

Sequential Quadriplex Real-Time PCR for Identifying 20 Common emm Types of Group A Streptococcus

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ABSTRACT We developed a sequential quadriplex real-time PCR-based method for rapid identification of 20 emm types commonly found in invasive group A Streptococcus (iGAS) strains recovered through the Centers for Disease Control and Prevention's Active Bacterial Core surveillance. Each emm real-time PCR assay showed high specificity and accurately identified the respective target emm type, including emm subtypes in the United States. Furthermore, this method is useful for rapid typing of GAS isolates and culture-negative specimens during outbreak investigations.

KEYWORDS group A Streptococcus, M protein, vaccine, emm typing, multiplex realtime PCR

roup A Streptococcus (GAS) causes both mild infections (pharyngitis, anitis, and lacksquare impetigo) and invasive diseases (acute rheumatic fever, rheumatic heart disease, and necrotizing fasciitis) worldwide (1). In the United States, nearly 24,000 invasive GAS infections were estimated in 2018 (https://www.cdc.gov/abcs/reports-findings/ survreports/gas18.html). GAS is an important cause of severe, life-threatening illness among the elderly population, particularly those individuals residing in long-term-care facilities (LTCFs) (2) and skilled nursing facilities (SNFs) (3). Currently, multiple vaccines are in early stages of development to protect against this medically important pathogen (4).

The M protein, encoded by the emm gene, is a major GAS virulence factor traditionally targeted in serotyping GAS isolates (5). In-house antisera and agglutination methods were used to identify up to 80 classical emm serotypes (6). More than 25 years ago, a method for deducing M serotypes based on the 5' variable region sequences of emm genes was described (7). This method was subsequently standardized at the CDC and became widely used worldwide (5). To avoid confounding with emm-like genes, the CDC's current whole-genome-sequence-based approach relies upon identification of gene sequence that is linked to the 5'-situated primer 1 (7). This reference method, which relies upon a 180-bp emm sequence encoding the surface-exposed M protein N-terminal 50 residues and 10 residues of conserved signal peptide (8), has identified over 250 emm types and 2,200 subtypes (https://www2.cdc.gov/vaccines/biotech/ strepblast.asp). Recently, a new reverse primer (CDC3) was designed and validated to replace the less specific primer 2 of the first emm typing scheme (7), with a resultant improvement of amplification specificity (9). Sequencing and whole-genome sequencing (WGS)-based methods (2, 3, 5, 10) are relatively costly compared to PCR approaches, especially for resource-limited settings, and are not always suitable for outbreak situations, in which fast results are important.

Here, we describe a TaqMan-based quadriplex real-time PCR strategy to rapidly identify 20 emm types (emm1, -2, -3, -4, -6, -11, -12, -28, -49, -59, -75, -76, -77, -81, -82,

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-83, -87, -89, -92, and -118) that were the most common among invasive GAS (iGAS) recovered through CDC's year 2015 invasive GAS surveillance (11).

MATERIALS AND METHODS

Bacterial isolates. All bacterial isolates were cultured on Trypticase soy agar (TSA) with 5% sheep blood plates at 37°C with 5% CO_2 . Genomic DNA was extracted from isolates using the Qiagen DNA minikit (Qiagen, Valencia, CA) as previously described (12), with a prelysis step. Briefly, a loopful of bacteria grown overnight from a blood agar plate was suspended in 50 μ l of lysis solution (lysozyme, 0.08 mg/ml, and mutanolysin, 150 U/ml) and incubated at 37°C for 1 h, followed by steps described in the kit manufacturer's user manual.

A total of 253 bacterial isolates were used in this study to validate the typing scheme. The specificity and sensitivity of each of the five quadriplex assays were validated with respective emm type GAS isolates collected from Active Bacterial Core surveillance (ABCs). Twenty isolates of known S. pyogenes emm types, including emm1, -2, -3, -4, -6, -11, -12, -28, -49, -59, -75, -76, -77, -81, -82, -83, -87, -89, -92, and -118, were used in assay optimization. Additionally, 189 isolates representing 179 subtypes of 80 emm types, 4 nontypeable GAS, and 6 group G streptococci (GGS) (Streptococcus dysgalactiae subsp. equisimilis) (6 subtypes of 5 emm types) were tested (see Table S1 in the supplemental material). The assays were further challenged for specificity with 44 non-S. pyogenes streptococcal and nonstreptococcal isolates representing 41 species, including the streptococci Streptococcus acidominimus, S. agalactiae, S. bovis, S. canis, S. didelphis, Streptococcus dysgalactiae subsp. dysgalactiae, S. dysgalactiae subsp. equisimilis, S. entericus, Streptococcus equi subsp. equi, Streptococcus equi subsp. zooepidemicus, S. gordinii, S. hyointestinalis, S. hyovaginalis, S. iniae, S. intermedius, S. milleri, S. mitis, S. mutans, S. oralis, S. ovis, S. parauberis, S. phocae, S. pluranimalium, S. pneumoniae, S. porcinus, S. salivarius, S. sanguinis, S. sinensis, S. thoraltensis, S. uberis, S. urinalis, and S. pseudopneumoniae (n = 4), as well as Enterococcus faecalis, Enterococcus faecium, Haemophilus influenzae type b, Neisseria meningitidis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Pseudomonas aeruginosa. Streptococcal species were from the CDC reference collection and were identified using standard approaches (13).

Real-time PCR. The *emm* type-specific 5' region for each *emm* type was selected by aligning all the available subtypes of each *emm* type from the CDC's *emm* database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm) using CLC Genomics Workbench 10.0. The oligonucleotides for individual *emm* types were designed by using PrimerQuest software (https://www.idtdna.com/pages/tools/primerquest) to capture all the subtypes in each *emm* type. The primers and probes with different reporting dyes (6-carboxyfluorescein [FAM], 6-carboxy-2,4,4,5,7,7-hexachlorofluorescein [HEX], 6-carboxy-X-rhodamine [ROX], and 1,1'-bis(3-hydroxyproyI)-3,3',3'-tetramethylindodicarbocyanine [Cy5]) and appropriate quenchers were synthesized at the CDC Biotechnology Core Facility. The oligonucleotide sequences with their chemistry and optimal concentrations are provided in Table 1.

Optimal concentrations for primers and probes were determined by using SYBR green and TaqMan real-time PCR methods, respectively. Optimal concentrations of each oligonucleotide set were optimized with various concentrations (100, 200, 300, 400, and 500 nmol/liter) of each oligonucleotide against 10-fold dilutions of DNA from targeted *emm* type isolates in a Stratagene Mx3005P real-time PCR instrument (Agilent, Santa Clara, CA). The concentrations were optimized to obtain the highest DNA dilution yielding a cycle threshold (C_7) value of \leq 35. Lower limits of detection for each oligonucleotide set were determined in both singleplex and multiplex formats using 10-fold serial dilutions of the targeted *emm* type control DNA. In total, 20 individual assays targeting 20 *emm* types were grouped into five quadriplex reactions (Table 2) based on their distribution in the United States (10). The reaction mixture (25 μ l) contained 12.5 μ l of 2× PerfeCTa multiplex qPCR supermix, QuantaBio (VWR, Radnor, PA), optimal concentrations of oligonucleotides (Table 1), and PCR grade water and DNA (5 μ l). Amplification was carried out with the following cycling conditions: 1 cycle of 95°C for 10 min and 40 cycles of 94°C for 15 s and 60°C for 1 min.

We also designed a synthetic DNA plasmid (Text S1) (Thermo Fisher Scientific; GeneArt), as described elsewhere (14–16), that was used as a positive control for all real-time PCRs. The synthetic DNA was designed to contain primer and probe binding regions for all 20 *emm* types, *S. pyogenes*-specific target *spy* as an internal positive control, and the *Erwinia* xeno assay for laboratory contamination control, all concatenated in a single plasmid (14).

RESULTS

The sequential real-time PCR scheme consisted of five 4-plex reactions to identify 20 *emm* types, inclusive of all known subtypes within each type (see ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm/ for a current listing of *emm* subtypes). Optimal concentrations of both primers and probes (Table 1) were determined for each assay. Monoplex real-time PCR of DNA from 106 GAS isolates from the 20 *emm* types showed that the assays specifically amplified all subtypes assigned to the 20 *emm* types, with the caveat that the *emm49* reaction coidentified the closely related deletion derivative of *emm49*, *emm151* (Table 1). No amplification was observed for the additional 66 *emm* types or for any of the 4 GAS *emm* nontypeable isolates or the 44 non-*S. pyogenes* species used to challenge the specificity of the assays. When 4

