**Cell transfection**

ASK RAGINI

**Total RNA extraction**

ASK RAGINI (standard Trisure protocol?)

**DNAse treatment of RNA samples**

### Total RNA samples were DNAse-treated treated using a TURBO DNA-free™ Kit (Ambion #AM1907) for 30 minutes at 37°C and according to manufacturer’s instructions.

**Reverse transcription**

250ng of DNAse-treated RNA were reverse transcribed with random hexamers (Invitrogen #N8080127) using SuperScript IIITM Reverse Transcriptase (Invitrogen #18080093) in a final volume of 20μl at 50°C for one hour. Control reactions lacking enzyme were systematically run in parallel as negative controls.

**Semi-quantitative PCR**

Semi-quantitative PCR reactions were performed on 1μl of diluted RT samples (1/2) with primers PI12\_F: GCTCACTCTCTTCCGCATC and PI12\_R: CTTGGCGTTCGGAGGATG using One TaqR DNA polymerase (New England Bioloabs #M0486S) and run on a thermo cycler with the following conditions: 1. Initial denaturation 2’ at 94°C; 2. {26 cycles} D 30” at 94°C, A 30” at 58°C, E 30” at 68°C; 3. Final extension 4’ at 68°C. PCR products were immediately loaded on a medium-sized 2% agarose gel pre-stained with Ethidium Bromide at a final concentration of 0.5 μg/mL, and run at 80V for one hour. Images were acquired on a BioRad Gel Doc system with exposure optimized for “faint bands” and ensuring not to overexpose the signal.

**Cloning and Sanger sequencing of PCR products**

PCR amplicons were excised from the gel, extracted using a ZymoCleanTM Gel Recovery Kit (Zymo Research #D4001), cloned into a TOPO vector (TOPOTM TA Cloning Kit for sequencing #450030) and Sanger-sequenced at Genewiz using a T7 fwd primer.