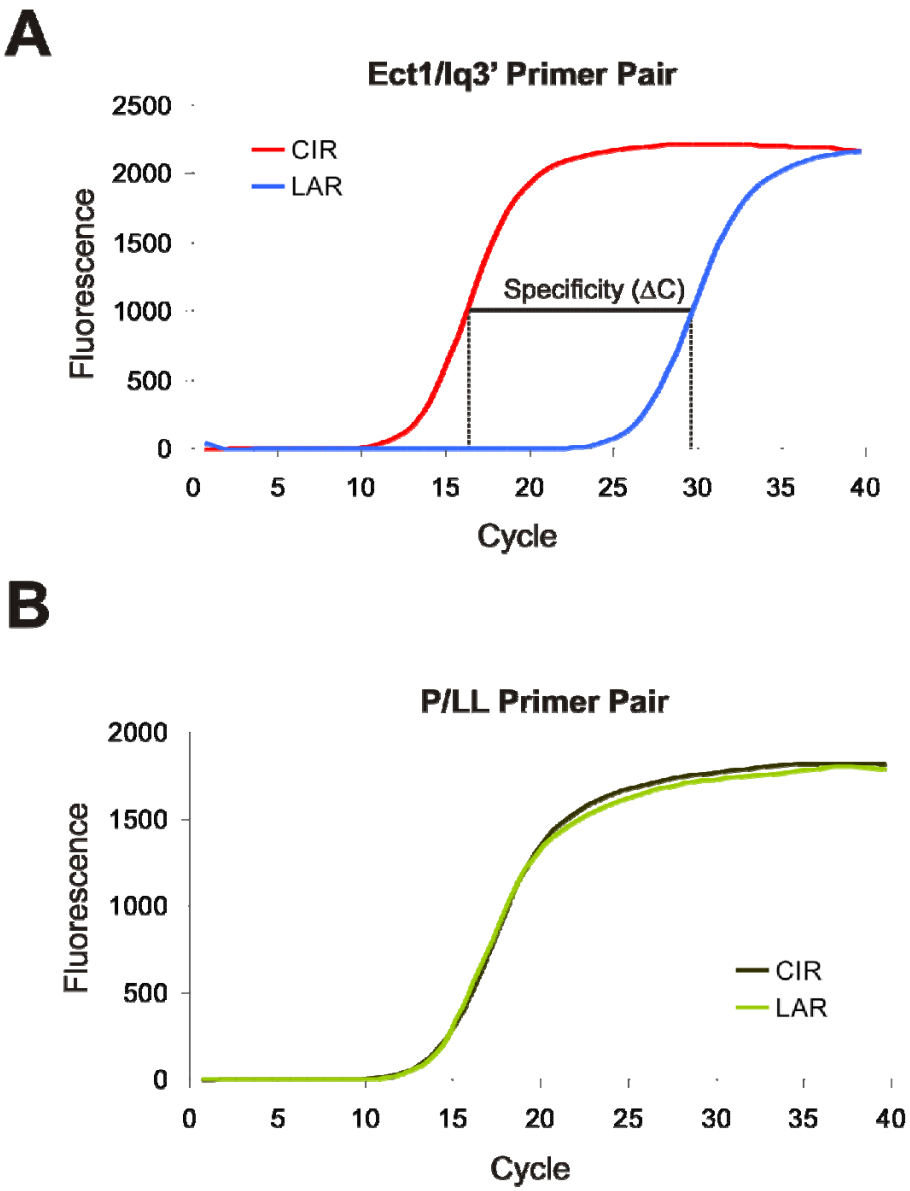
Supplementary Table 1. Oligonucleotides used in this work

