

## 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	TTTCATCCCTATGTGTTATAG	300				wzy (CR931632)	9875-10035	161	Pimenta et al., (2013)
1-P	TGCCAAAGCCAGCCAT	100	FAM	LNA	BHQ1				
1-R	GCTTTAGAAGGTAGAGTTAACAAC	300							
2-F	TGTTATCCCATATAAGAACCAGTGT	300				wzy (CR931633)	10342-10452	111	Pimenta et al., (2013)
2-P	TTGCAATT"T"CAATTTTTTGCCCAATCTC	200	ROX	"T"=BHQ1	BHQ2				
2-R	AAAATTACCCCAAAGCTATCCAA	300							
3-F	CCACTAAAGCTTTGGCAAAGAAA	300				galU (CR931634)	8564-8648	85	Pimenta et al., (2013)
3-P	TTGTAGACCGCCCCACAA"T"TCATTTTGT	200	HEX	"T"=BHQ1	BHQ1				
3-R	CCCGAACGTAAGCTTCTTCA	300							
4-F	GCTTCTGCTGAAGTGTGTGC	300				wzy (CR931635)	10521-10734	214	Pimenta et al., (2013)
4-P	TTCCACAAAAGAAGCCTACAGGTAACCCCA	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	GTTTGCCTAGAGTATGGGAAGG	200				wzy (CR931638)	8818-8949	132	Pimenta et al., (2013)
6A/6B/6C/6D -P	TGTTCTGCCCT"T"GAGCAACTGGTCTTGTATC	200	FAM		BHQ1				
6A/6B/6C/6D -R	TAGCCTTCTGAAACATTTAGCG	200							
6C/6D-F	TTGGGATGATTGGTGGTATTAG	200				wciN (CR931638)	6474-6562	89	Pimenta et al., (2013)
6C/6D-P	CCACGCAATT CGCCATC	100	ROX		BHQ2				
6C/6D-R	CTCTCAATTAGTCTTCCAGTTCTG	200							
6B/6D-F	GCA TTG CTA GAG ATG GTT CCT	300				wciNβ (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<pdU><pdU>A<pdU><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	AGAAAAGATAAATAGATTATCAAAACAATTGCGCAGA	200	CY5		BHQ3				
6A/6B-R	AACGTTCTCTATCCAATTAATTTCTC	300							
7C/7B1-F	GTGAAAAAAGTAGTACGTTACATAG	200				wzy (CR931642)	11978-12072	95	Velusamy et al., (2020)
7C/7B1-P	AGTACGTTACATATAGGACTTATCTTTTTTGATTGT	200	HEX		BHQ1				
7C/7B1-R	GGTACTAAATTAAGAAGTTTTTACTCA	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	ATGAAGGCTTTGGTTGACAGG	200				wzy (CR931643)	14101-14204	104	Pimenta et al., (2013)
7F/7A-P	ACACCACTATAGGCTGTTGAGACTAACGCACA	100	ROX		BHQ2				
7F/7A-R	ATTCTGCCATCAATTGCATATT	200							
8-F	ATTCTAATTACTACATTACTGCTTTACTA	200				wzy (CR931644)	10982-11083	109	
8-P	ATGTTTACTTTACGAGTTGGTTTATGTTGATT	300	FAM		BHQ1				

8-R	TCTTCTTAAATCATAATGAATCGTACC	200							Velusamy et al., (2020)	
9L/9N-F	CGTGGAATTTTCTACTGCAATAGG	200								
9L/9N-P	CAGCAATTTCTTAGCCGGATTCTCTCAC	100	CY5			BHQ3	wzy (CR931647)	11762-11876	115	Velusamy et al., (2020)
