**List of oligonucleotides used in pneumococcal serotype deduction by quadriplex real time PCR.**

**Reference**: Velusamy et al. (2020) Expanded sequential quadriplex real-time polymerase chain reaction (PCR) for identifying pneumococcal serotypes, penicillin susceptibility, and resistance markers. Diagnostic Microbiology and Infectious Disease 97(2): 115037. doi: 10.1016/j.diagmicrobio.2020.115037. Epub 2020 Mar 12.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Primer/Probe  Name | Sequence (5’-3’) | Conc.  (nM) | Special Chemistry | | | Target gene  (Accession No.) | Coordinates | Size (bp) | Reference |
| Dye | Chemistry | Quencher |
| 1-F | TTTCATCCCTATGTGTGGTATAG | 300 |  |  |  | *wzy* (CR931632) | 9875-10035 | 161 | Pimenta et al., (2013) |
| 1-P | TGCCAAAGCCAGCCAT | 100 | FAM | LNA | BHQ1 |
| 1-R | GCTTTAGAAGGTAGAGTTAACAAC | 300 |  |  |  |
| 2-F | TGTTATCCCATATAAGAACCGAGTGT | 300 |  |  |  | *wzy* ([CR931633](https://www.ncbi.nlm.nih.gov/nucleotide/68642292?report=genbank&log$=nuclalign&blast_rank=4&RID=V2PUKPZ3016)) | 10342-10452 | 111 | Pimenta et al., (2013) |
| 2-P | TTGCAATT"T"CAATTTTTTTGCCCCAATCTC | 200 | ROX | “T”=BHQ1 | BHQ2 |
| 2-R | AAAATTACCCCAAAAGCTATCCAA | 300 |  |  |  |
| 3-F | CCACTAAAGCTTTGGCAAAAGAAA | 300 |  |  |  | *galU* ([CR931634](https://www.ncbi.nlm.nih.gov/nucleotide/89994180?report=genbank&log$=nuclalign&blast_rank=11&RID=V2PW4F4P016)) | 8564-8648 | 85 | Pimenta et al., (2013) |
| 3-P | TTGTAGACCGCCCCACAA"T"TCATTTTGT | 200 | HEX | “T”=BHQ1 | BHQ1 |
| 3-R | CCCGAACGTAAAGCTTCTTCA | 300 |  |  |  |
| 4-F | GCTTCTGCTGTAACTGTTGTGC | 300 |  |  |  | *wzy* (CR931635) | 10521-10734 | 214 | Pimenta et al., (2013) |
| 4-P | TTCCACAAAAGAAGAGCCTACAGGTAACCCCA | 100 | ROX |  | BHQ2 |
| 4-R | CACCACCATAGTAACCAAAGTTCC | 300 |  |  |  |
| 5-F | CATGATTTATGCCCTCTTGCAA | 300 |  |  |  | *wzy* (CR931637) | 7001-7082 | 82 | Pimenta et al., (2013) |
| 5-P | TCTTCTTCTCA"T"CGTTTCCGCATGCTTTT | 200 | FAM | “T”=BHQ1 | BHQ1 |
| 5-R | GACAGTATAAGAAAAAGCAAGGGCTAA | 300 |  |  |  |
| 6A/6B/6C/6D -F | GTTTGCACTAGAGTATGGGAAGG | 200 |  |  |  | *wzy* (CR931638) | 8818-8949 | 132 | Pimenta et al., (2013) |
| 6A/6B/6C/6D -P | TGTTCTGCCC"T"GAGCAACTGGTCTTGTATC | 200 | FAM |  | BHQ1 |
| 6A/6B/6C/6D -R | TAGCCTTTCTGAAAACATTTAGCG | 200 |  |  |  |
| 6C/6D-F | TTGGGATGATTGGTCGTATTAG | 200 |  |  |  | *wciN* (CR931638) | 6474-6562 | 89 | Pimenta et al., (2013) |
| 6C/6D-P | CCACGCAATTCGCCATC | 100 | ROX |  | BHQ2 |