TABLE 1 Oligonucleotides used in the multiplex real-time PCR emm typing assays

Enmil-P GTGATGGTARTCCTAGGRAACTT 400 Enmil-P FAMPUREGGGA BMOIGH TGTTGCTGCAGADNSpc6 00 Enmil-R CATTGCATTCCTAATCTGCTTT 400 Enmil-R CATTGCATTCCTAATCTGCTTT 400 Enmil-R CATTGCATTCCTAATCTGCACTGCAGADNSpc6 200 Enmil-R CGCAGACGTAAGCGCAGATG 300 Enmil-R CCCGACATTAGCTGCAGACT 300 Enmil-R CCCGATTTAGCTGCAGACT 300 Enmil-R CCCGATTTAGCTGCAGACT 300 Enmil-R CCCGATTTAGCTCCGAACT 300 Enmil-R CCCGATTGCACCTGARC 300 Enmil-R CCCGATTGCACCTGARC 300 Enmil-R CCCGAATTAGANCAGCGAACT 300 Enmil-R CCCGAATTAGANCAGCGAACGAACGACCAACCGCCGACGACGACGACGACGA	Name	Oligonucleotide sequence (5'-3')	Optimal concn (nM)
Enmil-P FAMUTRCGGGA BHQ1dT-GTTTGCTCCAMGADR/Spc6 100 Enmil-R CATCTAMGGGAALACTCAGATAACACCA 200 Enmil-P CASTTAMGGCGAACAGTAAGAACCC 200 Enmil-R CASTTAMGGCGAACGTAGAGACGAATA 200 Enmil-R GCCTCTTTCAACTTTACTCAATTTCCGATA 200 Enmil-R GCCTCTTTACACTTATACTCAATTCCGATA 200 Enmil-R GCCTCTTTCAACTTACCTGAGCGAGATG 300 Enmil-R CTGTTTCAACTTCACTTGAGCGGGAGAGG 300 Enmil-R CTGTTTCAACTTCACCTGAGCGGGAGAGG 300 Enmil-R CTGTTCACCTGAGAGCGGTAAGGGGTAGGGGAGGG 300 Enmil-R TCCCCTTCACCTGAGAGCGGGTAGGGGAGGG 300 Enmil-R TCCCCATCGCAATAGAGGGGAGGGGAGGG 300 Enmil-R TCCCCCATCGCAATAGAGGGGAGGGAGGGGAGGG 300 Enmil-R TCCCCCATCGCAGAACGGGGAGGGGAGGG 300 Enmil-R TCCCCCATCGCAGAACGGGGAGGGGAGGGGAGGG 300 Enmil-R TCCCCCATCGCAGAACGGGAGGGGAGGGGAGGGGAGGGG	Emm1-F	GTGATGGTARTCCTAGGRAAGTT	400
Emm.1-R CATTCCATTCCTATCTCCGTTT 400 Emm.2-F CATTCAGTCCACACGATAGACCC 200 Emm.2-R CCTATAGCGCACACGTAGACCCCCCACACCCCCCCCCC 200 Emm.2-R CCTCCTTCTCACACTCCCACTCCCCACACCCCCCCCCC	Emm1-P	FAM/TRCGGGA-BHQ1dT-TGTTTGCTGCAAGADR/Spc6	100
Emm.2-F. CASTIAAGGCGAACATAAGAACC 200 Emm.2-P. CySIAATTAG-BIAJCATGAACCAGAATTACATGACAAASpc6 200 Emm.2-R GCCTCTTTCAACTTACTATATTCCCGAATA 200 Emm.3-R GCCAGACAGTAAGGCAGATG 300 Emm.3-R CCTGATTCAACTTACTCAATTCC 300 Emm.3-R CCTGATTCAACTTCCACTTC 300 Emm.4-R CTGATTCAACGCYGGAACT 300 Emm.4-R CTGATTCAACGCYGGAACT 300 Emm.4-R TCACGCTTGCAAUTTCAACGCYGTGASTTVASGAAAATGAGG/Spc6 300 Emm.4-R TCCACTTCCACTTGGAAUTTAAAACAGG 300 Emm.4-R TCCACTTCCACTTGGAAUTTAATAGCT 300 Emm.4-R CS/STGAAGTTAEAAGGCGCAACGCGTACGCASpc6 300 Emm.4-R CGCACATTAATTAGCT 300 Emm.1-R TCACCCATTCACAGCACAATTAATTAGCG 300 Emm.1-R CGCCACATTAATTAGCT 300 Emm.1-R AGATCATTAGTGCACAGCACATTAATTAGCG 300 Emm.1-R CACTCCCATACGCACACACATTAATTAGCGC 300 Emm.1-R CACTCCCCATACGCACACACACACACGCASpc6 300 Emm.1-R AGATCATTAG	Emm1-R	CATTGCATTCTCTAATCTCGCTTT	400
Enm.2.P. Cy5/AANTTAAG-BHQ2GT GAAGCAGATTACTACTGACAATASpc6 200 Enm.3.P. GCAGACAGTAAAGCAGAATG 300 Enm.3.P. CGCAGACAGTAAGCCAGATG 300 Enm.3.P. RDX/TRGGAGTGT-BHQ2dT-AATGVAGACHTYCTAGRCA/Spc6 300 Enm.3.P. CCTGATTHTACAAGTTCTCAGATTC 300 Enm.4.F CCTGATTHTACAAGTTCTCAGTTC 300 Enm.4.P CYS/CTBAARAA.BHQ2dT-ATAACGYGTASTTVASGAAAATGAGG/Spc6 300 Enm.4.F TATTCGCTTGGAACTACGGCAGT 300 Enm.4.R TATTCGCTTGAACAACAGAGACG 300 Enm.4.R TATTCGCTTAGACAACAGAGAG 300 Enm.4.R TATTCGCTTAGACAGAGAGG 300 Enm.1.F TATTCGCTTAGACGAACGGAAGG 300 Enm.1.P HEXTYCTAAAGS-BHQ1dT-AMAAACGGACGCASpc6 300 Enm.1.P HEXTYCTAAAGCAAGAGAGG 300 Enm.1.P ATAGTTTGCTAAGCAGAGCAGGCAGGAGG 300 Enm.1.P HEXTYCTAAAGCAAGAGAGCOTTCAGSpc6 300 Enm.1.P HEXTYCTAAAGCAAGAAGCAGTTAGGAGCAGG 300 Enm.1.P HAMAGTTGGGGTGTGTAGGAGGAGG 300 Enm.1.P<	Emm2-F	CAGTTAAGGCGAACAGTAAGAACCC	200
Enm.2-R GCTCTTCAACTTACTCAATTCCGAATTA 200 Enm.3-F GCACACGTAATAAGGCCAGATG 300 Enm.3-P ROXTREGGAGTGT-BHQ21T-AATGVAGAGTTTYCTAGBCA/Spc6 300 Enm.3-R CCTGATTTACACGVGGAACT 300 Enm.4-F CTGRTTCAACCTTCACCTTCAC 300 Enm.4-F CTGRTTCACCTGVGGAACT 300 Enm.4-F CTGRTTCACCTGAGACTAC 300 Enm.4-R TYCACGTTCAACCTTGACG 300 Enm.4-F CTGRTTCACCTGAGACACGGAGTAGCGAGAGG 300 Enm.4-F CYSTGAAAATTAAAAACAGG 300 Enm.4-F CSSTGCAGAATTATTATACCT 300 Enm.1-F GCCTTGCAAATTATATACGCGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	Emm2-P	Cy5/AAATTAAG-BHQ2dT-GAAGCAGAATTACATGACAAA/Spc6	200
Emm3-P GCA6ACAGTAAAGCCA6ATG 300 Emm3-P RDX/TRG6AGTGF*PH02/T-AATCACKACAGTTYC/LGRCA/Spc6 300 Emm3-R CCTGATHTAACAAGTTCTCAGTTC 300 Emm4-P Cy/GCTBAARAA.BH02/0T-ATAACGYGTASTTVASGAAAATGAGG/Spc6 200 Emm4-P Cy/GCTBAARAA.BH02/0T-ATAACGYGTTASTTVASGAAAATGAGG/Spc6 300 Emm6-P CY/GCTBAARAA.BH02/0T-CAACAGACGYGTTYCTRGGG/Spc6 100 Emm6-P TATTCCCTTGAACAGCAGACGYGTTYCTRGGG/Spc6 300 Emm1-R TGTTAASTGTCATHATAGCTT 300 Emm1-R TGTAASTGTCATHATAGCT 300 Emm1-R TGTACACCATAGGACGATTTATAGGG 300 Emm1-R TGTACACCATAGGACGATTTATAGGG 300 Emm1-R HEXTYCTCAAAGS-BH02/1-GCAACACACGCDTCG/Spc6 300 Emm1-R PGCTAGAGATTTAGTGAAGGTT 300 Emm1-R ATAGTTTTCGTAGAGTTAGTTAGGACGGT 400 Emm2-R FAMAGYTGM-BH02/HC-GCAACACRGCDTCG/Spc6 100 Emm2-R FAMAGATTAGCAGATTAGTTCGTGTCTCTTGTAGGACGAGT 400 Emm3-R CCTACTCTWCCGAAGGATTTAGTCGACGAGTAGTTAGTCG/Spc6 100 Emm3-R TGCAGAGAATAACGGTBH0	Emm2-R	GCTCTTCTTCAACTTTATCTAATTTCTCGAATA	200
Emm3-P ROX/TRGGACTGT-BH02/TF.ATGVAGACTTYCTAGRCA/Spc6 300 Emm3-F CCGRTTCAGCCYGGAACT 300 Emm4-F CGRTTCAGCCYGGAACT 300 Emm4-F CGRTTCAGCCYGGAACT 300 Emm4-F CYGCTCAACAACAACGACTTAGCYCTASTTVASGAAAATGAGG/Spc6 300 Emm4-F TYCACGTTCAACTTCAACCYGTASTTVASGAAAATGAGG/Spc6 300 Emm4-F CYS/TGAAARA-MATMAAAACAGG 300 Emm6-F CTGCAATTAGCAACGAGAG 300 Emm1-F GGCTTGCAAATATTATAACCGCAGACGACGACSpc6 300 Emm1-F GGCTTGCAAATATTATAGCGCAGAA 300 Emm1-F GGCTTGCAAATATATATAGCGCAGAA 300 Emm1-F GGCTTGCCAATAGCGAACGACGACGACGACSpc6 300 Emm1-F ROX/TTGCAATGGAAGCGAGACGACGACGACSpc6 300 Emm1-F GGCTTGCCAATGGAAGCGACGACGACGACGACGACGACGACGACGACGACG	Emm3-F	GCAGACAGTAAAGGCAGATG	300
Emm3.R CCTGATHTAACAAGTTCTCAGTTTC 300 Emm4.P CYSCTRAARAA.BHO2DLATAACGYGTASTTVASGAAAATGAGG/Spc6 200 Emm4.P CYSCTRAARAA.BHO2DLATAACGYGTASTTVASGAAAATGAGG/Spc6 300 Emm4.P CYSCTGRAARAA.BHO2DLATAACGYGTASTTVASGAAAATGAGG/Spc6 300 Emm6.P CYSCTGRATTAGAAAATTAAAAATCAGG 300 Emm6.P CYSCTGAGTTAGAAAATTAACCTT 300 Emm6.P CYSCTGATTATAGCTT 300 Emm1.P HEXTYCTAAACGS-BH02HT-GCAACAGYCGTTYCTGGGG/Spc6 300 Emm1.P HEXTYCTTAAAGG-BH02HT-GCAACAGYCGTYCTGGGG/Spc6 300 Emm1.P HEXTYCTTAAAGG-BH02HT-GCAACAGYCGTYCTGG/GG/Spc6 300 Emm1.P HEXTYCTGAAGGACGATTATATGGGACGATTA 300 Emm1.P HEXTYCTGAAGGACGATATATGGGACGATTA 300 Emm1.P RCTCATCCGAACGACACAGACGACDTT 300 Emm1.P FAM/AGYTGM-BH01HT-GYATACACACACAGSCGTTACTG/Spc6 300 Emm2.P FAM/AGACGACHTACGATATGGACTATGCTACTG/Spc6 300 Emm2.P FAM/AGACACGACATTAGGACTGGATTATATGCG/ACCC/Spc6 300 Emm3.P.P FAM/AGACACGACTTACGTGTATATGGACGACT/Spc6 300	Emm3-P	ROX/TRGGAGTGT-BHO2dT-AATGVAGAGTTTYCTAGRCA/Spc6	300
Enmah-F CTGRITTCAGCCYGGAACT 300 Emmah-P CyS/CTRAARAARAHACAGC 300 Emmah-R TYCAGCTTCAACTTARC 300 Emmah-R TYCAGCTTCAACTTARC 300 Emmah-R CyS/CTGAATARAACTAARCAGCG 300 Emmah-P CyS/TGAAGTTAG-BH02dT-GAAGAGCGTTTCTGRGG/Spc6 300 Emm1-P HEXTYCTCAACGAAGTATATATACTT 300 Emm1-P HEXTYCTCAACGAGAGS-BH01dT-AMAAACGTGAKCGCA/Spc6 300 Emm1-P GGCTTTGCAAVCAACGAAG 300 Emm1-P RAGTCATACTGACTATTATATAGGA 300 Emm1-P RAGTCATACTGACTATTATATAGGACGACACGACGACGACGACGACGACGACGACGACGAC	Emm3-R	CCTGATHTAACAAGTTCTCAGTTTC	300
Emm4-P CyS/CTRAARAA-BHO2AT ATAACGYGTTASTIVASGAAAATGAGG/Spc6 200 Emm4-F TATTCGCTTAGAAATTAAAAACAG 300 Emm6-F CYS/CTAAGTTAGABHO2dT-GCAAGAGYGTTY/CTRGGG/Spc6 300 Emm6-P CyS/CTAAGTTAGBHO2dT-GCAAGAGYGTTY/CTRGGG/Spc6 300 Emm1-F GGCTTTGCGAAVCAAACAGAAG 300 Emm11-P HEX/TYCTAAGS BH02dT-GAAGAGYGTTY/CTRGGG/Spc6 300 Emm11-F GGCTTTGCGAAVCAAACAGAG 300 Emm11-F GGCTTTGCAAACGAGUCAAGAG 300 Emm12-F AGATCAIAGTGATTTAGTCGCAGAA 300 Emm12-F AGATCAIAGTCAATTAGTCGAGGAGUCATGAGAGAGUCATGAGAGAGUCAGAGAGUCAGAGAGUCAGAGAGUCAGAGUCAGAGUCAGAGUCAGAGUCAGAGUCAGAGUCAGUC	Emm4-F	CTGRTTCAGCGYGGAACT	300