OLIGONUCLEOTIDE		EXPERIMENTAL USE	REFERENCE
NAME	*SEQUENCE*		
dVI-ΔA	5′-CCTCCGGCCT_CTCACCCCTG-3′	Construction of ΔA mutant *	This work
dVI-AC	5′-CCTCCGGCCT**C**CTCACCCCTG-3′	Construction of AC mutant *	This work
dVI-pU	5′-GTTCTGCGAG**T**AGGCCGGCTG-3′	Construction of pU mutant *	This work
A330C	5′-ACCGGCGTAG**C**GGGGGCGTGA-3′	Construction of CL2 mutant *	This work
EBS3C	5′-GACCGGCGTA**C**AGGGGGCGTG-3′	Construction of EBS3C mutant *	This work
SacBbr	5′-GGGAGCTCACGTGCCTCGTTTTCATCGATGAGA-3′	Construction CL1 mutant **	21
CL/DN	5′-ATAACGACGCG**GGGCT**GGAGGGTTCACTTTCGTTC-3′	Construction of CL1 mutant **	This work
CL/UP	5′-GTGAACCCTCC**AGCCC**CGCGTCGTTATGAACGTAA-3′	Construction of CL1 mutant **	This work
pT7R	5′-TCGACCCTATAGTGAGTCGTATTACTCGAG-3′	Construction of CL1 mutant **	This work
5Q_CAT	5′-TGTACCTATAACCAGACCGTTCAG-3′	Construction of γ- γ’ mutant **	22
γC-rv	5′-TACGGGGCGATC**G**GCCCCAGTCTCCTGCATC-3′	Construction of γC mutant **	This work
γC-fw	5′-GACTGGGGC**C**GATCGCCCCGTATGAGCGTCGAGG-3′	Construction of γC mutant **	This work
γ’G	5′-CACCTAGGCCAGG**C**GTGAGTAGG-3′	Construction of γ’G mutant **	This work
6QR_LACZ	5′-GATGTGCTGCAAGGCGATT-3′	Construction of γ- γ’ mutant **	22
Ect1	5′-CACCTGCTCGGATCTCGTC-3′	Reverse transcription and qPCR	17
P	5′-TGAAAGCCGATCCCGGAG-3′	Primer extension and PCR lariat/circle detection	23
LL	5′-GAGGTTCACGCACCGTTCTG-3′	PCR lariat/circle detection	17
ICI	5′-GGATGACGAAACGGTCCTG-3′	Reverse transcription	This work
Iq3’	5′-CTCCTCCGGCCTACTCACC-3′	qPCR	This work

* The mutants were generated with the Altered Sites II *in vitro* Mutagenesis pAlter-1 System (Promega)