| 6C/6D-R | CTCTTCAATTAGTTCTTCAGTTCG | 200 |  |  |  |
| 6B/6D-F | GCA TTG CTA GAG ATG GTT CCT | 300 |  |  |  | *wciNß* (AB795236) | 1020-1123 | 104 | Velusamy et al., (2020) |
| 6B/6D-P | <HEX>A<pdC><pdU>G<pdU><pdC><pdU><pdC>A <pdU>GA<pdU>A A<pdU><pdU>A<pdU><pdU> <BHQ1> | 300 | HEX |  | BHQ Plus |
| 6B/6D-R | CGATACAAGACCAGTTGCTCA | 300 |  |  |  |
| 6A/6B-F | CTGATAAAGTTTCGGATAGAAATAA | 300 |  |  |  | *wciP* (CR931639) | 8718-8826 | 109 | Velusamy et al., (2020) |
| 6A/6B-P | AGAAAAGATAAATAGATTATCAAAACAATTTGCGCAGA | 200 | CY5 |  | BHQ3 |
| 6A/6B-R | AACGTTCTCTATCCAATTAATTTCTC | 300 |  |  |  |
| 7C/7B1-F | GTGAAAAAAAGTAGTACGTTACATAG | 200 |  |  |  | *wzy* (CR931642) | 11978-12072 | 95 | Velusamy et al., (2020) |
| 7C/7B1-P | AGTACGTTACATATAGGACTTATTCTTTTTTTGATTGT | 200 | HEX |  | BHQ1 |
| 7C/7B1-R | GGTACTAAATTAAAGAAGTTTTTACTCA | 200 |  |  |  |
| 7C/7B2-F | TTGAGCATAACGGAGCGATA | 200 |  |  |  | *wchF* (CR931642) | 7013-7092 | 80 | Velusamy et al., (2020) |
| 7C/7B2-P | TGTTCCGAATATTGGTCCAGCTCGAG | 300 | FAM |  | BHQ1 |
| 7C/7B2-R | AGCAGCTATATCATAAGCAATCG | 200 |  |  |  |
| 7F/7A-F | ATGAAGGCTTTGGTTTGACAGG | 200 |  |  |  | *wzy* (CR931643) | 14101-14204 | 104 | Pimenta et al., (2013) |
| 7F/7A-P | ACACCACTATAGGCTGTTGAGACTAACGCACA | 100 | ROX |  | BHQ2 |
| 7F/7A-R | ATTCTCGCCATCAATTGCATATTC | 200 |  |  |  |
| 8-F | ATTCTAATTACTACATTACTGCTTTATACTA | 200 |  |  |  | *wzy* (CR931644) | 10982-11083 | 109 | Velusamy et al., (2020) |
| 8-P | ATGTTTACTTTACGAGTTGGTTTATGTTGATT | 300 | FAM |  | BHQ1 |
| 8-R | TCTTCTTAAATCATAATGAATCGTACC | 200 |  |  |  |
| 9L/9N-F | CGTGGAATTTTCTATACTGCAATAGG | 200 |  |  |  | *wzy* (CR931647) | 11762-11876 | 115 | Velusamy et al., (2020) |
| 9L/9N-P | CAGCAATTCTTAGCCGGATTCTCTCAC | 100 | CY5 |  | BHQ3 |
| 9L/9N-R | CTACTGCTACGATACCATATTCTACAG | 200 |  |  |  |
| 9V/9A-F | AGGTATCCTATATACTGCTTTAGG | 300 |  |  |  | *wzx* (CR931648) | 11767-11920 | 154 | Pimenta et al., (2013) |
| 9V/9A-P | ACACATTGACAACCGCT | 100 | HEX | LNA | BHQ1 |
| 9V/9A-R | CGAATCTGCCAATATCTGAAAG | 300 |  |  |  |
| 10A-F | TAGTGTCGGCAGACAAATTAT | 400 |  |  |  | *wcrG* (CR931649) | 12439-12546 | 108 | Velusamy et al., (2020) |
| 10A-P | TTGAGCATGGTCTCTGATGAGATTT | 200 | CY5 |  | BHQ3 |
| 10A-R | CACGCTCATACACTTTATTTGA | 400 |  |  |  |
| 10F-F | ATAAATTATGATGTTAATCCTAATGTGAC | 200 |  |  |  | *wcrC* (CR931652) | 8881-9026 | 146 | Velusamy et al., (2020) |
| 10F-P | TAAAATTAACGGAGAAATTAAGGGATTATATTAAAAGAGAA | 200 | ROX |  | BHQ2 |