9L/9N-R	CTACTGTACGATACCATATTCTACAG	200								
9V/9A-F	AGGTATCTATATACTGCTTTAGG	300								
9V/9A-P	ACACATTGACAACCGCT	100	HEX	LNA		BHQ1	wzx (CR931648)	11767-11920	154	Pimenta et al., (2013)
9V/9A-R	CGAATCTGCCAATATCTGAAAG	300								
10A-F	TAGTGTGCGCAGACAAATTAT	400								
10A-P	TTGAGCATGGTCTCTGATGAGATTT	200	CY5			BHQ3	wcrG (CR931649)	12439-12546	108	Velusamy et al., (2020)
10A-R	CACGCTCATAACATTTATTTGA	400								
10F-F	ATAAATTATGATGTTAATCCTAATGTGAC	200								
10F-P	TAAAATTAACGGAGAAATTAAGGGATTATATTAAGAGAGAA	200	ROX			BHQ2	wcrC (CR931652)	8881-9026	146	Velusamy et al., (2020)
10F-R	GGGGTTTAGAAAAAATCACTTTAATTT	200								
11A/11D/11E-F	AAATGGTTTGGATATGGTTTGTGG	300								
11A/11D/11E-P	ATTCCAACCTCTCCAATTTCTGCCACGG	100	ROX			BHQ2	wzy (CR931653)	12015-12121	107	Pimenta et al., (2013)
11A/11D/11E-R	AGTGCTAACTGTAAACTTGATTATGAG	300								
11B/11C-F	CCGCTATCAAATTTGGCGTATTG	100								
11B/11C-P	TCCGTGGCAAGATTCTGGTGCTAA	100	HEX			BHQ1	wzy (CR931655)	12516-12653	111	Velusamy et al., (2020)
11B/11C-R	AGTGATTATGAGCATAGTTGATCC	100								
12F/44-F	TTCGGAGGGTCCGATTATATTT	200								
12F/44-p	AAATGTAGTCCACGGAACCTGGA	200	CY5			BHQ3	wciI (CR931660)	5218-5366	149	Velusamy et al., (2020)
12F/44-R	CTTTGGTAATCCACTGTTCTGG	200								
13-F	AGACTACCATTTTTGATCAGTTAGATT	200								
13-P	AAGCAGCACTTCCAAGTCGTAATCTACC	100	FAM			BHQ1	wzy (CR931661)	13163-13298	136	Velusamy et al., (2020)
13-R	CAGAAAAACATATTTGTTCCATAAAATCCATC	200								
14-F	AGAGTGTATGAGGAATCC	300								
14-P	CGCCAAGTAACA"TT"TTCCATTCCATT	200	HEX	"T"=BHQ1		BHQ1	wzy (CR931662)	7920-8007	88	Pimenta et al., (2013)
14-R	ATATATCTACTGTAGAGGGAAT	300								
15A/15F-F	AATTGCCTATAAACTCATTGAGATAG	200								
15A/15F-P	CCCGCAAACCTCTGTCTT	100	FAM	LNA		BHQ1	wzy (CR931663)	7839-7968	130	Pimenta et al., (2013)
15A/15F-R	CCATAGGAAGGAAATAGTATTTGTTC	200								
15B/15C-F	CATAGTATTTGTAGTAATGGTTCAGATT	200								
15B/15C-P	ACTTCAATTAATAAGCGGATGATTGTAGCGT	200	FAM			BHQ1	wzy (CR931664)	7761-7847	87	Velusamy et al., (2020)
15B/15C-R	AGCAATATAAGAGGTATAGTTGGATAA	200								
16F-F	TAATGTTATGACCTTGGAATCTTCCC	300								
16F-P	AGCCATAAGTCT"TT"CCAAATGCTTAACCGCT	100	HEX	"T"=BHQ1		BHQ1	wzy (CR931668)	12016-12214	199	Pimenta et al., (2013)
16F-R	TCCCAAAGGATAATCAATAACTTTTAGAAG	300								