** The mutants were constructed by two-step PCR, with the Triple Master™ PCR System (Eppendorf)

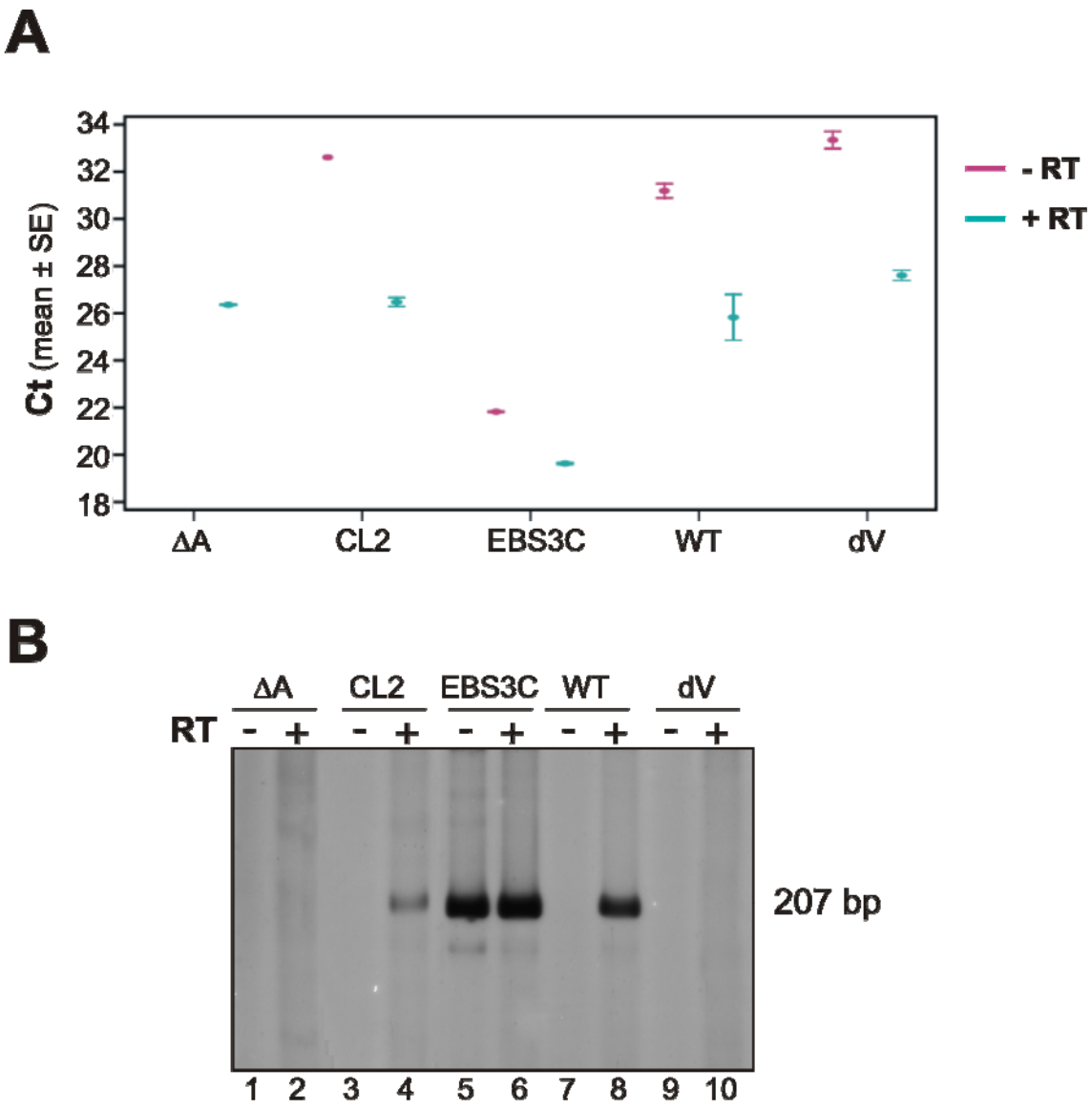
SUPPLEMENTARY FIGURES



Supplementary Fig. 1

Supplementary Figure 1. RT-qPCR specificity determination. The amplification plot represents fluorescence as a function of cycle number. Fluorescence was measured at the end of the elongation step of each cycle, in six replicates running on the same and on

separate plates, providing a measurement of variability within and between runs. The amplification refractory mutation system (ARMS) is based on the difference in efficiency of PCR primer extension between conditions in which the 3' end is matched and those in which it is mismatched to a template. The specificity of an ARMS test can be defined as the difference in C_ts (ΔC_t) observed on qPCR when the test is performed on equal amounts of template. Higher ΔC_t values are obtained for more specific, discriminatory tests. qPCR was performed with serial dilutions of DNA from two different templates: CIR and LAR, corresponding to PCR fragments containing the ligation point for the 5´and 3´ends and the lariat branch region, respectively. The amplification kinetics of the 1.45 x 10^7 dilution is shown. (**A**) Ect1/Iq3' primer pair: Ect1 hybridises with both templates, but Iq3' is complementary to the last six nucleotides of the intron; thus Iq3' efficiently amplifies the CIR template but not the LAR template. (**B**) P/LL primer pair: both oligonucleotides hybridise with the CIR and LAR templates. The two primer pairs are similarly efficient when CIR molecules are used as a template. (qPCR parameters: E > 95% and $R^2 > 0.990$)



Supplementary Fig. 2

Supplementary Fig. 2. Presence of DNA templates with linked intron 5′and 3′ends in RNA preparations. (**A**) Quantification in samples for constructs containing WT, domain V, EBS3C and representative mutants of the coordination loop and branch-site regions. The graph shows the threshold cycle (C_t) for the indicated constructs. Values are represented as means ± standard errors of three replicates from the same plate. qPCR

data for RNA samples not treated with reverse transcriptase were compared with the results obtained for cDNA samples. RT- samples showed amplification profiles closer to the final PCR cycles. A C_t value of 40 indicates a negative reaction, so the ΔA RT- level could not be shown on the graph. Most of the constructs displayed at least a 30-fold difference between RT- and RT+ amplification levels. Smaller differences were found for the EBS3C mutant, indicating the presence of DNA templates in which the 5´and 3´extremities are linked in RNA preparations. (qPCR parameters: E = 80.7% and R^2 = 0.994) (**B**) The migration pattern of products from the above RT-qPCR is shown, for correlation of the level of fluorescence with the appearance of amplicons. No band is observed in samples not treated with reverse transcriptase, other than the EBS3C mutant, for which a 207 bp band is visible. This same band is detected in RT+ lanes for WT intron RNA and CL2 and EBS3C mutants. No band appears in the dV or ΔA RT+ lanes.