| 10F-R | GGGGTTATAGAAAAAATCACTTTAATTT | 200 |  |  |  |
| 11A/11D/11E-F | AAATGGTTTGGATATGGTTTGTTTGG | 300 |  |  |  | *wzy* (CR931653) | 12015-12121 | 107 | Pimenta et al., (2013) |
| 11A/11D/11E-P | ATTCCAACTTCTCCCAATTTCTGCCACGG | 100 | ROX |  | BHQ2 |
| 11A/11D/11E-R | AGTGCTAACTGTAAAACTTGATTATGAG | 300 |  |  |  |
| 11B/11C-F | CCGCTATCAAATTTGGCGTATTG | 100 |  |  |  | *wzy* (CR931655) | 12516-12653 | 111 | Velusamy et al., (2020) |
| 11B/11C-P | TCCGTGGCAAGATTCTGGTGCTAA | 100 | HEX |  | BHQ1 |
| 11B/11C-R | AGCTGATTATGAGCATAGTTGATCC | 100 |  |  |  |
| 12F/44-F | TTCGGAGGGTCCGATTATATTT | 200 |  |  |  | *wciI* (CR931660) | 5218-5366 | 149 | Velusamy et al., (2020) |
| 12F/44-p | AAATGTAGCTCCACGGAACTTGGA | 200 | CY5 |  | BHQ3 |
| 12F/44-R | CTTTGGTAATCCACTGTTCTGG | 200 |  |  |  |
| 13-F | AGACTACCATTTTTTGATCAGTTAGATT | 200 |  |  |  | *wzy* (CR931661) | 13163-13298 | 136 | Velusamy et al., (2020) |
| 13-P | AAGCAGCACTTCCAAGTCGTAATCTACC | 100 | FAM |  | BHQ1 |
| 13-R | CAGAAAACATATTTTGTTCATAAATCCATC | 200 |  |  |  |
| 14-F | AGAGTGTATGAGGAATCC | 300 |  |  |  | *wzy* (CR931662) | 7920-8007 | 88 | Pimenta et al., (2013) |
| 14-P | CGCCAAGTAACA"T"TTCCATTCCATT | 200 | HEX | “T”=BHQ1 | BHQ1 |
| 14-R | ATATATCTACTGTAGAGGGAAT | 300 |  |  |  |
| 15A/15F-F | AATTGCCTATAAACTCATTGAGATAG | 200 |  |  |  | *wzy* (CR931663) | 7839-7968 | 130 | Pimenta et al., (2013) |
| 15A/15F-P | CCCGCAAACTCTGTCCT | 100 | FAM | LNA | BHQ1 |
| 15A/15F-R | CCATAGGAAGGAAATAGTATTTGTTC | 200 |  |  |  |
| 15B/15C-F | CATAGTATTTGTAGTAATGGTTCAGATT | 200 |  |  |  | *wzy* (CR931664) | 7761-7847 | 87 | Velusamy et al., (2020) |
| 15B/15C-P | ACTTCAATTAATAAGCGGATGATTGTAGCGT | 200 | FAM |  | BHQ1 |
| 15B/15C-R | AGCAATATAAGAGGTATAGTTGGATAA | 200 |  |  |  |
| 16F-F | TAATGTTATGACCTTGGTAATCTTCCC | 300 |  |  |  | *wzy* (CR931668) | 12016-12214 | 199 | Pimenta et al., (2013) |
| 16F-P | AGCCATAAGTCT"T"CCAAATGCTTAACCGCT | 100 | HEX | “T”=BHQ1 | BHQ1 |
| 16F-R | TCCCAAAGGATAATCAATAACTTTTAGAAG | 300 |  |  |  |
| 17F-F | CGGAATATCATGGAGCCTATTA | 200 |  |  |  | *wciP* (CR931670) | 10574-10703 | 130 | Velusamy et al., (2020) |
| 17F-P | TGTTTGCTGATCAGGATGATATCTGG | 200 | HEX |  | BHQ1 |
| 17F-R | AACGTTCTAATTTGTCCACATC | 200 |  |  |  |
| 18C/18F/18B/18A-F | TCGATGGCTAGAACAGATTTATGG | 200 |  |  |  | *wzy* (CR931673) | 12934-13081 | 148 | Pimenta et al., (2013) |
| 18C/18F/18B/18A-P | AGGGAGTTGAATCAACCTATAATTTCGCCCC | 100 | CY5 |  | BHQ3 |
| 18C/18F/18B/18A-R | CCATTGTCCCTGTAAGACCATTG | 200 |  |  |  |
