

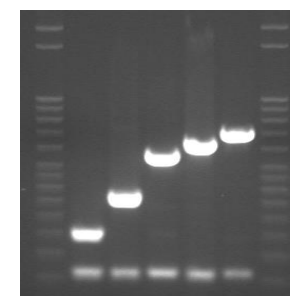
**Protocol for Multiplex PCR - *S. pneumoniae* SEROTYPING - clinical specimens and pneumococcal isolates (USA set)**  
 (da Gloria Carvalho et al., Revisiting pneumococcal carriage by use of broth enrichment and PCR techniques for enhanced detection of carriage and serotypes. J Clin Microbiol. 2010 May;48(5):1611-8. doi: 10.1128/JCM.02243-09. Epub 2010 Mar 10.)  
 Purpose: *S. pneumoniae* serotyping - USA set

Reagent	Final Conc.	Volume (µL)	Total Rxns	Final Volume*
PCR H <sub>2</sub> O	vary	<b>3.7</b>	20	<b>74</b>
2X PCR Buffer - QIAGEN Multiplex PCR Kit (cat#206143)		<b>12.5</b>	20	<b>250</b>
Fwd Primers	vary	see below		
Rev Primers	vary	see below		
Clinical sample DNA [QIAamp DNA Mini Kit (cat# 51306)] (see extraction protocol)	n/a	<b>5.0</b>	20	
<b>Total</b>		25.0	20	500.0

Number of Rxns:		<b>20</b>	
Thermal Cycling Parameters		Post-Reaction Electrophoresis	
1X	95°C for 15min	2% Nusieve Agarose (cat# 50090)	1.6g
		1X TAE	80mL
35X	94°C for 30sec	0.5µg/µL ethidium bromide	5.0µL
	54°C for 90sec	Heat/mix gel completely.	
	72°C for 60sec	Mix 10µL PCR Product + 2µL of loading buffer	
1X	72°C for 10min	Load 10µL of sample and 15µL of 50-bp ladder (cat# 70538-3)	
∞	4°C	<b>Run at 100V for 1:30 h</b>	

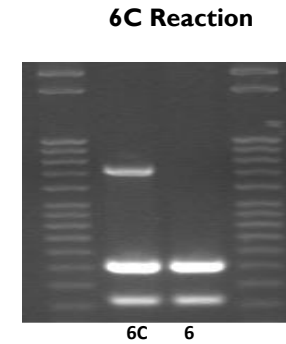
Primer Info		To get 25µM:		Primer Concentrations (µM)			bp
Reaction I (Primer ID)	Original Conc. (µM)	Primer (µL)	Water (µL)	Conc. (µM) I rxn	Per X Rxns (µM)	Amount to add (µL)	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
6A/6B/6C/6D-F (914837)	479.21	13.04	236.96	0.3	20	6.0	250
6A/6B/6C/6D-R (914838)	442.67	14.12	235.88	0.3	20	6.0	
3-F (914831)	415.41	15.05	234.95	0.3	20	6.0	371
3-R (914832)	444.44	14.06	235.94	0.3	20	6.0	
19A-F (914855)	444.55	14.06	235.94	0.3	20	6.0	566
19A-R (914856)	447.73	13.96	236.04	0.3	20	6.0	
22F/22A-F (903763)	404.13	15.47	234.53	0.5	20	10.0	643
22F/22A-R (903764)	331.63	18.85	231.15	0.5	20	10.0	
16F-F (918138)	484.05	12.91	237.09	0.4	20	8.0	717
16F-R (918139)	424.71	14.72	235.28	0.4	20	8.0	
				3.8		76.0	