EnmA+RTYCAGCTTCANCTTCARC300EnmA+RTYTCGCTTCANCTTACANCAGA300EnmA+RCySTCANGTTAG-BHQ2AT-GCANGAGYCTTYCTRGGG/Spc6100EnmA+RTGTAASYTGCTATATTACCTT300Enm11-PGGCTTTGCANAYCANCAGAAG300Enm11-RTTGCATASCAGAAGAGAG300Enm11-RTTGCATAGCGATTATTATATAGG300Enm11-RTTCATCCCATAGCGAATTATATAGG300Enm12-RAGATCATAGTGATATTAGCGACAAA300Enm12-RRAATCATAGTGATTATTAGCGACAAA300Enm12-RRAATCATAGTGACGACAAA300Enm12-RRAATCATAGTGACGACAAA300Enm12-RRAATCATAGTGACGACAAA300Enm28-PFAWAGYTGAMAGCTGATTA400Enm28-PFAWAGYTGAMAGCTGATTA400Enm29/151-PHEX/CGTATAGY-BHQ14F-CTTTTCTGCTGCTGGCACGC/Spc6300Enm49/151-PHEX/CGTATAGY-BHQ14F-CTTTTCTGCTGCTGGCACGC/Spc6300Enm49/151-PFAMAAATACGA-BHQ14F-CTTTCTCTGCTGCTGGCACGC/Spc6300Enm59-PFAMAAATACGA-BHQ14F-CATTGCTCCTCTGCAA300Enm59-PFAMAAATACGA-BHQ14F-CATTGCTGCTCTCTGGCAACGC/Spc6300Enm59-PFAMAAATACGA-BHQ14F-CATTGCTGCTAGGTATAGGTCT/Spc5100Enm57-FTATGAGCACGTCTTAGTGCCCTCCTGCA300Enm57-FTATGAGCACGTCTTAGCCTGCAACGAACGAACTAAGGTCT/Spc6300Enm77-PROXTGAGCACGCACGTATAGCCTTAGATAGGTT/Spc6300Enm77-PROXTGAGCACGCCTTAGCTAGACTAGAGTTAGGTCAGGCACGSpc6300Enm77-PROXTGAGCACGCAGGTATTGCC300Enm77-PROXTGAGCACGGAGGTATTGCC3	Emm4-P	Cy5/CTRAARAA-BHQ2dT-ATAACGYGTTASTTVASGAAAATGAGG/Spc6	200
Enmi6-F TATCSCTTAGAAATTAAAACAGG 300 Emmi6-F CySrGAAGTAGACAAGCAAGAGYCHTYCTBGGUSpc6 300 Emmi1-F TGTTAASYTGTCATTATTAGCT 300 Emmi1-F GGCTTTGCAAAGCAAAGCAAGAG 300 Emmi1-F HEXTYCTAAGS #RUQIT-AMAACGGCGACGCASpc6 300 Emmi1-F HCATCCCATAGCGAATTATAGG 300 Emmi1-F HCATCGCATAGCGAATTATAGG 300 Emmi1-F AGATCATTAGGCAACGAATTAGGCGCAGAA 300 Emmi1-F HCATCCCATAGCGAAGAACGGCATTATAGGCACCAGA 300 Emmi1-F HCATCCATAGCGCAAGAACGGCATTAGGCACGACGACGACGACGACGAACGA	Emm4-R	TYCACGTTCTACCTTGARC	300
Emm6-P Cys/TGAAGTTAG-BH024T-GCAAGAGY0TTYCTR/GGG/Spc6 100 Emm64 TGTTAASYTCATTATTAG/CTT 300 Emm11-F GGCTTGCAAAYCAAACAAAG 300 Emm11-F GGCTTGCAAAYCAAACAAAG 300 Emm11-R TTCATCCCATAGCGAATTATATAGG 300 Emm11-P TTCATCCCATAGCGAATTATATAGG 300 Emm11-P TTCATCCCATAGCGAATTATATAGG 300 Emm12-P AQXITTGVAAGCA-BH024T-GAAACGCCOTTCAG/Spc6 300 Emm12-P ACTCATCWCVGAATTCATGAACGTAGDDTT 300 Emm28-P FAM/AQYTGWABQCTGGTAG 400 Emm29-P FAM/AQYTGWBH01dT-GYATACAACACATTGCTTACTG/Spc6 300 Emm39-P FAM/AQYTGWBH01dT-GYATACAACACGTGCSpc6 300 Emm99/151-P TECCGAGAATAACGTGTMTA 400 Emm99/151-P TEXCCAAACGAAAGAGAGATAAGGTTAGG 300 Emm99/151-P TECCGAGAATAACGTGTMTA 400 Emm99/151-P TEXCGAGATAACGATAAGGTAGAGAATAAGGTTAGG 300 Emm99/151-P TEXCGAGATAAGGTGTAGTAG 300 Emm99/151-P TEXCGAGATAAGGTATAGAGATAAGGTTAGGAGATAGGTTAGAGATAGGTTAGAG 300 </td <td>Emm6-F</td> <td>TATTCGCTTAGAAAATTAAAAACAGG</td> <td>300</td>	Emm6-F	TATTCGCTTAGAAAATTAAAAACAGG	300
Emm6-R TOTAASYTGTCATTATAGCTT 300 Emm11-F GGCTTGCAAAGCAAG 300 Emm11-P HEXITYCTAAAGS BHQ1dT-AMAAACGTGGAKCGCA/Spc6 300 Emm11-R TTCATCCCATAGCGAATTATATAGG 300 Emm12-F AGATCATAGTGATTAGTGGCAGAAG 300 Emm12-P ROXITTGVAAGC-BHQ2dT-GAAAGTGGCDTCAG/Spc6 300 Emm23-P FAMMASYTGAAGC-BHQ2dT-GAAAGTGGCDTCAG/Spc6 100 Emm28-F GRCTTTGCAAAGTAGCDTT 400 Emm29-F FAMMASYTGAAGC-BHQ2dT-GAAACGCGC/Spc6 100 Emm39/151-P HEXCGTATAGY-BHQ1dT-CTTTTCTCTGCGAACGC/Spc6 300 Emm99/151-P HEXCGTATAGY-BHQ1dT-CTTTTCTCTGCGAACGC/Spc6 300 Emm99/151-P HEXCGTATAGY-BHQ1dT-GYTTTCCTGTCT 400 Emm99/151-P HEXCGTATAGY-BHQ1dT-GATATGTGCAACGC/Spc6 300 Emm99/151-P HEXCGTATAGY-BHQ1dT-GAAAGCAAGGAAGTTAAG 200 Emm99/151-P HEXCGTATAGY-BHQ1dT-GAAAGCAAGGAAGTTAAGTCTAAGGCT/Spc6 100 Emm39-F CTTAGCAAACGAAACGAAGTTAAGGCAAGGAAGTTAAGTTAAGTC/Spc6 300 Emm39-F TAATTAAAGCCAAGACGAAAGTAAGGTTAAGGTTAAGTTAAGTSpc6 300	Emm6-P	Cy5/TGAAGTTAG-BHQ2dT-GCAAGAGYGTTTYCTRGGG/Spc6	100
Emm11-F GGCTTGCAAAYCAAACAGAAG 300 Emm11-P HEX/TCTAAGCGAATTATATAGG 300 Emm11-R TTCATCCCATAGCGAATTATATAGG 300 Emm12-F AGATCATAGTGCAATTAGTCGACGAA 300 Emm12-F AGATCATAGTGCATTAGTCGCAGAA 300 Emm12-R ROXTTTGCAAAGGAAGTAGDDT 300 Emm22-R GRCTTYGCTAATGGARCTGRT 400 Emm22-R GRCTTYGCTAATGGARCTGRT 400 Emm22-R ACTCATCWCAGGTTCCTCATGT 400 Emm49/151-P TGCGGAGAATAACGTGTMTA 400 Emm49/151-P TGCGGAGAATAACGTGTMTA 400 Emm49/151-P TGCGGAGAATAACGTGTMTA 400 Emm49/151-P TGCGAGAATAACGTGTMTA 400 Emm49/151-P TTGCAAACCAAAGAGAAGATAAGCTGTAGT 200 Emm59-F CTTTGCTAATAGCTAATGTGAAGTAGAGAGTAAGGCT/Spc6 300 Emm59-F TAGTAACCAAAGAAGAGAGTTAAGG 300 Emm59-F TAGTAACCAAAGAAGAGAGTTAAGG 300 Emm59-F TAGTACAACCAAAGGAAGGTTAAGG 300 Emm59-F TAGTACAACCAAAGGAGAGTTAAGG 300	Emm6-R	TGTTAASYTGTCATTATTAGCTT	300
Emm11-P HEX/TYCTAAGS-BH01dT-AMAAKGCTGAKCGCA/Spc6 300 Emm11-R TTCATCCCATAGCGAATTATATGG 300 Emm12-P ROX/TTGVAAGGACBH02dT-GAAKGCGCTTCAG/Spc6 300 Emm12-R ATAGTATTCGTGAAGGTAGACTGRT 400 Emm22-R GRCTTYTGCTAATGGARCTGRT 400 Emm22-R GRCTTYTGCTAATGGARCTGRT 400 Emm23-R ACTCATCWCYGAGTTTCTCATGT 400 Emm39/15-P TEXCGAGACATACGTGTMTA 400 Emm49/151-P TCGGGAGAATACGTGTMTA 400 Emm49/151-P TCGGGAGAATACGTGTMTA 400 Emm39/F CTATCATCWCYGAGTTTCTCATGT 400 Emm59/F CTATGCAACCAAACGAAGTTAAG 200 Emm59/F CTATGCAACGAAAGGTAAG 300 Emm59/F TAATTAAATAGTTATCTCTCTCTTCTAATGGAGAATAAGTTMAGT 200 Emm59/F TAGGACAYATAACAGTAAGGTGAAGAGTTAAGG 300 Emm59/F TAGCACCAAACGAAGCATGGG 300 Emm59/F TAGCACCAAACGAAGAGTTAAGG 300 Emm59/F TAGCACACAACACAGAGATTAGG 300 Emm79/F TGCAACCAAACCAAAGAGTTAAGG	Emm11-F	GGCTTTGCAAAYCAAACAGAAG	300
Emm11-R TCATCCATAGCGAATTATATAGG 300 Emm12-F AGATCATAGCGAATTAGTCGCAGAA 300 Emm12-P ROXITTGVAAGAC-BHQ2dT-GAAACRGCDTTCAG/Spc6 300 Emm12-R ATAGTATTGCTGAAGGTAGDDTT 300 Emm12-R ATAGTATTGCTGAAGGACACATTGRT 400 Emm28-P FAMVAGYTGM-BHQ1dT-GYATACAACACATTGCTTACTG/Spc6 100 Emm29/151-F* TGCGGAGAATTAACGTGTMTA 400 Emm49/151-F* TGCGGAGAATTAACGTGTMTA 400 Emm49/151-F* TGCGGAGAATTAACGTGTMTA 400 Emm49/151-F* TCTGCGACAATAACGTGTMTA 400 Emm39/F FAMVAATTAGX-BHQ1dT-CTTTCTTCTTGTGCAACGC/Spc6 200 Emm39/F CTATTGCGAACCAAACAGAAGTAAG 200 Emm39/F FAMVAATACGA-BHQ1dT-GYATGACTAAGGTAGAGTT/Spc6 100 Emm59-F TATGAACCAYARTACAGAAGCATGG 300 Emm75-F TATGAACCAAACAGAAGTTAAGG 300 Emm75-F TATGAACCAAACAGAAGATTAAGG 300 Emm75-F TATGAACCAAACAGAAGATTAAGG 300 Emm75-F TATGAACCAAACAGAAGTTAAGG 300 Emm75-F	Emm11-P	HEX/TYCTAAAGS-BHO1dT-AMAAACGTGAKCGCA/Spc6	300
Emm12-F ACATCATAQCTEATTTAGTECGCAGAA 300 Emm12-F ROXTTTOVAAGC.=HDQ2dT-GAAACGDTTCAG/Spc6 300 Emm12-R ATAGTATTGCTGAAGGTAGDDTT 300 Emm28-F GRCTTTGCTGAAGGTAGDDT 400 Emm28-F GRCTTTGCTGAAGGTAGDDT 400 Emm28-F FAMIACSTIGHTAGARCTGRT 400 Emm49/151-P TGCGGACAGATACGCTGTATGT 400 Emm49/151-P TGCGGACAGATACGCTGTGTATTAT 400 Emm49/151-P TGCGGACAGATACGCTGTGTATTATCTCGTCTCTCTGCACGC/Spc6 300 Emm49/151-P TGCGGACAGATACGCTAFAGTGTATGGACAACGACGC 400 Emm59-F CTTATCACAAPABQ1CTCTCTCTCTCTCTCTGCACGC/Spc6 300 Emm59-F FAMIAATACGCABPIQ1dT-CYATTGACTAATGGACATAAGGTCT/Spc6 300 Emm75-F TATGAGCACAPRATACGAABQ11C-CYATTGACTAAGGTAGGACGACGACGACGACGACGACGACAPAGACACCAAACGAAGAGAAGTAAGGACCTTAGGACACTTAGGACAACGACAGACA	Emm11-R	TTCATCCCATAGCGAATTATATAGG	300
