TECHNICAL NOTE FOR SARS CoV2 OMICRON PRIMERS

The primer sequences designed by Bionivid specifically target a 9bp deletion in the N gene of SARS CoV2 Omicron variant (B1.1.529). This deletion has been observed in most (> 98%) of the 700 Omicron sequences (Source: GISAID) analyzed at Bionivid.

The designing of the primers is based on two selection methods - **Positive selection** and **Negative selection** method.

Assay Specificity for Positive selection

This primer set specifically targets the deleted region in N gene of Omicron variant and does not bind to any other SARS-CoV2 variants.

Target Genome	Target Gene	Primer Name	Forward Primer(5'-3')	Reverse Primer(3'-5')	Size(bp)
SARS-CoV2 (B1.1.529)	N	SARS-CoV2-OM-BIONIVID-P1	TAACCAGAATGGTGGGGCGCGATCA (Tm~67)	CTGGCCCAGTTCCTAGGTAG (Tm~58.2)	266
SARS-CoV2 (B1.1.529)	N	SARS-CoV2-OM-BIONIVID-P2	AGTAACCAGAATGGTGGGGCGCGAT (Tm~67)	CTGGCCCAGTTCCTAGGTAG (Tm~58.2)	266

Note: Since the forward primers have a higher Tm value (due to dominating GC regions), care has been taken to design primers that are specific to Omicron, are stable and have low propensity for primer dimer formation. We suggest setting an annealing temperature of 55-58°C for RT-PCR reactions and extension temperature around 72°C**.** Due to higher Tm value for positive primers, extended duration of Denaturation step i.e 95°C for 15-20 seconds is recommended.

Assay Specificity for Negative selection

In the negative selection method, the primers will bind to all SARS-CoV2 variants but not to Omicron sequences. So, a positive signal will be received for all SARS-CoV2 variants except Omicron.

Target Genome	Target Gene	Primer Name	Forward Primer(5'-3')	Reverse Primer(3'-5')	Size(bp)
SARS-CoV2 (B1.1.529)	N	SARS-CoV-2 Bionivid N1	AGTAACCAGAATGGAGAACGCAGT (Tm~62.5)	CTGGCCCAGTTCCTAGGTAG (Tm~58.8)	277
SARS-CoV2 (B1.1.529)	N	SARS-CoV-2 Bionivid N2	ACCAGAATGGAGAACGCAGT (Tm~59.7)	CTGGCCCAGTTCCTAGGTAG (Tm~58.8)	273
SARS-CoV2 (B1.1.529)	N	SARS-CoV-2 Bionivid N3	CAGTAACCAGAATGGAGAACGC (Tm~61.0)	CGGGTGCCAATGTGATCTTT (Tm~63.1)	372
SARS-CoV2 (B1.1.529)	N	SARS-CoV-2 Bionivid N4	CAGTAACCAGAATGGAGAACGC (Tm~61.0)	CTGGCCCAGTTCCTAGGTAG (Tm~58.8)	278

Note: The set of primers can be used individually or in combination with other existing and standard SARS CoV2 specific primers for detection of the Omicron variant.

***in silico* Validation:**

in silico primer tests were performed on two websites, idtdna.com and genome.ucsc.edu. On idtdna.com (https://sg.idtdna.com/pages/tools/oligoanalyzer), the secondary structure of primers and amplicon and the self-and heterodimerization tendencies of each primer set were predicted. We performed *in silico* PCR on the genome.ucsc.edu site (https://genome.ucsc.edu/cgi-bin/hgPcr). *in silico* tests have also been performed on the ~700 Omicron sequences deposited in GISAID (https://www.gisaid.org/).

DISCLAIMER

The sequences are intended to be used for the purposes of respiratory virus surveillance and research. The recipient agrees to use them in compliance with applicable laws and regulations. The sequences are *in silico* validated and subject to wet lab validation. Every effort has been made to assure *in silico* based accuracy of the sequences but Bionivid cannot provide any warranty regarding their accuracy.

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