

Supplementary table: Primers included in the enrichment PCRs*

Primer name	Specificity	Sequence**
Primers included in the enrichment PCR for PPSV 23 and related serotypes		
1Forward	Serotype 1	GGG ACT TTA ATT TTA TGC AGT G
1Reverse	Serotype 1	CAC AAT GGC TTT AGA AGG TAG AG
2Forward	Serotype 2	CGG CAT TGT ATT CTT TAT ATC G
2Reverse	Serotype 2	CGT TTG TAT CCA TTT AAC TGC ATC
3Forward	Serotype 3	TTG ATA TTC CCC TTG ACA ATA G
3Reverse	Serotype 3	GGA GGC TTC AAG ATT CAA CTC
4Forward	Serotype 4	CCG TCT ATA TTT ATA TGG GTT TGC
4Reverse	Serotype 4	CCT GAT AAT TTT GTA CTT CTG AAT G
5Forward	Serotype 5	GTT TTC CCA ATA GTA GTT TGC G
5Reverse	Serotype 5	CCC ACA GCC AAA TAG AGT TG
6B6AForward	Serogroup 6 (6A, 6B, 6C, 6D)	TCA ACC TGC AGT AAT TTT AAC A
6B6AReverse	Serogroup 6 (6A, 6B, 6C, 6D)	CTA CTT TCT GAA TTT CAC GGA TAT AAA G
7F7AForward	Serotypes 7F, 7A	GCA AGT GTT TCA ATG GGA GTA
7F7AReverse	Serotypes 7F, 7A	ACG GAG GGA CCA TAC AAT AAG
8Forward	Serotype 8	GAT GTT AGT TTC TTC GAT TCC AG
8Reverse	Serotype 8	TCA TAA TGA ATC GTA CCA ATC AAC
9N9LForward	Serotypes 9N, 9L	TCA ATG GCG ACT TTA TTT GC
9N9LReverse	Serotypes 9N, 9L	AGT CTA TTA TCT CCT GTA GGG TGC
9V9AForward	Serotypes 9V, 9A	AAC TAT ATT TAC CCT ACT CTC CAC AG
9V9AReverse	Serotypes 9V, 9A	ACC GGA AAA AGC AAT TGA G
10A10BForward	Serotypes 10A, 10B	TGA GCT ATT TAA GGA CCT GGG
10A10BReverse	Serotypes 10A, 10B	GCA AGC GTC ACT TTC TTG A
11A11DForward	Serotypes 11A 11D	GAA ATA TCG CCA TTC ATC AG

11A11DReverse	Serotypes 11A, 11D	CAA CAG CAA CTG TGC CAC T
124446Forward	Serotypes 12F, 12A,12B, 44,46	TGA ATA TGG ACG GTG GAG
124446Reverse	Serotypes 12F, 12A,12B, 44,46	AGC AAA GAA AGC CGA AAG
14Forward	Serotype 14	CCT ACT TCC AAA ACA GTT TAT GC
14Reverse	Serotype 14	GTC AAT ATT GAT TGG CAT TTT C
15B15CForward	Serotypes 15B, 15C	TAA TAA GCG GAT GAT TGT AGC G
15B15CReverse	Serotypes 15B, 15C	TAT ACT GAT TAA CTT TCC AGA TGG G
17FForward	Serotype 17F	AGA GGG ATT GTT GAA GGT ATT C
17FReverse	Serotype 17F	AGT AGT CTC GCA TTT CTA TCA TCC
18Forward	Serogroup 18	AAT TGT TCT TTT CCT GTA CTC AGT C
18Reverse	Serogroup 18	CGA ACC ATT GAA ACT ATC ATC TG
19AForward	Serotype 19A	TGT ATT TGC CCT TAT TAA TGT GC
19AReverse	Serotype 19A	AAG TGC AAG ATT ATG AAT CTC TCT C
19FForward	Serotype 19F	TCA GTA TTT GCA CTG GTT AAT TC
19FReverse	Serotype 19F	AAG AAC AAG GTT GTA TAT TTC CTT C
20Forward	Serotype 20	CTT TAT CAG GAA TAC GCC AAT C
20Reverse	Serotype 20	CTG TAT AAT AAC GAG AAC CAA CG
22F22AForward	Serotypes 22F, 22A	AGG ATG CAG TAG ATA CCA GTG G
22F22AReverse	Serotypes 22F, 22A	TAT AAA CGG AGG TTG TTG TCC
23FForward	Serotype 23F	TGA TAG TGA ACT TGG GAT TGT C
23FReverse	Serotype 23F	CTT TAT CGG TAA GGT GGA TAA