| 19A-F | CGCCTAGTCTAAATACCA | 200 |  |  |  | *wzy* (CR931675) | 9492-9580 | 89 | Pimenta et al., (2013) |
| 19A-P | TATCAATGAGCCGATCCGTCACTT | 100 | ROX |  | BHQ2 |
| 19A-R | GAGGTCAACTATAATAGTAAGAG | 200 |  |  |  |
| 19F-F | TGAGGTTAAGATTGCTGATCG | 300 |  |  |  | *wzy* (CR931678) | 11131-11350 | 221 | Pimenta et al., (2013) |
| 19F-P | CGCACTGTCAATTCACCTTC | 100 | ROX | LNA | BHQ2 |
| 19F-R | CACGAATGAGAACTCGAATAAAAG | 300 |  |  |  |
| 20-F | AAAGATACTGGCTGAGGAGCTATCTATT | 200 |  |  |  | *wciL* (CR931679) | 10132-10223 | 92 | Velusamy et al., (2020) |
| 20-P | AGGATAAGGTCTACTTTGTGGGAGTTC | 200 | FAM |  | BHQ1 |
| 20-R | AGTCAAAAGTACTCAACCATTCTGATATATTC | 200 |  |  |  |
| 21-F | GGTTTAAATATCGCTCCGGGTAT | 100 |  |  |  | *wzy* (CR931680) | 12355-12441 | 87 | Velusamy et al., (2020) |
| 21-P | TGTGAATTGGACACGTTATGGAGC | 100 | ROX |  | BHQ2 |
| 21-R | CAAAAAAAGGGCTTGTAGACGAA | 100 |  |  |  |
| 22A/22F-F | CTTGGGACTTCTCTATTTGTTATAGG | 200 |  |  |  | *wzy* (CR631682) | 12961-13048 | 88 | Velusamy et al., (2020) |
| 22A/22F-P | TCCCGAAACCAAATTGCTATCCCTCC | 200 | FAM |  | BHQ1 |
| 22A/22F-R | AATATGAGTTACCGCCAACTTT | 200 |  |  |  |
| 22A-F | CCCAGGACAATCACAAGAACTA | 300 |  |  |  | *wcwA* (CR931681) | 8693-8776 | 84 | Velusamy et al., (2020) |
| 22A-P | TTTGGAGTTGGTTTCTGATCCAGA | 100 | ROX |  | BHQ2 |
| 22A-R | TGATGCTTGGCCAAATTGGAG | 300 |  |  |  |
| 22F-F | CTTGTCAAGTATGCTGAGGATTTG | 200 |  |  |  | *wcwA* (HE651300) | 39-120 | 82 | Velusamy et al., (2020) |
| 22F-P | ACTCAACAAGCTACAGATGGACATGAAGT | 200 | CY5 |  | BHQ3 |
| 22F-R | AGATTTCTCCTGGATATAATGCGAT | 200 |  |  |  |
| 23A-F | CTCCCCTCCATTACCCATTTGG | 200 |  |  |  | *wzy* (CR931683) | 8626-8711 | 86 | Pimenta et al., (2013) |
| 23A-P | AGCTAGAAC”T”CCCACACTCCCTACTCCCA | 200 | ROX | “T”=BHQ1 | BHQ2 |
| 23A-R | TGAAGAAAGTGCTGTTTGTGAACC | 200 |  |  |  |
| 23B-F | TTGAAGAAATTGCTCCAGAAACAT | 300 |  |  |  | *wzx* (CR931684) | 13656-13733 | 78 | Velusamy et al., (2020) |
| 23B-P | TAGAGCTATTTATCTTTCGTGGTTTT | 200 | CY5 |  | BHQ3 |
| 23B-R | CCAAAAGACTAGCCTCAACCACTAA | 300 |  |  |  |
| 23F-F | GACAGCAACGACAATAGTCATCTC | 300 |  |  |  | *wzy* (CR931685) | 9049-9274 | 226 | Pimenta et al., (2013) |
| 23F-P | ATTGTGTCCA"T"AACCCTTCGTCGTATTTCCAAAG | 200 | ROX | “T”=BHQ1 | BHQ2 |
| 23F-R | TCCATCCCAACCTAACACACTTC | 300 |  |  |  |
| 24F/24A/24B-F | GGAGCGGGATATATTTCTTCTAGTC | 200 |  |  |  | *wzy* (CR931688) | 12323-12415 | 93 | Velusamy et al., (2020) |