**USA Reaction I**

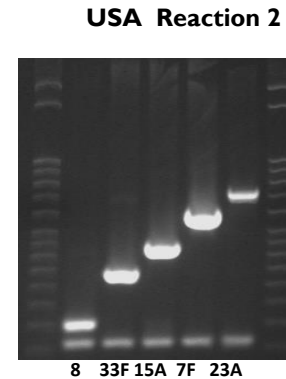


6 3 19A 22F 16F

Primer Info		To get 25 $\mu$ M:		Primer Concentrations ( $\mu$ M)			bp
Reaction 6C (Primer ID)	Original Conc. ( $\mu$ M)	Primer ( $\mu$ L)	Water ( $\mu$ L)	Conc. ( $\mu$ M) 1 rxn	Per X Rxns ( $\mu$ M)	Amount to add ( $\mu$ L)	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
6A/6B/6C/6D-F (914837)	479.21	13.04	236.96	0.3	20	6.0	250
6A/6B/6C/6D-R (914838)	442.67	14.12	235.88	0.3	20	6.0	
6C/6D-F (914839)	418.28	14.94	235.06	0.5	20	10.0	727
6C/6D-R (914840)	458.03	13.65	236.35	0.5	20	10.0	
				<b>1.8</b>		<b>36.0</b>	

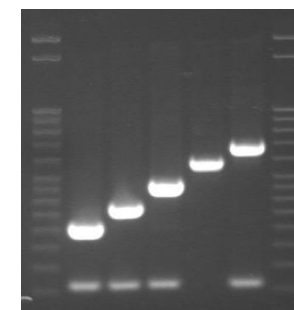


Primer Info		To get 25 $\mu$ M:		Primer Concentrations ( $\mu$ M)			bp
Reaction 2 (Primer ID)	Original Conc. ( $\mu$ M)	Primer ( $\mu$ L)	Water ( $\mu$ L)	Conc. ( $\mu$ M) 1 rxn	Per X Rxns ( $\mu$ M)	Amount to add ( $\mu$ L)	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
8-F (911983)	440.26	14.20	235.80	0.2	20	4.0	201
8-R (911984)	474.20	13.18	236.82	0.2	20	4.0	
33F/33A/37-F(807842)	469.65	13.31	236.69	0.3	20	6.0	338
33F/33A/37-R (807843)	462.94	13.50	236.50	0.3	20	6.0	
15A/15F-F (914847)	458.05	13.64	236.36	0.3	20	6.0	434
15A/15F-R(914848)	472.92	13.22	236.78	0.3	20	6.0	
7F/7A-F (918151)	381.91	16.37	233.63	0.4	20	8.0	599
7F/7A-R (918152)	429.74	14.54	235.46	0.4	20	8.0	
23A-F (903765)	519.51	12.03	237.97	0.5	20	10.0	722
23A-R (903766)	482.31	12.96	237.04	0.5	20	10.0	
				<b>3.6</b>		<b>72.0</b>	



Primer Info		To get 25µM:		Primer Concentrations (µM)			bp
Reaction 3 (Primer ID)	Original Conc. (µM)	Primer (µL)	Water (µL)	Conc. (µM) l rxn	Per X Rxns (µM)	Amount to add (µL)	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
19F-F (903759)	381.06	16.40	233.60	0.5	20	10.0	304
19F-R (903760)	403.38	15.49	234.51	0.5	20	10.0	
12F/12A/44/46-F (914843)	440.29	14.20	235.80	0.5	20	10.0	376
12F/12A/44/46-R (914844)	417.99	14.95	235.05	0.5	20	10.0	
11A/11D-F (914841)	457.45	13.66	236.34	0.3	20	6.0	463
11A/11D-R (914842)	440.9	14.18	235.82	0.3	20	6.0	
38/25F/25A-F (807850)	453.44	13.78	236.22	0.3	20	6.0	574
38/25F/25A-R (807851)	412.76	15.14	234.86	0.3	20	6.0	
35B-F (914863)	446.26	14.01	235.99	0.5	20	10.0	677
35B-R (918606)	431.88	14.47	235.53	0.5	20	10.0	
				4.4		88.0	