17F-F	CGGAATATCATGGAGCCTATTA	200								
17F-P	TGTTTGTGATCAGGATGATATCTGG	200	HEX			BHQ1	wciP (CR931670)	10574-10703	130	Velusamy et al., (2020)
17F-R	AACGTTCTAATTTGTCCACATC	200								
18C/18F/18B/18A-F	TCGATGGCTAGAACAGATTTATGG	200								
18C/18F/18B/18A-P	AGGGAGTTGAATCAACCTATAATTTGCCCC	100	CY5			BHQ3	wzy (CR931673)	12934-13081	148	Pimenta et al., (2013)
18C/18F/18B/18A-R	CCATTGTCCCTGTAAGACCATTG	200								
19A-F	CGCCTAGTCTAAATACCA	200								
19A-P	TATCAATGAGCCGATCCGTCACCTT	100	ROX			BHQ2	wzy (CR931675)	9492-9580	89	Pimenta et al., (2013)
19A-R	GAGGTCAACTATAATAGTAAGAG	200								
19F-F	TGAGGTTAAGATTGCTGATCG	300								
19F-P	CGCACTGTCAATTCACCTTC	100	ROX	LNA		BHQ2	wzy (CR931678)	11131-11350	221	Pimenta et al., (2013)

19F-R	CACGAATGAGAAGCTCGAATAAAAG	300							
20-F	AAAGATACTGGCTGAGGAGCTATCTATT	200							
20-P	AGGATAAGGTCTACTTTGTGGGAGTTC	200	FAM		BHQ1	wciL (CR931679)	10132-10223	92	Velusamy et al., (2020)
20-R	AGTCAAAAGTACTCAACCATTCTGATATATTC	200							
21-F	GGTTTAAATATCGCTCCGGGTAT	100							
21-P	TGTGAATTGGACACGTTATGGAGC	100	ROX		BHQ2	wzy (CR931680)	12355-12441	87	Velusamy et al., (2020)
21-R	CAAAAAAAGGGCTTGAGACGAA	100							
22A/22F-F	CTTGGGACTTCTCTATTTGTTATAGG	200							
22A/22F-P	TCCCGAAACCAATTGCTATCCCTCC	200	FAM		BHQ1	wzy (CR631682)	12961-13048	88	Velusamy et al., (2020)
22A/22F-R	AATATGAGTTACCGCCAACCTTT	200							
22A-F	CCCAGGACAATCACAGAAGCTA	300							
22A-P	TTTGGAGTTGGTTTCTGATCCAGA	100	ROX		BHQ2	wcwA (CR931681)	8693-8776	84	Velusamy et al., (2020)
22A-R	TGATGCTTGGCCAAATTGGAG	300							
22F-F	CTTGCAAGTATGCTGAGGATTGG	200							
22F-P	ACTCAACAAGCTACAGATGGACATGAAGT	200	CY5		BHQ3	wcwA (HE651300)	39-120	82	Velusamy et al., (2020)
22F-R	AGATTTCTCCTGGATATAATGCGAT	200							
23A-F	CTCCCTCCATTACCCATTTGG	200							
23A-P	AGCTAGAAC"TT"CCCACACTCCCTACTCCCA	200	ROX	"T"=BHQ1	BHQ2	wzy (CR931683)	8626-8711	86	Pimenta et al., (2013)
23A-R	TGAAGAAAGTGCTGTTTGTGAACC	200							
23B-F	TTGAAGAAATTGCTCCAGAAACAT	300							
23B-P	TAGAGCTATTTATCTTTCTGGTTTT	200	CY5		BHQ3	wzx (CR931684)	13656-13733	78	Velusamy et al., (2020)
23B-R	CCAAAAGACTAGCCTCAACCACTAA	300							
23F-F	GACAGCAACGACAATAGTCATCTC	300							
23F-P	ATTGTGTCCA"TT" AACCTTCGTCGTATTTCCAAAG	200	ROX	"T"=BHQ1	BHQ2	wzy (CR931685)	9049-9274	226	Pimenta et al., (2013)