| 24F/24A/24B-P | TCTATTGTTACYGGTCCATTAGGACGT | 200 | CY5 |  | BHQ3 |
| 24F/24A/24B-R | CAAACCTACATCGCTTGGATAAT | 200 |  |  |  |
| 28F/28A-F | TTTAGTTCGTGGAGGTAGACT | 200 |  |  |  | *wzy* (CR931692) | 10658-10753 | 96 | Velusamy et al., (2020) |
| 28F/28A-P | ACCAATTTCAATTCCAGGAGCGAA | 100 | CY5 |  | BHQ3 |
| 28F/28A-R | ACATTCCCAATACCTATAAATAGCC | 200 |  |  |  |
| 31-F | AGGTTGGGACAAACCTTGC | 200 |  |  |  | *wzy* (CR931695) | 9382-9496 | 115 | Velusamy et al., (2020) |
| 31-P | CCCTTAGTGACATCTGTAATGCTATCTTCT | 200 | CY5 |  | BHQ3 |
| 31-R | CGTAAGAGAGCCTTCTCAATAGTC | 200 |  |  |  |
| 33A/33F/37-F | GGAACTGGTTCAGCAACTATACG | 500 |  |  |  | *wzy* (CR931698) | 11392-11537 | 146 | Pimenta et al., (2013) |
| 33A/33F/37-P | CCCCAAATAGGAC"T"TTTCTGCCATGCCAAA | 300 | HEX | “T”=BHQ1 | BHQ1 |
| 33A/33F/37-R | GGTTCTAAGACCGTCTGAAATACC | 500 |  |  |  |
| 34-F | CGTGGAAGTTTCTCGCAAATAA | 200 |  |  |  | *wzy* (CR931703) | 7683-7820 | 138 | Velusamy et al., (2020) |
| 34-P | TTTACTGAAGACTTAGTCGGATTGGG | 200 | HEX |  | BHQ1 |
| 34-R | CACGTAAGAAATAGGAGATATGAAGC | 200 |  |  |  |
| 35A-F | TTCCTGATTATGTTGAGATTTGGC | 200 |  |  |  | *wzy* (CR931704) | 7389-7467 | 79 | Velusamy et al., (2020) |
| 35A-P | ACCAGAGTTAGACACTATCTTGGTTTCC | 300 | HEX |  | BHQ1 |
| 35A-R | AGCGTTGATGGAAGTAATGAATATC | 200 |  |  |  |
| 35B-F | GAAAGGTATGGAGAAGTTGAGAATG | 200 |  |  |  | *wzy* (CR931705) | 8168-8256 | 89 | Velusamy et al., (2020) |
| 35B-P | ATTCCTTACGTAGAACTGTAAGGGAAGG | 200 | HEX |  | BHQ1 |
| 35B-R | TCCATCTCTATTATTCATATTAAACCCTATTA | 200 |  |  |  |
| 35F/47F-F | GTGGTCGTATATACTTGATGAATAAATCG | 200 |  |  |  | *wzy* (CR931707) | 7694-7834 | 141 | Velusamy et al., (2020) |
| 35F/47F-P | TCCATTCAACTGGTCGTCCGAATAATCC | 200 | FAM |  | BHQ1 |
| 35F/47F-R | ACATACAAATTATCAACATACAGATAGGTC | 200 |  |  |  |
| 37-F | GATGCCAGCATTTATTCACACC | 200 |  |  |  | *tts* (AJ131985) | 2220-2310 | 91 | Velusamy et al., (2020) |
| 37-P | AGAGACGCCAGTGAGTATCAAGGAGT | 200 | FAM |  | BHQ1 |
| 37-R | GGCATTCAATACGACTAATACCAATAC | 200 |  |  |  |
| 38-F | TGATGAGCTTCCAAATTCTTTT | 200 |  |  |  | *wcyV* (CR931710) | 18262-18344 | 83 | Velusamy et al., (2020) |
| 38-P | TGATGAAAACCAATCATGATAGTGGCAGTATTA | 200 | CY5 |  | BHQ3 |
| 38-R | TCAAGTCTTCTTTGTTTTTTACAAT | 200 |  |  |  |
| 39-F | TGCGCTAAGGTATATTCCGTATTT | 200 |  |  |  | wzy (CR931711) | 11321-11431 | 111 | Velusamy et al., (2020) |
| 39-P | TGATGATGGAGCCTATCATTATAAAGCAGC | 200 | CY5 |  | BHQ3 |
| 39-R | GACATCAAGTTCCCAAACCAATC | 200 |  |  |  |