEP352 FWD: GATGTGCCCGATCGAAACC

MEP353 REV: GCGTACACCGACAGCGA

WT Amplicon: 66

TCACGTGAGCGTGCCGGGCTGGAGGTCCGCGACGTGCACCCGTCGCACTACGGCCGGATGTGCCCGATCGAAACCCCTGAGGGGCCCAACATCGGTCTGATCGGCTCGCTGTCGGTGTACGCGCGGGTCAACCCGTTCGGGTTCATCGAAACGCCGTACCGCAAGGTGGTCGACGGCGTGGTTAGCGACGAGATCGTGTA

I491F Amplicon: 66

TCACGTGAGCGTGCCGGGCTGGAGGTCCGCGACGTGCACCCGTCGCACTACGGCCGGATGTGCCCGATCGAAACCCCTGAGGGGCCCAACATCGGTCTGTTCGGCTCGCTGTCGGTGTACGCGCGGGTCAACCCGTTCGGGTTCATCGAAACGCCGTACCGCAAGGTGGTCGACGGCGTGGTTAGCGACGAGATCGTGTA

Reagents to order…

([Theoretical](http://biotools.nubic.northwestern.edu/OligoCalc.html) Tm difs based on NN, 50mM salt, 250nM primer)

I491F Segment Set A: 27 bases, 0.2C Tm diff

WT: CCTGAGGGGCCCAACATCGGTCTGATC 65.46

WT RC: GATCAGACCGATGTTGGGCCCCTCAGG

I491F: CCTGAGGGGCCCAACATCGGTCTGTTC 65.26

I491F Segment Set B: 28 bases, 0.22C Tm diff

WT: CCTGAGGGGCCCAACATCGGTCTGATCG 67.5

WT RC: CGATCAGACCGATGTTGGGCCCCTCAGG

I491F: CCTGAGGGGCCCAACATCGGTCTGTTCG 67.28

I491F Segment Set C: 29 bases, 0.22C Tm diff

WT: CCTGAGGGGCCCAACATCGGTCTGATCGG 68.39

WT RC: CCGATCAGACCGATGTTGGGCCCCTCAGG

I491F: CCTGAGGGGCCCAACATCGGTCTGTTCGG 68.17

I491F Segment Set D: 30 bases, 0.23C Tm diff

WT: CCTGAGGGGCCCAACATCGGTCTGATCGGC 69.66

WT RC: GCCGATCAGACCGATGTTGGGCCCCTCAGG

I491F: CCTGAGGGGCCCAACATCGGTCTGTTCGGC 69.43