Emm12-P ROX/TTGV/AGAC-BHQ2dT-GAAACRGCDTTCAG/Spc6 300 Emm12-R ATAGTATTGCTGAAGGTAGDDT 300 Emm28-F GRCTTTTGCTAATGGAACTGRT 400 Emm28-P FAM/AGYTGM-BHQ1dT-GYATACAACACATTGCTTACTG/Spc6 100 Emm28-R ACTCATCWCYGAGTTTCTCCATGTT 400 Emm49/151-P TEXCGGAGAATAACCTGTMTA 400 Emm49/151-P TEXCGGAGAATAACCGTGTMTA 400 Emm49/151-R CCTATTCTTTCTAGATATTCTCCTGTTCTTGCAACGC/Spc6 300 Emm59-F CTTTGCAAACCAAACAGAAGTAAG 200 Emm59-F CTTTGCAAACCAAACAGAAGTAAG 300 Emm59-F FAM/AAATAGC3-BHQ1dT-GYATTGACTAATGAGATTAACGTC/Spc6 300 Emm59-F FAM/AATAGCA-BHQ1dT-GYATAGAGATTAACGCT/Spc6 300 Emm75-F TATGAAGCAYRATACGAAGGACCTTGCTCA 300 Emm75-F TAGAAGCAAACAGAAGAGTAAGG 300 Emm76-F TCGAAACCAAACAGAAGAGTAGG 300 Emm77-F TGCAAACCAAACGAGAGTTAGG 300 Emm77-F TGCAAACCAAACGAGAGTTAGG 300 Emm77-F TGCAAACCAAACGAGAGTTAGGTAGGACACGC/Spc6 300	Emm12-F	AGATCATAGTGATTTAGTCGCAGAA	300
Emm12:RATAGTATTGCTGAAGGTAGDDT300Emm28:FGRCTTYTGCTAATGGARCTGRT400Emm28:PFAM/ACYTGM-BHQ1dT-CYATACAACACATTGCTTACTG/Spc6100Emm49/151:PTGCGGAGAATACGTGTTGTTATA400Emm49/151:PTGCGGAGAATACGTGTTATTAT400Emm49/151:PHEX/CGTATAGY-BHQ1dT-CTTTTICTCTTGCTACGC/Spc6300Emm49/151:PCCTATCTTTCTTCTAGATTATCCGT400Emm49/151:PCCTATCTTCTTCATAGT-BHQ1dT-CTTTTICTCTTGTGCACGC/Spc6300Emm49/151:PCCTATCTTCTTCAGATAGTAAG200Emm59:FCTTAGAAGCAAACAGAAGTAAG300Emm59:FTAATTAAATTACAAGCASTGG300Emm75:PFAM/AATACGA-BHQ1dT-GTCTTGCTCTCTCTCTA300Emm75:PFAM/AATACGAAGGAAGTAAGGG300Emm75:PFAM/AATACCAAACAGAAGTAAGGC300Emm75:PTGCAAACCAAACAGAAGTAAGGC300Emm76:PTGCAAACCAAACAGAAGTAAGG300Emm77:PTGCAAACCAAACAGAAGTAAGG300Emm77:PROX/TGASCTACAGGC-BHQ2dT-GAACCTGATAAGYT/Spc6300Emm77:PROX/AGTGAGTAAGGATTAGG300Emm77:PROX/AGTGAGTAAGGTTAGGG300Emm79:PHEXTGCGGG-BHQ1dT-GCAACCTACGAGAGAACCC/Spc6300Emm81:FTGCAAACCAAACGAAGTTAGGG300Emm81:FTGCAAACCAAACGAAGTTAGT300Emm81:RTTSAAGTTGGCTAGTATAT300Emm81:RTTSAAGTTGGCTAGTATATG300Emm81:RTTSAAGTTGGCTAGTATATG300Emm81:RTTGAAGCCGAAGGTTATGGCAGGCTGATACC400Emm81:RTTGAAGCCGGAGGTGTATCTA<	Emm12-P	ROX/TTTGVAAGAC-BHO2dT-GAAACRGCDTTCAG/Spc6	300
Emm28-F GRCTTYTGCTATAGGARCTGRT 400 Emm28-P FAW/AGYTGCTATGGARCTGAT 400 Emm28-P ACTCATCWCVGAGTTTCTCATGT 400 Emm49/151-F** TGCGGAGATAACGTGTMTA 400 Emm49/151-F** TGCGGAGATAACGTGTMTA 400 Emm49/151-R CCTATTCTTCTAGATATTCTCCGT 400 Emm49/151-R CCTATTCTTTCTAGATATTCTCCGT 400 Emm59-F CTTTGCAAACGAAACGAAAGTTAAG 200 Emm59-F FAW/AATACGA-BHQ1dT-GTATTGCTCTCTCTCTCTAGATAGGAATAAGTCT/Spc6 100 Emm59-R TAATTAAACGAVRATACAAAGAAGCATGG 300 Emm75-F TAGGAAGCAVRATACAAAGAAGGATGGA 300 Emm75-R CTATCTCGTAGTCTTAGATAGGA 300 Emm76-F TGCAAACCAAACGAAGGATTAAGG 300 Emm76-F TGCAAACCAAACGAAGTTAAGG 300 Emm77-F TGCAAACCAAACGAAGTTAAGG	Emm12-R	ATAGTATTGCTGAAGGTAGDDTT	300
Emm28-P FAWAGYTGM-BHOLDT-GYATACAACACATTGCTTACTG/Spc6 100 Emm28-R ACTCATCWCYGAGTTTCTCATGTT 400 Emm49/151-P TGCGGAGAATAACGTGTIMTA 400 Emm49/151-P HEX/CGTATAGY-BHOLDT-CTTTTCTCTCTGCAACGC/Spc6 300 Emm49/151-R CCTATTCTTCTTCTAGATTATTCTCCTGT 400 Emm59-F CTTTGCAAACCAAACAGAGATAAG 200 Emm59-F FAWAAATACGA-BHOLDT-GYATAGCAATAGGAATAAGCT/Spc6 100 Emm59-F FAWAAATACGA-BHOLDT-GYATAGCAATAGGAATAAGTTTAATAC/Spc6 100 Emm59-F FAWAAATACGA-BAGGATTAAGGAGGAGTTAAGGAGTTAATGGAGATAAGGATCTCTCCTCTCTCT	Emm28-F	GRCTTYTGCTAATGGARCTGRT	400
Emm28-R ACTCATCWCYGAGTTTCTCATGTT 400 Emm48/151-F* TGCGGAGATIAACGTGTMTA 400 Emm49/151-P HEX/CGTIATGAW-BHQ1dT-CTTTTCTCTTCTTGCAACGC/Spc6 300 Emm49/151-R CCTATTCTTTCTAGATATTCTCCGT 400 Emm59-F CTTGCAAACCAAACGAAGACAGAGTTAAG 200 Emm59-R CTATACAAACCAAACGAACGACAGACTTAAG 200 Emm59-F CTTGCAAACCAAACGAACGACAGAGATTAAG 200 Emm59-F TATGAAACGAAACGAACGACAGAGAGTTAAG 300 Emm35-R TAATTAAAGTTATCTCTCTCTCTCTCAA 200 Emm75-F TATGAAGACYRATACAAAGCAGTTAGGATAGGTTAATCC/Spc6 300 Emm75-F TAGCAAACCAAACGAAGGTTAAGG 300 Emm76-F TGCAAACCAAACGAAGTTAAGG 300 Emm76-F TGCAAACCAAACGAAGTTAAGG 300 Emm77-F TGCAAACCAAACGAAGGTTAAGG 300 Emm77-F TGCAAACCAAACGAAGAGTTAAGG 300 Emm77-F TGCAAACCAAACGAAGAGTAAGG 300 Emm77-F TGCAAACCAAACGAAGAGAGTTAAGG 300 Emm77-F TGCAAACCAAACGAAGAGAGAGTTAGG 300 Emm77-F	Emm28-P	FAM/AGYTGM-BHO1dT-GYATACAACACATTGCTTACTG/Spc6	100
Emm49/151-F ^{an} TGCGGAGAATAACGTGTMTA 400 Emm49/151-P HEX/CGTATAGYSHQ1dT-CTTTTTCGCTACGCACGC/Spc6 300 Emm49/151-R CCTATTCTTTCTGAATCCTCCGT 400 Emm59-F CTTTGCAAACCAAACGAAGTTAGC 200 Emm59-F CTTTGCAAACCAAACGAAGTTAGC 200 Emm59-F FAM/ANATCGA-BHQ1dT-GYATTGACTAGGAATAAGTCT/Spc6 100 Emm59-F FAM/ANATACGA-BHQ1dT-GYATTGACTAGGAATAAGTTA/Spc6 300 Emm75-F FAM/ANATAGA-BHQ1dT-GACATGGACCTTAGATAAGGTTAATGC/Spc6 300 Emm75-F FAM/ANTA-BHQ1dT-GACAAGGACCTTAGATAAGTTAATG/Spc6 300 Emm76-F TGGAAACCAAACGAAGTTAAGG 300 Emm77-F TGCAAACCAAACAGAAGTTAAGG 300 Emm77-F TGCAAACCAAACAGAAGTTAAGG 300 Emm77-F TGCAAACCAAACAGAAGTTAGG 300 Emm77-P ROX/AGTGAATGCGATHAGG 300 Emm77-P ROX/AGTGAATGCGATHAGG 300 Emm77-P ROX/AGTGAATGCGATTAGG 300 Emm77-P ROX/AGTGTAATGCGATTATGT 300 Emm77-R AAGGTCTGTAAGTGTATGT 300 E	Emm28-R	ACTCATCWCYGAGTTTCTCATGTT	400
Emm49/151-P HEX/CGTATAGY-BHQ1dT-CTTTTCTCTTCTGCAACGC/Spc6 300 Emm49/151-R CCTATTCTTTCTAGATATTCTCCGT 400 Emm59-F CTTGCAAACCAAACGAAGTAAG 200 Emm59-F CTTGCAAACCAAACGAAGTAAG 200 Emm59-F TATTAAATAGTATCTCTCTCTCTAA 200 Emm59-F TATGAAACCAAACGAAGTAAGGAGTAGGAGTAAGGTC/Spc6 100 Emm75-F TATGAAACCAAACGGAGGG 300 Emm75-F TATGAAACCAAACGGAAGTTAAGG 300 Emm75-F TGCAAACCAAACGGAAGTTAAGG 300 Emm76-F TGCAAACCAAACGGAAGTTAAGG 300 Emm77-F TGCAAACCAAACGAAGTTAAGG 300 Emm77-F TGCAAACCAAACGGAAGTTAGG 300 Emm77-F TGCAAACCAAACGGGATAGGTAGTAGG 300 Emm81-F TGCAAACCAAACGGGTATAGG 300 Emm81-F TGCAAACCAAACGGGGTATGTG 300 <td>Emm49/151-F^a</td> <td>TGCGGAGAATAACGTGTMTA</td> <td>400</td>	Emm49/151-F ^a	TGCGGAGAATAACGTGTMTA	400
Emm49/151-RCCTATTCTTTCTAGATATTCTCCGT400Emm59-FCTTTGCAAACCAAAGTTAAG200Emm59-FFAWAAATACGA-BPQ10T-GYATTGACTAATGAGAATAAGTCT/Spc6100Emm59-RTATGAAGCAYRATACGAAGCAGGAGTGAG300Emm75-FTATGAAGCAYRATACGAAGCATGG300Emm75-PFAWMAATTA-BHQ10T-AGAARGACCTTAGATAAGTTAATAC/Spc6100Emm75-RCTAATCTCGTAGTCTTACCTGCTCA300Emm76-FTGCAAACCAAACGAAGTTAAGG300Emm76-FTGCAAACCAAACGAAGTTAAGG300Emm76-FTGCAAACCAAACGAAGTTAAGG300Emm76-RGTTCAGCCATAGCTCACGGC-BHQ2dT-GAACATGATAAGYT/Spc6300Emm77-FTGCAAACCAAACAGAAGTTAAGG300Emm77-PROX/IGASCTGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm77-RACGCTCGTATAGCCGGTTATCT300Emm77-RTGCAAACCAAACAGAAGTTAAGG300Emm81-FTGCAAACCAAACGGGTTATCT300Emm81-FTGCAAACCAAACGAGAGTTAAGG300Emm81-RTTGCAAGCCAAACAGAAGTTAAGG300Emm81-RTTGCAAGCCAAACGAGAGTTAAGG300Emm81-RTTGCAAGCGGAAGTGTATCTA300Emm82-FATCACTGAGGCAGTGTATCTAC300Emm83-RCCAGCAGAAGTTAAGGCTGATTACC300Emm83-RCCAGACGAAGTTAAGCCTGATAGCCAGAGCA/Spc6300Emm83-RCCAGACGAAAGTACACCAAGGSRCRC/Spc6300Emm83-RCCAGACGGAAGTAACCCAGGA200Emm83-RCCAGACGGAAGTAACCCAGGAA200Emm83-RCCAGACGGACATACCCAAGCGAA200Emm83-RCCAGACGGACATACCCAAGCGAA	Emm49/151-P	HEX/CGTATAGY-BHO1dT-CTTTTCTCTTCTTGCAACGC/Spc6	300
Emm39-F CTTTGCAAACCAAACAGAAGTTAAG 200 Emm39-P FAMVAAATACGA-BHQ1dT-GYATTGACTAATGAGAATAAGTCT/Spc6 100 Emm59-R TAATTAAATAGTTACTCTCTCTCTTCTTCTAA 200 Emm75-F TATGAAGCAYRATACAAAGCATGG 300 Emm75-R TATGAAGCAYRATACAAAGCATGG 300 Emm75-R TATGAAGCAYRATACAAAGCATGG 300 Emm75-R CTAATCTCGTAGTCTACCTTGCTCA 300 Emm76-F TGCAAACCAAACAGCAGAGTTAAGG 300 Emm76-F TGCAAACCAAACAGCBCBHQ2dT-GAACATGATAAGYT/Spc6 300 Emm77-P ROX/TGASCTACAGGCBHQ2dT-GAACAGAAACCC/Spc6 300 Emm77-F TGCAAACCAAACAGAAGTTAAGG 300 Emm77-R AGGTCTGTAATGCGGTTATGT 300 Emm81-F TGCAAACCAAACAGAAGTTAAGG 300 Emm81-R TSGAAACGAAACAAGAAGARAATGTACYG/Spc6 300 Emm81-P HEX/TGGGG-BHQ1dT-YCAGAAGAGAAGAAGAACC/Spc6 300 Emm81-R TSAAGTTGGCAATGGCTGTATCTA 300 Emm81-R TSAAGTTGGCATGTCAFAGGCTGATAAC 400 Emm82-R TTCCAAGCAAGAAGTAACCCAAAGAGGAGAA/Spc6 200	Emm49/151-R	CTATICTICTAGATATICTCCGT	400