		G
33F33A37Forward	Serotypes 33F,33A, 37	TCA ACT AGT CAA GGA TTT GAT GG
33F33A37Reverse	Serotypes 33F,33A, 37	CGT ATC AGA TTT GCG ATT TC
Primers included in the enrichment PCR for the additional serotypes		
7B7C40Forward	Serotypes 7B, 7C, and 40	AAA ACT CAA GTA TCT GTG YCA CCT T
7B7C40Reverse	Serotypes 7B, 7C, and 40	CAT CTC TAT TCG ACC TTG CGT TA
10F10CForward	Serotypes 10F and 10C	TAG TTT TGG TTA CGT AGT TGT TGA CT
10F10CReverse	Serotypes 10F and 10C	GCA ATR AAT ACT GTA GCA TAC GAT AGT T
11F11A11DForward	Serotypes 11F, 11A, and 11D	GAA ATA TCG CCA TTC ATC AG
11FReverse	Serotype 11F	GAT TGT ACC CCA TCA CCG
11B11CForward	Serotypes 11B and 11C	TCT GGT GCT AAG GGG ATC AA
11B11CReverse	Serotypes 11B and 11C	CCA ATT ACT CCA TTA TCT ATT GCT AAT
13Forward	Serotype 13	GAT GGG AAA ATA CGA TAT GCT C
13Reverse	Serotype 13	GAA AAT CGT AAC ATG GAA AAA GTA A
15F15AForward	Serotypes 15F and 15A	TAT TTC CTT CCT ATG GGA CAA C
15F15AReverse	Serotypes 15F and 15A	GCA AGC ATT TTA CCA AGT TCA TAA A
16FForward	Serotype 16F	TTG TTC TTA CAT TTA GCC GTA GTG
16FReverse	Serotype 16F	TCG TCG TTG AAA ACA ATT TCT TAC
16AForward	Serotype 16A	CCG CTC ACG GTA TGG ACT A
16AReverse	Serotype 16A	CCA GCA ATA TAC TCA GGA AAT AAT TC
17AForward	Serotype 17A	GTA GAC TTC TTA GAG CCT ATT GTG G

17AReverse	Serotype 17A	CAT TCG ACC AGA TAT AGG TAC GAT
19B19CForward	Serotypes 19B and 19C	AGA ATT CGG AGA TTT GTG GTA T
19B19CReverse	Serotypes 19B and 19C	CAA TCC ACC TCC ATA AAC GA
21Forward	Serotype 21	CAA TTC TAC TGA GTC CAT ATT ATG AAA
21Reverse	Serotype 21	ACC ATC GTA CCT GCA CCA TAA
23AForward	Serotype 23A	TTT ACT TTA ATT TAT AGC TTT TTG GCT AA
23AReverse	Serotype 23A	GGT GCA TGA GTT AGG AGA AAG TG
23BForward	Serotype 23B	GGA TCG TTG TTC ATA GCG G
23BReverse	Serotype 23B	GAT AAT AAA GAA ATT ACT AAC CAT GTC GT
24F24A24BForward	Serotypes 24F, 24A, and 24B	TCA ACA CTT ATG ATG RTG CCT G
24F24A24BReverse	Serotypes 24F, 24A, and 24B	GCA GAA ACA AAR GTA AGA ATT ATA GAT ATC
25F25A38Forward	Serotypes 25F, 25A, and 38	GAC TAC AAA CTG CGG TAG TAG AAA TG
25F25A38Reverse	Serotypes 25F, 25A, and 38	TGG AAC AAT TCT AAT CGT TAA TAC G
27Forward	Serotype 27	GCA GCC ACC TCT TCT CAT TC
27Reverse	Serotype 27	GGA AGG AAC AAC CCA ACA AT
28F28AForward	Serotypes 28F and 28A	CAG AGT TTG GTC GAG GTT CCT A
28F28AReverse	Serotypes 28F and 28A	GCT CAA CTT TAT TTC TCT AGA ATA AAC A
29Forward	Serotype 29	CCG AAA ATT GTT CAC AGG ATA C
29Reverse	Serotype 29	AGC TTT CTT TTG TAC GAC TCT TTT A
31Forward	Serotype 31	TGA AAA TCC CTT AGT GAC ATC TG
31Reverse	Serotype 31	GCC ATA ATC AAA AAT AAG TTA GAC ATA A

