RRM2B-Q284X Sequencing Strategy

Mutation Details

RRM2B WT

GCTTATTTTATTGTTATTTTTTTGTTCCTTATTCTGATTTTGTTTGCATTCCAGGAGTTTTTAACAGAAG
CCTTGCCTGTTGGCCTCATTGGAATGAACTGTGTTTTGATGAAA**c**AGTACATTGAGTTTGTAGCTGAC
AGAtTaCTTGGAGAACTTGGATTCTCAAAGGTAAATAAAGTGTTTTAAAACATGTGACTAGTGGTTTT
AGTTACTGTTCCATTGCTGTGAAAAGATGCAAGGCAGCTTATCAAAGAAAACACGTGGTGGAGCCTT

RRM2B-Q284X-EM1-B6

GCTTATTTTATTGTTATTTTTTTGTTCCTTATTCTGATTTTGTTTGCATTCCAGGAGTTTTTAACAGAAG
CCTTGCCTGTTGGCCTCATTGGAATGAACTGTGTTTTGATGAAA**t**AGTACATTGAGTTTGTAGCTGAC
AGAcTtCTTGGAGAACTTGGATTCTCAAAGGTAAATAAAGTGTTTTAAAACATGTGACTAGTGGTTTT
AGTTACTGTTCCATTGCTGTGAAAAGATGCAAGGCAGCTTATCAAAGAAAACACGTGGTGGAGCCTT

Nucleotide change highlighted and in lower-case = nominated change, silent changes are only in lower case.

PCR is performed using KAPA fast Taq polymerase, although alternatives may be used.

RRM2B-Q284X amplification primers

Rrm2b_F1	GGTGCCCCGAGAATGTGAAAT
Rrm2b_R1	GGCCACAAACTTTCGATCCTTAG

PCR mix

KAPA Taq PCR master mix	5µl
Rrm2b_F1	0.5µl
Rrm2b_R1	0.5µl
H_2O	3.0µl
DNA	1µl

60TM30FA

1. 95ºC 1min.
2. 95ºC 10sec.
3. **60**ºC 10sec.
4. 72ºC 1sec.
5. Go to 2 for 29 cycles
6. 72ºC 30sec.
7. 16 ºC forever
8. end

RRM2B-Q284X sequence based primers

Rrm2b_F2	TCTGGCATACCTTGCCTTGAGT
Rrm2b_R3	AAACCTCTTGCGGTTTGCAGCG

PCR products selected for sequencing are to be purified and sent to Geneservice (Source Bioscience)

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