Emm39-P FAM/AAATACGA-BHQ IdT-GYATTGACTAATGAGAATAAGTCT/Spc6 100 Emm39-R TAATTTAAATAGTTATCTCTCTCTCTCTAA 200 Emm75-F TATGAAGCAYRATACAAAGCAGG 300 Emm75-F FAM/AATTA-BHQ IdT-AGAARGAAGTAAGGTTTAATAC/Spc6 100 Emm75-F TGCAAACCAAACCATACGG 300 Emm76-F TGCAAACCAAACCAACGAGTAAAGG 300 Emm76-F TGCAAACCAAACACAACAGAGAGTTAAGG 300 Emm76-F TGCAACCAAACAGAAGTAAGG 300 Emm77-F TGCAAACCAAACAGAAGTARGG 300 Emm77-F TGCAAACCAAACAGAAGTARGG 300 Emm77-F TGCAAACCAAACAGAAGTATAGG 300 Emm77-P ROX/AGTGATGCA-BHQ2dT-CGAACCTACAGAAACC/Spc6 300 Emm81-F TGCAAACCAAACAGAAGTATAAGG 300 Emm81-F TGCAAACCAAGAAGAGTATAAGG 300 Emm81-F TGCAACCAAGAAGAGTATAGG 300 Emm81-F TGCAAACCAAGAAGAGTATAAGG 300 Emm81-F TGCAAACCAAGAAGAGTATAGG 300 Emm81-F TGCAATGCAGGGTTATCTA 300 Emm82-F ATCACTGAGGCAGTGTATACT	Emm59-F	CTTTGCAAACCAAACAGAAGTTAAG	200
EmmS9-RTAATTTAAATAGTTATCTCTCTCTCTCTAA200Emm75-FTATGAAGCAYRATACAAAGCATGG300Emm75-FFAM/AATTA-BHQACCTTAGCATAAGTTTAATAC/Spc6100Emm75-RCTAATCTCGTAGTCTTACCTTGCTCA300Emm76-FTGCAAACCAAACGAGAAGTTAAGG300Emm76-PROX/TGASCTACAGGC-BHQ2dT-GAACATGATAAGTTYAATAC/Spc6300Emm76-RGTTCAGYCAATAGCTCMTYAT300Emm77-FTGCAAACCAAACGAAGTARGG300Emm77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACTGCACSpc6200Emm77-RAAGGTCTGTAATGCGGTTAATG300Emm81-FTGCAAACCAAACGAAGTTAAGG300Emm81-PHEX/TGCGGGEBHQ1dT-YCAGAAGGAAGTAAGG300Emm82-PCy5/TGGAAGAGCTGTATGCG300Emm82-PCy5/TGGAAGAG-GTGTATCTCA300Emm82-RTTCAAGCCGGTGTATCTA300Emm83-RTCCAACGAAGAG-BHQ2dT-CTAAC300Emm83-RCCCAGCAGGTGTATCTA300Emm83-RCCCAGCAGAGAG-BHQ2dT-AACCAAAGGSSRCRC/Spc6300Emm83-RCCCAGCAGAAGTTAAGC400Emm83-RCCACAGAAGATTAAGCCGTGATAAC200Emm83-RCCACAGAAGTAATCGAAGCA200Emm83-RCCACAGAAGTAATCCARCGAA300Emm87-PFAM/TGGYTGC-BHQ2dT-AACACAAGRGSRCRC/Spc6300Emm87-PCY5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6300Emm87-PFAM/TGGYTGC-BHQ2dT-CAACCAAGAGAAA/Spc6200Emm87-PFAM/TGGYTGC-BHQ2dT-AACACAAGRGSRCRC/Spc6300Emm87-PFAM/TGGYTGC-BHQ1dT-CRAAGATAATGAAAAGAATTACATAACAVA/Spc6300 <t< td=""><td>Emm59-P</td><td>FAM/AAATACGA-BHO1dT-GYATTGACTAATGAGAATAAGTCT/Spc6</td><td>100</td></t<>	Emm59-P	FAM/AAATACGA-BHO1dT-GYATTGACTAATGAGAATAAGTCT/Spc6	100
Emm75-FTATGAAGCAYRATACAAAGCATGG300Emm75-PFAM//AATTA-BHQ1dT-AGAARGACCTTAGATAAGTTTAATAC/Spc6100Emm75-RCTAATCTGATAGTTAGCTGCTCA300Emm76-FTGCAAACCAAACAGAAGTTAAGG300Emm76-PROX/TGASCTACAGG-BHQ2dT-GAACATGATAAGYT/Spc6300Emm77-FTGCAAACCAAACAGAAGTTARGG300Emm77-FTGCAAACCAAACAGAAGTTARGG300Emm77-PROX/AGTGATGC-ABHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm77-RAGGCTGTTATGTGGGTTATGT300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-PHEX/TGCGGG-BHQ1dT-YCAGAAGARAATGTACYG/Spc6300Emm81-RTTSAAAGTTGGCTATGTATTCC300Emm82-PCy5/TGGAAGAG-BHQ2dT-AGGCAGAGCA/Spc6300Emm83-RTTTCAAGCTCGTTGCTTATTC300Emm83-PCy5/TGGAAGGCAGGTGTATCTA300Emm83-PCCAGACAGAAGTAAGGCTGATAAC400Emm83-PCY5/CAATGCAG-BHQ2dT-ACACAAGRGSRCRC/Spc6200Emm83-RTCCCATTCATTCGAAGCAAACGGAA200Emm83-RCCACTATCACAAGTAAACCAARGGAAA/Spc6200Emm87-PFAM/TIGGYTGC-BHQ1dT-TCAAGGAAGAAA/Spc6300Emm87-RCCAGGCGGACAGTGACAAT300Emm87-RCCATCTCTCTGABHQ1dT-TCAAGGAAAAAGAAAA/Spc6300Emm89-PHEX/TGTCTCTGABHQ1dT-CRAAGGTGAAAA/Spc6300Emm89-PHEX/TGTTTACCAAAT300Emm89-RCATCTATGAGCGAAGTAACCAAGGTGACACAYCA/Spc6300Emm89-RCATCTATTAGCGAATA300Emm8	Emm59-B	ΤΑΑΤΤΤΑΑΑΤΑGTTΑΤCTCTCTCTCTAA	200
Emm75-PFAM/AATTA-BHQ1dT-AGAARGACCTTAGATAAGTTTAATAC/Spc6100Emm75-RCTAATCTCGTAGTCTTACCTTGCTCA300Emm76-FTGCAAACCAAACGAAGTTAAGG300Emm76-PROX/TGASCTACAGGC-BHQ2dT-GAACATGATAAGYT/Spc6300Emm77-PROX/ACCAAACGAAGTTARGG300Emm77-PROX/ACGAAACGAAGTTARGG300Emm77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6300Emm81-FTGCAAACCAAACGAAGTTAAGG300Emm81-PHEX/TGCGGG-BHQ1dT-YCAGAAGAGAATGTACYG/Spc6300Emm81-PHEX/TGCGGG-BHQ1dT-YCAGAAGAGACA/Spc6300Emm82-FATCACTGAGCAGTGTATCT300Emm82-FCyS/TGGAAGAG-BHQ2dT-ARGTTGATGCAGGA/Spc6300Emm83-FCCAGACAGAGAGTTAAGCTGATAAC400Emm83-FCCAGACAGAAGTTAAGCTGATAAC400Emm83-FCCAGACAGAAGTTAAGCGAAGTCAACC200Emm83-FCCAGACAGAAGTTAAGCGAAG200Emm83-FCCAGACAGAAGTTAAGCGAAG200Emm83-FCCAGACAGAAGTAACCAAAGGSCRCC/Spc6200Emm83-FCYAGAGAAGTAACCAAAGTGAGAAA/Spc6200Emm87-FFAM/TGGYTGC-BHQ1dT-CAATGGGAAGAAA/Spc6300Emm89-FHEX/TGTCTCTG-BHQ1dT-CAAGGGAGACA/Spc6300Emm89-FCATGACTGGAACGTGACAAT300Emm89-FCATGACTGGGACAGTGACAAT300Emm89-FAGGCCACCATGGCAAAT300Emm89-FCATGACGGGACAGTGACAAT300Emm89-FCATGACGGGACAGTGACAACA300Emm89-F	Emm75-E	TATGAAGCAYRATACAAAGCATGG	300
Emm75-RCTAATCTCGTAGTCTTACCTTGCTCA300Emm75-FTGCAAACCGAACGAGAGTTAAGG300Emm76-FTGCAAACCGAACGGCBHQ2dT-GAACATGATAAGYT/Spc6300Emm76-RGTTCAGYCAATAGCTCMTYAT300Emm77-RTGCAAACCGAACGGABQ2dT-GAACCTACAGAAACCC/Spc6200Emm77-RROX/AGTGGTGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6300Emm77-RAAGGTCTGTAATGCGGTTATGT300Emm81-FTGCAAACCGAAACGAAAGTTAAGG300Emm81-FTGCAAACCGAACGGAAGGTTATGT300Emm81-RTTSAAGTTTGGCTATGTATGCCGGTTATCTA300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm83-FCCAGACAGAGGTGTATCTA300Emm83-FCCAGACAGAGGTGTATCTC300Emm83-FCCAGACAGAGGTGTATCTA300Emm83-FCCAGACAGAGGTGTATCTA300Emm83-FCCAGACAGAGGTGTATCTC300Emm83-FCCAGACAGAGGTGTATCTA300Emm83-FCCAGACAGAGAGTAAGGCTGATAAC400Emm83-FCCATTCATGGAAGABU2dT-AACACAAGRGSRCC/Spc6200Emm83-FCCATTCATTCAGGAGAACTACCARGGAA200Emm87-FFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAAAASpc6300Emm87-FTAAGGCGGACAGTGACAAT300Emm87-FTAAGGCGGACAGTGACAAT300Emm87-FTAAGGCGGACAGTGACAATCC200Emm87-FGATGACCGGACAGTAATCCA200Emm87-FTAAGGCGAGGACGTATCCC300Emm87-FTAAGGCGGACAGTAGTCCC300Emm87-FTAAGGCGGACGAGTATCCAC <td>Emm75-P</td> <td>FAM/AATTA-BHO1dT-AGAARGACCTTAGATAAGTTTAATAC/Spc6</td> <td>100</td>	Emm75-P	FAM/AATTA-BHO1dT-AGAARGACCTTAGATAAGTTTAATAC/Spc6	100
Emm 76-FTGCAAACCAAACAGAAGTTAAGG300Emm 76-PROX/TGASCTACAGGC-BHQ2dT-GAACATGATAAGYT/Spc6300Emm 76-RGTTCAGYCAATAGCTCMTYAT300Emm 77-FTGCAAACCAAACAGAAGTTARGG300Emm 77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm 77-RAAGGTCTGTAATGCGGTTATGT300Emm 81-FTGCAAACCAAACAGAAGTTAAGG300Emm 81-PHEX/TGCGGG-BHQ1dT-YCAGAAGAAGTACTGACYG/Spc6300Emm 82-PATCACTGAAGCAGGTGTATCTA300Emm 82-PCy5/TGGAAGAG-BHQ2dT-ARGTTTGATGCAGAGCA/Spc6300Emm 82-PCy5/TGGAAGAG-BHQ2dT-ARGTTTGATGCAGAGCA/Spc6300Emm 83-PCy5/CAATGCAGGTGATAAC400Emm 83-PCy5/CAATGCAG-BHQ2dT-ARGTTGGGAGAGCA/Spc6200Emm 83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm 83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm 83-PCY5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm 83-PCYAGAGAAGTARCCARCGAA200Emm 83-PCYAGAGAAGTARCCARCGAGA200Emm 83-PCYAGAGAAGTARCCARCGAGGATAATCC200Emm 87-PFAM/TGGYTGC-BHQ1dT-CARTGYGGAAGAAA/Spc6300Emm 97-PHEX/TGCTCTG-BHQ1dT-CRARGTGAGAGCACAYCA/Spc6300Emm 97-PHEX/ATAGTGG-BHQ1dT-CACAGGTGAGCACAYCA/Spc6300Emm 89-PHEX/TGTCTCTG-BHQ1dT-CAGCGTGAGCACAYCA/Spc6300Emm 89-PHEX/ATAGGB-BHQ1dT-AGCGTGAGACACYCA/Spc6300Emm 92-PHEX/ATAGGB-BHQ1dT-AGCGTGAGACACAYCA/Spc6300Emm 92-PHEX/	Emm75-R	CTAATCTCGTAGTCTTACCTTGCTCA	300