32F32AForward	Serotypes 32F and 32A	GGT ATG CTT ACA ATG AGA CGC
32F32AReverse	Serotypes 32F and 32A	AAT TCG TTC CCG GAT AAG ATG
33B33D33CForward	Serotypes 33B, 33D, and 33C	TCG TTG GAT GAC AAA ACT CTT AC
33B33D33CReverse	Serotypes 33B, 33D, and 33C	CCT CCC TGA GCC AAA ATA AC
33CForward	Serotype 33C	CGA ATC GTC ATA AGG CAA AA
33CReverse	Serotype 33C	AAT AGG AGT AAC AAA GAG AAG CCT AA
34Forward	Serotype 34	TTA AAA GTA TTA TTG GTA GTG ATT CTT TTG
34Reverse	Serotype 34	TGT AAA GAC ATT CCC TGT AGG C
35F47FForward	Serotypes 35F and 47F	ATA AAA AGA AAG TCT TTG CCA GAG
35F47FReverse	Serotypes 35F and 47F	CAA CTT TTG GAA GAT ACT GAA CAT AA
35A35C42Forward	Serotypes 35A, 35C, and 42	GGA GAC TRT TAA AAC TTT TTT CGT TC
35A35C42Reverse	Serotypes 35A, 35C, and 42	TTA AGT AAG TCT TCG CAA TCC AG
35BForward	Serotype 35B	CTA ATT TGG CTA TGA AGC TAA TCC C
35BReverse	Serotype 35B	TAA CTT AAA TAG GCA TTA ACA AAA TAG GT
36Forward	Serotype 36	CAA TTT CCC CTT ATT CTG TAG TTC
36Reverse	Serotype 36	TGG AGA TCC CCA AGA GAA AAT A
39Forward	Serotype 39	ATT GGT TTG GGA ACT TGA TGT C
39Reverse	Serotype 39	GCA ATA AGG CAA ATA AGG GAT AAT TA
41F41AForward	Serotypes 41F and 41A	TGA CAC TAT TTA TAA TTG CTT TAT CCT T
41F41AReverse	Serotypes 41F and	TAG CGA GAA ACT ATC TGC ATC

	41A	TTG
43Forward	Serotype 43	AGA TCA AAT GGT GGT ATT AGG AA
43Reverse	Serotype 43	GGA ATA GAT CAT TAA CCC TAA TGA AT
45Forward	Serotype 45	TAT GCA GGA AAT ATC CGA GAA GG
45Reverse	Serotype 45	CCG TGA AAC AGA AAC GCT ATG
47AForward	Serotype 47A	TAT TTG CCA TAA CGG ACT CTA GAA C
47AReverse	Serotype 47A	GCT AAA ATA ATA AAT AGC GAA CTT ACT ACA
48Forward	Serotype 48	GCA TTT GGA GTT ATT GCC CTA C
48Reverse	Serotype 48	CGA CGG AAT CAA TAT AAA TAA GTG ATA
LytA primers included in the multiplex PCR for direct sample and sweep colony detection and serotyping		
LytAForward	LytA-Viridans group of <i>Streptococcus</i> to include <i>S.pneumoniae</i>	ATCGAACTCTTRCGYAATCTA
LytAReverse	LytA-Viridans group of <i>Streptococcus</i> to include <i>S.pneumoniae</i>	TCAAACCTTRTCTTTTGGATAAGAR C

* Original sequences for the primers in the two enrichment PCRs except for the lytA specific ones are from supplementary references 1 and 2

**All primers would have the sequence TCTATTGGGCTATGTCAC attached to the 5' end

Supplementary references

- Kong, F, Brown, M, Sabananthan, A, Zeng, X, Gilbert, GL.** 2006. Multiplex PCR-based reverse line blot hybridization assay to identify 23 *Streptococcus pneumoniae* polysaccharide vaccine serotypes. *J. Clin. Microbiol.* **44**:1887-1891.

2. **Zhou, F, Kong, F, Tong, Z, Gilbert, GL.** 2007. Identification of Less-Common *Streptococcus pneumoniae* Serotypes by a Multiplex PCR-Based Reverse Line Blot Hybridization Assay. J Clin Microbiol. **45**:3411-3415.