Sample/Template	Details	Checklist
Source	If cancer, was biopsy screened for adjacent normal tissue?	
Method of preservation	Liquid N2/RNAlater/formalin	
Storage time (if appropriate)	If using samples >6 months old	
Handling	fresh/frozen/formalin	
Extraction method	TriZol/columns	
RNA: DNA-free	Intron-spanning primers/no RT control	
Concentration	Nanodrop/ribogreen/microfluidics	
RNA: integrity	Microfluidics/3':5' assay	
Inhibition-free	Method of testing	
Assay optimisation/validation		
Accession number	RefSeq XX_1234567	
Amplicon details	exon location, amplicon size	
Primer sequence	even if previously published	
*Probe sequence**	identify LNA or other substitutions	
In silico	BLAST/Primer-BLAST/m-fold	
empirical	primer concentration/annealing temperature	
Priming conditions	oligo-dT/random/combination/target-specific	
PCR efficiency	dilution curve	
Linear dynamic range	spanning unknown targets	
Limits of detection	LOD detection/accurte quantification	
Intra-assay variation	copy numbers not Cq	
RT/PCR		
Protocols	detailed description, concentrations, volumes	
Reagents	supplier, Lot number	
Duplicate RT	ΔCq	
NTC	Cq & melt curves	
NAC	ΔCq beginning:end of qPCR	
Positive control	inter-run calibrators	
Data analysis		
Specialist software	e.g., QBAsePlus	
Statistical justification	e.g., biological replicates	
Transparent, validated normalisation	e.g., GeNorm summary	