Emm 76-PROX/TGASCTACAGGC-BHQ2dT-GAACATGATAAGYT/Spc6300Emm 76-RGTTCAGYCAATAGCTCMTYAT300Emm 77-RGCAAACCAAACAGAAGTTARGG300Emm 77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm 77-RAAGGTCTGTAATGCGGTTATGT300Emm 81-FTGCAAACCAAACAGAAGTTAAGG300Emm 81-PHEX/TGCGGG-BHQ1dT-YCAGAAGAAGTGTACYG/Spc6300Emm 81-RTISAAGTTIGGCTATGTATKTCCG300Emm 82-PATCACTGAAGGCAGGTGTATCTA300Emm 82-PCy5/TGGAAGAG-BHQ2dT-ARGTTGATGCAGAGCA/Spc6300Emm 83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm 83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm 83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm 83-FCY5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm 83-FCYAGAGAAGTTAACGCAAGAAGTCGAAGC400Emm 83-FCYAGAGAAGTARCCARCGAA200Emm 87-FCYAGAGAAGTARCCARCGAA200Emm 87-FCYAGAGAAGTARCCARCGAA200Emm 87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAATTACATAACAVA/Spc6300Emm 87-PHEX/TGTCTCTG-BHQ1dT-CRATGGAGACAATTACATAACAVA/Spc6300Emm 87-PHEX/TGTCTCTG-BHQ1dT-CRCT300Emm 87-PGATGACCGGACGTTACCA200Emm 87-RCATCTATTTTGTCTAGAGTGACACAYCA/Spc6300Emm 87-RCATCTATTTTGTCTAGAGTGACACAYCA/Spc6300Emm 87-RCATCTATTTTGTCTAGAGTGGAGCACAYCA/Spc6300Emm 92-PHEX/TGTCCTGT-BHQ2dT-GCAAAGCGTATACACCAATA/Spc6300Emm 92-P	Emm76-F	TGCAAACCAAAAGTTAAGG	300
Emm76-RGTTCAGYCAATAGCTCMTYAT300Emm77-FTGCAAACCAAACAGAAGTTARGG300Emm77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm77-RAAGGTCTGTAATGCGGTTATGT300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-RTTSAAGTTTGGCTATGTTATGT300Emm81-RTTSAAGTTGGCTATGTATKTCCG300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-FCy5/TGGAAGAG-BHQ2dT-ARGTTGATGCAGAGCA/Spc6300Emm82-RCTTCCAGACGAGGTGTATCTC300Emm83-RCCAGACAGAG-BHQ2dT-ARGTTGATGCAGAGCA/Spc6300Emm83-FCCAGACAGAGTTAAGGCTGATAAC400Emm83-FCCAGACAGAGTAACGCGGAAGCTGATAAC400Emm83-FCCAGACAGAGAGTAACGCAGAG200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-CARTGYGGAAGAAA/Spc6300Emm87-PFAM/TTGGYTGC-BHQ1dT-CCARTGYGGAAGAAA/Spc6300Emm89-PHEX/TGTCTCTG-BHQ1dT-CCARTGYGGAAGAAA/Spc6300Emm89-PHEX/TGTCTCG-BHQ1dT-CRAAGGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-PHEX/TGTCTCG-BHQ1dT-CRCAGGAGCACAYCA/Spc6300Emm89-RCATCTATTTTGTCTAGATGTTCTCCT300Emm89-RGATGACCGAGCGTTTCTAC200Emm92-PHEX/ATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTAGCCAATA200Emm92-RAGCTCACCATGTTAGCCAATA200Emm92-R </td <td>Emm76-P</td> <td>ROX/TGASCTACAGGC-BHO2dT-GAACATGATAAGYT/Spc6</td> <td>300</td>	Emm76-P	ROX/TGASCTACAGGC-BHO2dT-GAACATGATAAGYT/Spc6	300
Emm77-FTGCAAACCAAACAGAAGTTARGG300Emm77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm77-RAAGGTCTGTAATGCGGTTATGT300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-FTGCAAACCGAACAGAGAGTTAAGG300Emm81-RTTSAAGTTIGGCTATGTATKTCCG300Emm82-FATCACTGAGGCAGGTGTATGTATKTCCG300Emm82-FATCACTGAGGCAGGTGTATGTT300Emm83-FCCAACAGAAGAAGABHQ2dT-ARGTTGATGCAGAGCA/Spc6300Emm83-FCCAGACAGAGGTGTATGCTATTC300Emm83-FCCAGACAGAGTAAGCTGATTAAC400Emm83-FCCAGACAGAGAGTAAGCTGATAAC400Emm83-FCCAGACAGAGTARCCARCGAAGC400Emm87-FCYAGAGAAGTARCCARCGAAGC200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-FCYAGAGAAGTARCCARCGAA300Emm87-FCYAGAGAAGTARCCARCGAA300Emm87-PFAWTTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6300Emm87-PFAWTTGGTTCTG-BHQ1dT-CRAAGGTAATCC300Emm89-FTAAGGCGACACTGACAAT300Emm89-FTAAGGCGAACATGACAAT300Emm89-FCATCTATTTTGTCTAGATGTCTCCT300Emm92-PHEX/ATATGG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm92-PHEX/ATATGG-BHQ1dT-ACCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-RCTCCGTTTTATCTGAAGGT-GAAAGCTATACAACCAAGCAAAT/Spc6300	Emm76-R	GTTCAGYCAATAGCTCMTYAT	300
Emm77-PROX/AGTGATGCA-BHQ2dT-CTGAACCTACAGAAACCC/Spc6200Emm77-RAAGGTCTGTAATGCGGTTATGT300Emm81-FTGCAAACCAAACGAAGTTAAGG300Emm81-FTGCAAACCAAACGAAGTTAAGG300Emm81-PHEX/TGCGGG-BHQ1dT-YCAGAAGARAATGTACYG/Spc6300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-FCy5/TGGAAGAG-BHQ2dT-ARGTTGATGCAGAGCA/Spc6300Emm82-RTTTCAAGCTCGTTTGCTCTATTC300Emm83-FCCAGACAGAAGTTAAGGCTGATACC400Emm83-FCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-FCYAGAGAAGTARCCARCGAAA200Emm87-FCYAGAGAAGTARCCARCGAAA200Emm87-FCYAGAGAAGTARCCARCGAAA200Emm87-FCYAGAGACGGACAGTGACAAT300Emm89-PHEX/TGTCTCTG-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-FCATCTATTTGTCTAGATGTTCCCT300Emm89-FGATGACCGGAACGTGACAAT300Emm89-RCATCTATTTGTCAGATGTTCCCT300Emm92-FGATGACCGGAGCGHCACTGAGCACAYCA/Spc6300Emm92-FGATGACCGGAGCGHCGAGGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm18-FGCGGACAGTAACGCG300Emm118-FGCGGACAGTAACGCG300Emm118-RCTCCGTTTTACTGTAAGGCTGACAACGACAAT/Spc6300 <td>Emm77-F</td> <td>TGCAAACCAAACAGAAGTTARGG</td> <td>300</td>	Emm77-F	TGCAAACCAAACAGAAGTTARGG	300
Emm77-RAAGGTCTGTAATGCGGTTATGT300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-PHEX/TGCGGA-BHQ1dT-YCAGAAGARAATGTACYG/Spc6300Emm81-RTTSAAGTTTGGCTATGTATKTCCG300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-RTTTCAAGCTCGTTTGCTCTATTC300Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-RTCCATTTCATGCAG-BHQ2dT-ARGTTTGAAGC400Emm83-RCCAGACAGAAGTTAAGGCTGATAAC400Emm83-RTCCATTTCATGTAACCAACAGRGSRCRC/Spc6200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-RCTTTCCTTCTTTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-FTAAGGCGGACAGTGACAAT300Emm89-PHEX/ATGTCTCTG-BHQ1dT-CRARGATAATGAAARAGAATTACATAACAVA/Spc6300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-FGATGACCGGABHQ1dT-CRACGGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm92-RAGCTCACCATGTTTAGCCAATA200Emm18-FGCGGACAGTAACGCG300Emm18-FCGCGGACAGTAACGCG300Emm18-RCTCCGTTTTAGCGAAAGCTATACAACACCAAAT/Spc6300Emm118-RCTCCGTTTTATGTGAAAGATGG300	Emm77-P	ROX/AGTGATGCA-BHO2dT-CTGAACCTACAGAAACCC/Spc6	200
Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-FTGCAAACCAAACAGAAGTTAAGG300Emm81-PHEX/TGCGGG-BHQ1dT-YCAGAAGARAATGTACYG/Spc6300Emm81-RTTSAAGTTTGGCTATGTATKTCCG300Emm82-FATCACTGAAGGCAGGTGATCTA300Emm82-PCy5/TGGAAGAG-BHQ2dT-ARGTTTGATGCAGAGCA/Spc6300Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-FCCAGACAGABHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-FCY5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGGAAGTARCCARCGAA200Emm87-FCYAGGAAGTARCCARCGAA200Emm87-FCYAGGCGACAGTGACAAT300Emm89-FTAAGGCGGACAGTGACAAT300Emm89-FTAAGGCGGACAGTGACAAT300Emm89-RCATCTATTTGGTAGAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTCABHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-FHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm92-RAGCTCACCATGTTTAGCCAATA200Emm18-FGCGGACAGTAACGCG300Emm18-FCCGCGACGTAACGCG300Emm118-PR0X/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6300Emm118-RCTCCGTTTTTATCGTAAGATCG300	Emm77-R	AAGGTCTGTAATGCGGTTATGT	300
Emm81-PHEX/TGCGGG-BHQ1dT-YCAGAAGARAATGTACYG/Spc6300Emm81-RTTSAAGTTGGCTATGTATKTCCG300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-PCy5/TGGAAGAG-BHQ2dT-ARGTTGATGCAGAGCA/Spc6300Emm83-RTTCCAAGCTCGTTAGCTCATATC300Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-RCCCATTCATGTAACAAATTCTGAAGC400Emm83-RCCCATTCATGTAACAAATTCTGAAGC400Emm83-RCCCATTCATGTAACAAATTCTGAAGC400Emm83-RCCCATTCATGTAACAAATTCTGAAGC200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-FTAAGGCGGACAGTGACAAT300Emm89-FGATGACCGGAGCGTTCTCAC200Emm92-FGATGACCGGAGCGTTTCAC200Emm92-FGATGACCGGABHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm92-FGATGACCGGABHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-FGATGACCGGABHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm18-FGCGGACAGTAACGG300Emm18-RCTCCGTTTTAGCGAAAGCTATACAACCAAAT/Spc6300Emm18-RCTCCGTTTTAGCGAAAGCTATACAACCAAAT/Spc6300Emm118-RCTCCGTTTTAGCAAAGGAAGCTATACAACCAAAT/Spc6300	Emm81-F	TGCAAACCAAACAGAAGTTAAGG	300
