Comparison of SARS-CoV2 N gene real-time RT-PCR targets and commercially available mastermixes

Julianne R Brown[a], Denise M. O'Sullivan[b], Divya Shah[a], Laura Atkinson[a], Rui PA Pereira[b], Alexandra S Whale[b], Eloise J Busby[b], Jim F Huggett[b,c], Kathryn Harris[a]

SUPPLEMENTARY METHODS

Preparation of RNA Transcript:

A genetic construct containing the partial sequence of the SARS-CoV2 nucleocapsid (N) gene (location: SARS-CoV-2 genome region 28274-29239 MN908974.3) was synthesised and inserted into a pEX-A128 plasmid vector by Eurofins MWG Operon. 1 µg of plasmid was linearised using the restriction enzyme *BamHI* (New England Biolabs) (0.4 units/µL), 1X CutSmart Buffer (New England Biolabs) and nuclease free water (Ambion) in a reaction volume of 50 µL following incubation at 37°C for 1 hour. The linearised plasmid was purified using the QIAquick PCR purification kit (Qiagen) according to manufacturer guidelines. The plasmid was analysed using the Agilent 2100 BioAnalyzer with DNA 7500 series II kit (Agilent) to confirm the expected fragment length of 3450 bp, and quantified using the High Sensitivity dsDNA assay with the Qubit® 2.0 Fluorometer (ThermoFisher).

An RNA transcript was prepared from the linearised plasmid by *in vitro* transcription using the T7 RNA polymerase (MEGAScript T7 kit, Ambion Life Technolgies) according to manufacturer's guidelines. Subsequent purification using the RNeasy Mini Kit for RNA clean up (Qiagen) was performed which included an on-column DNase I step (RNase-free DNase set, Qiagen). Dilutions of the *in vitro* transcribed RNA were prepared in RNA Storage solution (Thermo Fisher) and aliquots stored at -80°C for further use. This prepared material was analysed using the Agilent 2100 BioAnalyzer with RNA 6000 kit (Agilent) and quantified using the High Sensitivity RNA assay with the Qubit® 2.0 Fluorometer (ThermoFisher). Total molecular weight (MW, g/mol) of the single stranded RNA transcript was estimated by multiplying the number of each nucleotide present (A, C, G, U) by the respective MW. Mass per RNA molecule (g) was calculated using the Avogadro number (6.022×10^{23} mol^{-1}). Copy number concentration in the stock RNA solution was calculated using the Qubit results and the mass per RNA molecule in g. Dilutions of the RNA transcript were prepared in nuclease-free water for RT PCR.

Sequence of the RNA Transcript

Partial N gene (+3G from T7 promoter and BamHI sequence from anti-sense DNA strand)
GGGAUGUCUGAUAAUGGACCCCAAAAUCAGCGAAAUGCACCCCGCAUUACGUUUGGUGGACCCUCAG AUUCAACUGGCAGUAACCAGAAUGGAGAACGCAGUGGGGCGCGAUCAAAACAACGUCGGCCCCAAGG UUUACCCAAUAAUACUGCGUCUUGGUUCACCGCUCUCACUCAACAUGGCAAGGAAGACCUUAAAUUC CCUCGAGGACAAGGCGUUCCAAUUAACACCAAUAGCAGUCCAGAUGACCAAAUUGGCUACUACCGAAG AGCUACCAGACGAAUUCGUGGUGGUGACGGUAAAAUGAAAGAUCUCAGUCCAAGAUGGUAUUUCUA CUACCUAGGAACUGGGCCAGAAGCUGGACUUCCCUAUGGUGCUAACAAAGACGGCAUCAUAUGGGUU GCAACUGAGGGAGCCUUGAAUACACCAAAAGAUCACAUUGGCACCCGCAAUCCUGCUAACAAUGCUGC AAUCGUGCUACAACUUCCUCAAGGAACAACAUUGCCAAAAGGCUUCUACGCAGAAGGGAGCAGAGGC GGCAGUCAAGCCUCUUCUCGUUCCUCAUCACGUAGUCGCAACAGUUCAAGAAAUUCAACUCCAGGCA GCAGUAGGGGAACUUCUCCUGCUAGAAUGGCUGGCAAUGGCGGUGAUGCUGCUCUUGCUUUGCUGC UGCUUGACAGAUUGAACCAGCUUGAGAGCAAAAUGUCUGGUAAAGGCCAACAACAACAAGGCCAAAC UGUCACUAAGAAAUCUGCUGCUGAGGCUUCUAAGAAGCCUCGGCAAAAACGUACUGCCACUAAAGCA UACAAUGUAACACAAGCUUUCGGCAGACGUGGUCCAGAACAAACCCAAGGAAAUUUUGGGGACCAGG AACUAAUCAGACAAGGAACUGAUUACAAACAUUGGCCGCAAAUUGCACAAUUUGCCCCCAGCGCUUCA GCGUUCUUCGGAAUGUCGCGCAUUGGCAUGCCUAG