

ITEM TO CHECK	PROVIDED	COMMENT
	Y/N	
1. SPECIMEN		
Detailed description of specimen type and numbers	N	Not applicable to this study
Sampling procedure (including time to storage)	N	Not applicable to this study
Sample allocation, storage conditions and duration	N	Not applicable to this study
2. NUCLEIC ACID EXTRACTION		
Description of extraction method including amount of sample processed	N	Not applicable to this study
Volume of solvent used to elute/resuspend extract	N	Not applicable to this study
Number of extraction replicates	N	Not applicable to this study
Extraction blanks included?	N	Not applicable to this study
3. NUCLEIC ACID ASSESSMENT AND STORAGE		
Method to evaluate quality of nucleic acids	N	N/A
Method to evaluate quantity of nucleic acids (including molecular weight and calculations when using mass)	Y	See "Methods" section, "DNA samples" subsection
Storage conditions: temperature, concentration, duration, buffer, aliquots	Y	See "Methods" section, "DNA samples" and "Digital PCR" subsections
Clear description of dilution steps used to prepare working DNA solution	N	N/A
4. NUCLEIC ACID MODIFICATION		
Template modification (digestion, sonication, pre-amplification, bisulphite etc.)	N	Not applicable to this study
Details of resequencing following modification if performed	N	Not applicable to this study
5. REVERSE TRANSCRIPTION		
cDNA priming method and concentration	N	Not applicable to this study
One or two step protocol (include reaction details for two step)	N	Not applicable to this study
Amount of RNA added per reaction	N	Not applicable to this study
Detailed reaction components and conditions	N	Not applicable to this study
Estimated copies measured with and without addition of RT*	N	Not applicable to this study
Manufacturer of reagents used with catalogue and lot numbers	N	Not applicable to this study
Storage of cDNA: temperature, concentration, duration, buffer and aliquots	N	Not applicable to this study
6. qPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION		
Sequence accession number or official gene symbol	Y	See "Methods" section, "DNA samples" subsection
Method (software) used for design and <i>in silico</i> verification	Y	See "Methods" section, "Data analysis" subsection
Location of amplicon	N	N/A
Amplicon length	N	N/A
Primer and probe sequences (or amplicon context sequence)**	N	N/A
Location and identity of any modifications	N	N/A
Manufacturer of oligonucleotides	Y	See "Methods" section, "qPCR Assays"
7. qPCR PROTOCOL		
Manufacturer of qPCR instrument and instrument model	Y	See "Methods" section, "Digital PCR"
Buffer/kit manufacturer with catalogue and lot number	Y	See "Methods" section, "qPCR assays"
Primer and probe concentration	N	N/A
Pre-reaction volume and composition (incl. amount of template and if restriction enzyme added)	N	See "Methods" section, "Digital PCR"
Template treatment (initial heating or chemical denaturation)	Y	See "Methods" section, "Digital PCR" subsection
Polymerase identity and concentration, Mg ⁺⁺ and dNTP concentrations***	N	N/A
Complete thermocycling parameters	Y	See "Methods" section, "Digital PCR" subsection
8. ASSAY VALIDATION		
Details of optimisation performed	N	Not applicable to this study
Analytical specificity (vs. related sequences) and limit of blank (LOB)	N	Not applicable to this study
Analytical sensitivity/LoD and how this was evaluated	Y	See "Results" section, "Lower baseline, improved LoD, and more accurate MAF with real-time qPCR for EGFR 19del mutation detection assay" subsection
9. DATA ANALYSIS		
Testing for inhibitors (from biological matrix/extraction)	N	Not applicable to this study
Description of qPCR experimental design	Y	See "Methods" section
Comprehensive details negative and positive of controls (whether applied for QC or for estimation of error)	N	N/A
Partition classification method (thresholding)	Y	See "Results" section, "Lower baseline, improved LoD, and more accurate MAF with real-time qPCR for EGFR 19del mutation detection assay" subsection
Examples of positive and negative experimental results (including fluorescence plots in supplemental material)	N	
Description of technical replication	Y	See "Results" section, "Lower baseline, improved LoD, and more accurate MAF with real-time qPCR for EGFR 19 del mutation detection assay" subsection
Repeatability (intra-experiment variation)	N	N/A (not goal, comp instruments)
Reproducibility (inter-experiment/user/lab etc. variation)	N	N/A
Number of partitions measured (average and standard deviation)	Y	See "Methods" section, "Data analysis" subsection
Partition volume	N	N/A
Copies per partition (λ or equivalent) (average and standard deviation)	N	See "Methods" section, "Data analysis" subsection
qPCR analysis program (source, version)	Y	See "Methods" section, "Digital PCR" subsection
Description of normalisation method	N	N/A
Statistical methods used for analysis	Y	See "Methods" section, "Data analysis" subsection
Data transparency		

Table S1. dMIQE2020 checklist for authors, reviewers and editors. Authors should fill detail whether information is provided. Where 'yes' is selected use comment box to detail location of information or to include the information. Where 'no' is selected use comment box to outline rationale for omission. Sections 4 and 5 may not apply depending on experiment.

* Assessing the absence of DNA using a no RT assay (or where RT has been inactivated) is essential when first extracting RNA. Once the sample has been validated as DNA-free, inclusion of a no-RT control is desirable, but no longer essential.

** Disclosure of the primer and probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information when it is not available assay context sequences must be submitted (Bustin et al. Primer sequence disclosure: A clarification of the miqe guidelines. Clin Chem 2011;57:919-21.)

*** Details of reaction components is highly desirable, however not always possible for commercial disclosure reasons. Inclusion of catalogue number is essential where component reagent details are not available.