Emm81-RTTSAAGTTTGGCTATGTATKTCCG300Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-PCy5/TGGAAGAG-BHQ2dT-ARGTTTGATGCAGAGCA/Spc6300Emm82-RTTTCAAGCTCGTTTGCTCTATTC300Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm87-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-FTAAGGCGGACAGTGACAAT300Emm89-RCATCTATTTGTCTAGATGTTCTCCT300Emm92-FGATGACCGGAGGCGTTTCTAC200Emm92-RAGCTCACCATGTTAGCGAATA200Emm92-RAGCTCACCATGTTAGCGAATA200Emm18-FGCGGACAGTATCACCAATA200Emm18-FROX/TCTAGCGF-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm18-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACCAAACCAAAT/Spc6300Emm18-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACCAACCAAAT/Spc6300Emm18-RCTCCGTTTTATCTGTAAGATCG200Emm18-RCTCCGTTTTATCTGTAAGATCG200Emm18-RCTCCGTTTTATCTGTAAGATCG300Emm118-RCTCCGTTTTATCTGTAAGATCG300	Emm81-P	HEX/TGCGGG-BHQ1dT-YCAGAAGARAATGTACYG/Spc6	300
Emm82-FATCACTGAGGCAGGTGTATCTA300Emm82-PCy5/TGGAAGAG-BHQ2dT-ARGTTTGATGCAGAGCA/Spc6300Emm82-RTTTCAAGCTCGTTTGCTCTATTC300Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAGTARCCARCGAA200Emm87-FCYAGAGAGTARCCARCGAA200Emm87-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-FTAAGGCGGACAGTGACAAT300Emm89-RCATCTATTTGCTGABU1dT-TCARAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm92-FGATGACCGGAGGGTTTCTAC200Emm92-FGATGACCGGAGGGGTTTCTAC300Emm92-FGATGACCGGAGGAGTGTTCTAC200Emm92-RAGCTCACCATGTTTAGCCAATA200Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PRX/TCTAGGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6300Emm118-RCTCCGTTTTATCTGTAAGATCG300	Emm81-R	TTSAAGTTTGGCTATGTATKTCCG	300
Emm82-PCy5/TGGAAGAG-BHQ2dT-ARGTTTGATGCAGAGCA/Spc6300Emm82-RTTTCAAGCTCGTTTGCTCTATTC300Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm89-RCTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-RCATCTATTTTGTCTAGATGTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-RAGCTCACCATGTTAGCCAATA300Emm92-RGCGGACAGTAGCAATA300Emm92-RMEX/ATAGTGG-BHQ1dT-GCAAGACACAYCA/Spc6300Emm18-FGCGGACAGTAGCAATA200Emm18-FCCGGACGTTTCACC200Emm18-FCCGGACAGTAGCGAGCG300Emm18-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACCAACCAAAT/Spc6300Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6300Emm118-RCTCCGTTTTACTGTAAGATCG300	Emm82-F	ATCACTGAGGCAGGTGTATCTA	300
Emm82-RTTTCAAGCTCGTTTGCTCTATTC300Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-RCATCTATTTGCTAGAGTGATCATCC300Emm89-RCATCTATTTGTCTAGAGTGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-RAGCTCACCATGTTTAGCCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCGTGAGCACAYCA/Spc6300Emm18-FGCGGACAGTAACGCG300Emm18-FROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6300Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6300Emm118-RCTCCGTTTTATCTGTAAGATCG300	Emm82-P	Cy5/TGGAAGAG-BHQ2dT-ARGTTTGATGCAGAGCA/Spc6	300
Emm83-FCCAGACAGAAGTTAAGGCTGATAAC400Emm83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm89-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-PHEX/TGTCTCTG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTAGATGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RGATGACCGGAGCGTTTCTAC200Emm92-RAGCTCACCATGTTTAGCCAATA200Emm18-FGCGGACAGTAACGCG300Emm118-FGCGGACAGTAACGCG300Emm118-RCTCCGTTTTATCTGTAAGATCATAACAAACCAAAT/Spc6300Emm118-RCTCCGTTTTATCTGTAAGATCATAACAACCAAAT/Spc6300	Emm82-R	TITCAAGCTCGTTTGCTCTATTC	300
Emm83-PCy5/CAATGCAG-BHQ2dT-AACACAAGRGSRCRC/Spc6200Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm89-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-PHEX/TGCTCTG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTAGATGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RGATGACCGGAGCGTTTCTAC200Emm18-FGCGGACAGTGACAATA300Emm18-FGCGGACAGTAACGCG300Emm18-FCCGGACAGTAACGCG300Emm18-RCTCCGTTTTACCACAGCG300Emm118-RCTCCGTTTTACCACAGCG300Emm118-RCTCCGTTTTATCTGTAAGATCATACAACCAAAT/Spc6300	Emm83-F	CCAGACAGAAGTTAAGGCTGATAAC	400
Emm83-RTCCATTTCATGTAACAAATTCTGAAGC400Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm87-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-PHEX/TGTCTCTG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTAGATGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-RAGCTCACCGGAGCGTTTCTAC200Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PR0X/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm83-P	Cv5/CAATGCAG-BHO2dT-AACACAAGRGSRCRC/Spc6	200
Emm87-FCYAGAGAAGTARCCARCGAA200Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAA/Spc6200Emm87-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-PHEX/TGTCTCTG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTCAGAGTGATCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCGAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PR0X/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm83-R	TCCATTTCATGTAACAAATTCTGAAGC	400
Emm87-PFAM/TTGGYTGC-BHQ1dT-TCARTGYGGAAGAAA/Spc6200Emm87-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGCGGACAGTGACAAT300Emm89-PHEX/TGTCTCG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTCTAGATGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PR0X/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTATCTGTAAGATCG300	Emm87-F	CYAGAGAAGTARCCARCGAA	200
Emm87-RCTTTCCTTCTATTTCAGAGTAATCC200Emm89-FTAAGGCGGACAGTGACAAT300Emm89-PHEX/TGTCTCTG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTCTAGATGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm87-P	FAM/TTGGYTGC-BHO1dT-TCARTGYGGAAGAAA/Spc6	200
Emm89-FTAAGGCGGACAGTGACAAT300Emm89-PHEX/TGTCTCTG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6300Emm89-RCATCTATTTTGTCTAGATGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm87-R	CTITCCTTCTATTTCAGAGTAATCC	200
Emm89-P HEX/TGTCTCG-BHQ1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6 300 Emm89-R CATCTATTTTGTCTAGATGTTCTCCT 300 Emm92-F GATGACCGGAGCGTTTCTAC 200 Emm92-P HEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6 300 Emm92-R AGCTCACCATGTTTAGCCAATA 200 Emm118-F GCGGACAGTAACGCG 300 Emm118-P ROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6 300 Emm118-R CTCCGTTTTTATCTGTAAGATCG 300	Emm89-F	TAAGGCGGACAGTGACAAT	300
Emm89-RCATCTATTITGTCTAGATGTTCTCCT300Emm92-FGATGACCGGAGCGTTTCTAC200Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm89-P	HEX/TGTCTCTG-BHO1dT-CRAAGATAATGAAARAGAATTACATAACAVA/Spc6	300
Emm92-F GATGACCGGAGCGTTTCTAC 200 Emm92-P HEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6 300 Emm92-R AGCTCACCATGTTTAGCCAATA 200 Emm118-F GCGGACAGTAACGCG 300 Emm118-P ROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6 200 Emm118-R CTCCGTTTTTATCTGTAAGATCG 300	Emm89-R	CATCTATTTGTCTAGATGTTCTCCT	300
Emm92-PHEX/AATAGTGG-BHQ1dT-AGCGTGAGCACAYCA/Spc6300Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm92-F	GATGACCGGAGCGTTTCTAC	200
Emm92-RAGCTCACCATGTTTAGCCAATA200Emm118-FGCGGACAGTAACGCG300Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm92-P	HEX/AATAGTGG-BHO1dT-AGCGTGAGCACAYCA/Spc6	300
Emm118-F GCGGACAGTAACGCG 300 Emm118-P ROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6 200 Emm118-R CTCCGTTTTTATCTGTAAGATCG 300	Emm92-R	AGCTCACCATGTTTAGCCAATA	200
Emm118-PROX/TCTAGCGT-BHQ2dT-GCAAAGCTATACAACCAAAT/Spc6200Emm118-RCTCCGTTTTTATCTGTAAGATCG300	Emm118-F	GCGGACAGTAACGCG	300
Emm118-R CTCCGTTTTTATCTGTAAGATCG 300	Emm118-P	ROX/TCTAGCGT-BHO2dT-GCAAAGCTATACAACCAAAT/Spc6	200
	Emm118-R	CTCCGTTTTTATCTGTAAGATCG	300