23F-R	TCCATCCCAACCTAACCACTTC	300							
24F/24A/24B-F	GGAGCGGGATATATTTCTTCTAGTC	200							
24F/24A/24B-P	TCTATTGTTACYGGTCCATTAGGACGT	200	CY5		BHQ3	wzy (CR931688)	12323-12415	93	Velusamy et al., (2020)
24F/24A/24B-R	CAAACTACATCGCTTGGATAAT	200							
28F/28A-F	TTTAGTTCGTGGAGGTAGACT	200							
28F/28A-P	ACCAATTTCAATTCAGGAGCGAA	100	CY5		BHQ3	wzy (CR931692)	10658-10753	96	Velusamy et al., (2020)
28F/28A-R	ACATTCCTAACCTATAAATAGCC	200							
31-F	AGGTTGGGACAAACCTTGC	200							
31-P	CCCTTAGTGACATCTGTAATGCTATCTCT	200	CY5		BHQ3	wzy (CR931695)	9382-9496	115	Velusamy et al., (2020)
31-R	CGTAAGAGAGCCTTCTCAATAGTC	200							
33A/33F/37-F	GGAAGTGGTTCAGCAACTATACG	500							
33A/33F/37-P	CCCCAAATAGGAC"TT"TTTCTGCCATGCCAAA	300	HEX	"T"=BHQ1	BHQ1	wzy (CR931698)	11392-11537	146	Pimenta et al., (2013)
33A/33F/37-R	GGTTCTAAGACCGTCTGAAATACC	500							
34-F	CGTGGAAAGTTTCTCGCAAATAA	200							
34-P	TTTACTGAAGACTTAGTCGGATTGGG	200	HEX		BHQ1	wzy (CR931703)	7683-7820	138	Velusamy et al., (2020)
34-R	CACGTAAGAAATAGGAGATATGAAGC	200							
35A-F	TTCTGATTATGTTGAGATTTGGC	200							
35A-P	ACCAGAGTTAGACACTATCTTGGTTCC	300	HEX		BHQ1	wzy (CR931704)	7389-7467	79	Velusamy et al., (2020)
35A-R	AGCGTTGATGGAAGTAATGAATATC	200							
35B-F	GAAAGGTATGGAGAAGTTGAGAATG	200							
35B-P	ATTCTTTACGTAGAAGTGAAGGGAAGG	200	HEX		BHQ1	wzy (CR931705)	8168-8256	89	Velusamy et al., (2020)
35B-R	TCCATCTCTATTATTCATATTAACCCCTATTA	200							
35F/47F-F	GTGGTCGTATATACTTGATGAATAAATCG	200							
35F/47F-P	TCCATCAACTGGTCGTCCGAATAATCC	200	FAM		BHQ1	wzy (CR931707)	7694-7834	141	Velusamy et al., (2020)
35F/47F-R	ACATACAAATTATCAACATACAGATAGGTC	200							

37-F	GATGCCAGCATTATTACACACC	200				tts (AJ131985)	2220-2310	91	Velusamy et al., (2020)
37-P	AGAGACGCCAGTGAGTATCAAGGAGT	200	FAM		BHQ1				
37-R	GGCATTCAATACGACTAATACCAATAC	200							
38-F	TGATGAGCTTCCAAATTCITTT	200				wcyV (CR931710)	18262-18344	83	Velusamy et al., (2020)
38-P	TGATGAAAACCAATCATGATAGTGCCAGTATTA	200	CY5		BHQ3				
38-R	TCAAGTCTTCTTTGTTTTTTACAAT	200							
39-F	TGCGCTAAGGTATATCCGTATTT	200				wzy (CR931711)	11321-11431	111	Velusamy et al., (2020)
39-P	TGATGATGGAGCCTATCATTATAAAGCAGC	200	CY5		BHQ3				
39-R	GACATCAAGTCCCAAACCAATC	200							