Confirming circRNA expression by qPCR

Quantitative PCR was performed using SYBR Green Master Mix (Thermo Fisher) on an ABI 7900HT instrument (Applied Biosystems). The divergent primer pairs flanking the back-splice sit are designed using Prime3 online primer design web tool (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). To confirm the expression of lcRNAseq-derived circRNAs in dopamine neurons and pyramidal neurons, relative abundances of target circRNAs were evaluated by qPCR in human substantia nigra or temporal cortex samples, as well as in human fibroblast and PBMC samples. The human ubiquitin gene UBC was used as a reference to normalize RNA loading. Control samples lacking template and those lacking reverse transcriptase showed virtually no expression of these target circRNAs indicating that DNA contamination did not materially influence results. Expression values were analyzed using the comparative threshold cycle method63. All the quantitative PCR reactions were conducted in triplicate. Equal amplification efficiencies for target and reference transcripts were confirmed using melting curve analysis.