^aAssay targets both types emm49 and emm151.

assays were multiplexed together in 5 individual reactions, we also observed no cross-reaction with any of the other *emm* types/subtypes (Table S1) or the non-S. *pyogenes* species. The multiplex reactions did not produce any false-positive or false-negative results. Among 209 clinical sterile-site GAS isolates (from ABCs) used in the

TABLE 2 Quadriplex	reactions for	identification	of 20 emm	types	of iGAS commonly
found in the United	States				

emm typing reaction	emm types ^a
1	emm1, emm89, emm12, emm82
2	emm28, emm92, emm77, emm4
3	emm59, emm11, emm3, emm2
4	emm87, emm81, emm118, emm6
5	emm75, emm49/151, ^b emm76, emm83

^aEach target assay identifies known subtypes tested within the emm type.

^bemm49/151 is considered here as one type. emm151 is a rarely encountered 11-codon deletion derivative of emm49 that occurs within the same MLST type as emm49 (ST433).

validation, the real-time PCR *emm* typing assays correctly identified respective *emm* types and had 100% concordance with conventional PCR and sequencing and/or WGS results. While we only tested the subtypes for the 21 *emm* types (including *emm151*) that were available within ABCs, we infer based on *in silico* alignment of all available subtypes in the *emm* database for the primer and probe designs that the PCR assays would detect all known subtypes.

The sensitivity of the real-time PCR assays was determined with 10-fold serial dilutions of DNA extracted from all 21 positive-control isolates used in the validation. All newly validated assays had high sensitivity of detection (<10 genome copies/assay) in both monoplex and multiplex formats.

DISCUSSION

The CDC's Streptococcus Lab serves as a reference laboratory for streptococci and assists clinical and state public health laboratories by providing support and characterization of GAS, particularly from LTCFs and SNFs, where outbreaks among residents commonly occur (2, 3, 17, 18). Traditionally, PCR combined with sequencing (5) along with other genotyping methods, such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), have been extensively used to study the clonal relatedness of GAS isolates for epidemiological investigations (3, 17). Recently, WGS has been used for higher discriminatory genetic analysis using single nucleotide polymorphisms (SNPs) to increase resolution in GAS outbreak investigations (2, 18). While all these methods provide valuable information, they either are costly or take several days to complete, and many use equipment and reagents not readily available in most laboratories. The real-time-PCR-based emm typing method developed in this study has an advantage over other methods in that it is more rapid, with results generated in a few hours rather than days. Our method identifies four emm types in a single reaction and utilizes a sequential multiplex-approach which does not require post-PCR steps for confirmation, saving time and cost. Furthermore, it employs real-time PCR, which is a technology available in many clinical, public health, and reference laboratories. This approach can provide information on the relatedness of GAS isolates circulating in a particular setting and allows for a more rapid response. We have recently found this assay very useful for typing of GAS disease cluster isolates, and we find this approach quite useful prior to proceeding with higher-resolution genomic sequencing of strains sharing the same emm types (our unpublished data). Also, this PCR-based approach could further detect GAS from culture-negative or low-DNA-copy-number clinical specimens employing the real-time PCR for the GAS-specific target gene spy (19), in which many specimens would be projected to yield emm type information from included emm type-specific targets.

In summary, a sequential quadriplex real-time PCR scheme was developed that was highly sensitive and specific for the identification of the most frequently occurring 20 *emm* types covering ~93% of iGAS isolates in the United States collected through CDC's ABCs program in 2015 (10). Also, the current real-time PCR scheme overlaps 60% of *emm* types (18 of 30 *emm* types) included in a GAS vaccine (4, 20) that has good phase I clinical trial data and targets common M serotypes in the United States, Canada, and Europe. Most clinical and public health laboratories

routinely isolate and identify GAS but do not perform DNA-based *emm* typing, as the method is labor-intensive. This method will provide typing capability for these laboratories for outbreak investigation support where *emm* typing may be useful to determine genetic relatedness of isolates to help guide response efforts. A limitation of the method is that the real-time PCR assays do not target all known *emm* types and subtypes, and all subtypes within each of the 20 *emm* types targeted were not tested. However, while this method was developed using the most commonly occurring *emm* types in the United States, the assay also encompasses a large proportion of types globally (21–23) and can be adapted for use in any country where these *emm* types are common.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only. **SUPPLEMENTAL FILE 1**, PDF file, 0.04 MB.

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