

comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16. In one embodiment, the probe comprising the sequence of SEQ ID NO:13 further comprises a 5' FAM fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:14 further comprises a 5' CAL Fluor Orange 560 fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:15 further comprises a 5' CAL Fluor Red 610 fluorophore and a 3' BHQ-2 quencher, and the probe comprising the sequence of SEQ ID NO:16 further comprises a 5' Quasar Blue 670 fluorophore and a 3' BHQ-2 quencher.

Provided is an isolated oligonucleotide not more than 40 nucleotides in length for use in a multiplex composition for detecting and serotyping dengue virus by amplification of at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, comprising:

(a) a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:7 and 9-21;

(b) a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting of SEQ ID NOS:7 and 9-21 by up to three nucleotide changes, wherein the oligonucleotide is capable of hybridizing to and amplifying dengue virus nucleic acids; or

(c) complements of (a) and (b).

In another embodiment, the disclosure includes an isolated oligonucleotide not more than 40 nucleotides in length comprising a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21; or variants thereof comprising a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21 by up to three nucleotide changes, wherein the oligonucleotide is capable of hybridizing to and amplifying and/or detecting dengue virus nucleic acids; or complements thereof.

Oligonucleotides may further comprise a detectable label. For example, the detectable label may be a fluorophore such as, but not limited to carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'- tetrachloro-4-7-dichlorofluorescein (TET). The oligonucleotide may further comprise a quencher such as, but not limited to a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyI, and a minor groove binder (MGB). In certain embodiments, the oligonucleotide is selected from the group consisting of: an oligonucleotide consisting of a

nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21 or a complement thereof.

Provided is a primer set comprising two or more isolated oligonucleotides for use in a multiplex composition for detecting and serotyping dengue virus by amplification of at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, comprising a primer selected from the group consisting of:

(a) an oligonucleotide consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOS:7 and 9-12;

(b) an oligonucleotide complementary to an oligonucleotide of (a); and

(c) a combination of an oligonucleotide selected from (a) and (b).

In one aspect, there is provided a kit for detecting and serotyping dengue virus in a biological sample, the kit comprising:

written instructions for identifying the presence and serotype of dengue virus; and

a combination of primer pairs, each primer pair comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, wherein said forward and reverse primers are not more than about 40 nucleotides in length, and wherein said combination of primer pairs is selected from the group consisting of:

(a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;

(b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;

(c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;

(d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;

(e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;

(f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;

(g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;

(h) a forward primer and a reverse primer each comprising at least 18 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting of (a)-(g);

12. The method of claim 6, wherein amplifying comprises reverse transcriptase polymerase chain reaction (RT-PCR), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), or a fluorogenic 5' nuclease assay, or a combination thereof.

13. The method of claim 7, wherein the primers and probes that are used for detecting dengue virus in a biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, a primer comprising the sequence of SEQ ID NO:12, a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

14. The method of claim 6, further comprising serotyping the dengue virus present in the biological sample by detecting the presence of at least one target sequence selected from the group consisting of a dengue-1 target sequence, a dengue-2 target sequence, a dengue-3 target sequence, and a dengue-4 target sequence.

15. A kit for detecting and serotyping dengue virus in a biological sample, the kit comprising:

written instructions for identifying the presence and serotype of dengue virus; and a combination of primer pairs, each primer pair comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, wherein said forward and reverse primers are not more than about 40 nucleotides in length, and wherein said combination of primer pairs is selected from the group consisting of:

- (a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;
- (b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;
- (c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;
- (d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;
- (e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;
- (f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;
- (g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;
- (h) a forward primer and a reverse primer each comprising at least 18 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting of (a)-(g);

(i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting of (a)-(g) in that the primer has up to three nucleotide changes compared to the corresponding sequence, wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay; and

(j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting of (a)-(i); and

(k) a combination of primers selected from the group comprising (a)-(j).

16. The kit of claim 15, further comprising at least one probe for detecting dengue virus in a biological sample, wherein the at least one probe is selected from the group consisting of a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, a probe comprising the sequence of SEQ ID NO:16, a probe comprising the sequence of SEQ ID NO:17, a probe comprising the sequence of SEQ ID NO:18, a probe comprising the sequence of SEQ ID NO:19, a probe comprising the sequence of SEQ ID NO:20, and a probe comprising the sequence of SEQ ID NO:21.

17. The kit of claim 16 comprising a set of probes for detecting dengue virus in a biological sample, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

18. The kit of claim 15 comprising a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.