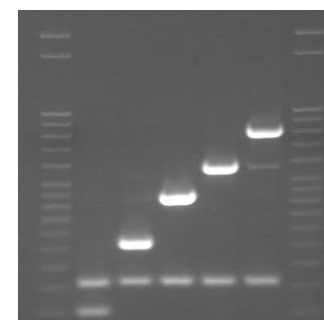
USA Reaction 3



19F 12F 11A 38 35B

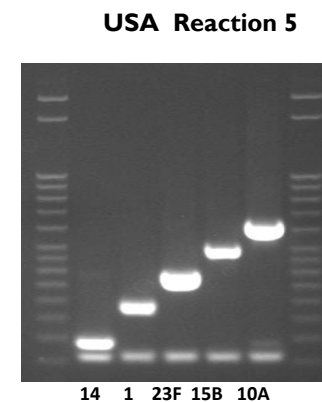
Primer Info		To get 25µM:		Primer Concentrations (µM)			bp
Reaction 4 (Primer ID)	Original Conc. (µM)	Primer (µL)	Water (µL)	Conc. (µM) l rxn	Per X Rxns (µM)	Amount to add (µL)	
24F/24A/24B-F (911995)	458.05	13.64	236.36	0.2	20	4.0	99
24F/24A/24B-R (911996)	453.69	13.78	236.22	0.2	20	4.0	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
7C/7B/40-F (807802)	397.69	15.72	234.28	0.3	20	6.0	260
7C/7B/40-R (807803)	406.43	15.38	234.62	0.3	20	6.0	
4-F (914833)	462.37	13.52	236.48	0.3	20	6.0	430
4-R (914834)	478.61	13.06	236.94	0.3	20	6.0	
18C/18F/18B/18A-F (914853)	449.23	13.91	236.09	0.3	20	6.0	573
18C/18F/18B/18A-R (914854)	419.56	14.90	235.10	0.3	20	6.0	
9V/9A-F (913426)	330.24	18.93	231.07	0.5	20	10.0	816
9V/9A-R (913427)	219.76	28.44	221.56	0.5	20	10.0	
				3.4		68.0	

USA Reaction 4

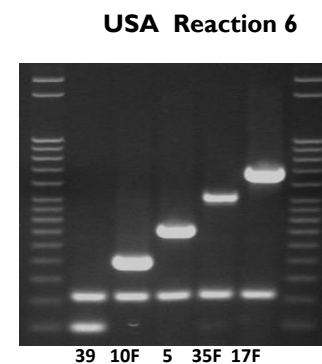


24 7C 4 18 9V

Primer Info		To get 25 $\mu$ M:		Primer Concentrations ( $\mu$ M)			bp
Reaction 5 (Primer ID)	Original Conc. ( $\mu$ M)	Primer ( $\mu$ L)	Water ( $\mu$ L)	Conc. ( $\mu$ M) l rxn	Per X Rxns ( $\mu$ M)	Amount to add ( $\mu$ L)	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
14-F (914845)	458.38	13.63	236.37	0.3	20	6.0	189
14-R (914846)	438.64	14.25	235.75	0.3	20	6.0	
1-F (914827)	419.17	14.91	235.09	0.3	20	6.0	280
1-R (914828)	496.11	12.60	237.40	0.3	20	6.0	
23F-F (905349)	306.9	20.36	229.64	0.5	20	10.0	384
23F-R (905350)	394.39	15.85	234.15	0.5	20	10.0	
15B/15C-F (914849)	478.59	13.06	236.94	0.3	20	6.0	496
15B/15C-R (914850)	427.81	14.61	235.39	0.3	20	6.0	
10A-F (906191)	433.65	14.41	235.59	0.5	20	10.0	628
10A-R (906192)	442.5	14.12	235.88	0.5	20	10.0	
				4.0		80.0	

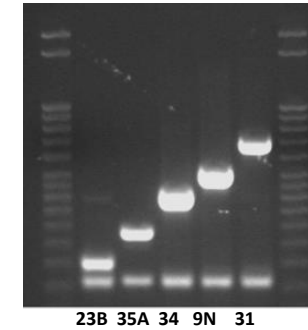


Primer Info		To get 25 $\mu$ M:		Primer Concentrations ( $\mu$ M)			bp
Reaction 6 (Primer ID)	Original Conc. ( $\mu$ M)	Primer ( $\mu$ L)	Water ( $\mu$ L)	Conc. ( $\mu$ M) l rxn	Per X Rxns ( $\mu$ M)	Amount to add ( $\mu$ L)	
39-F (911999)	416.04	15.02	234.57	0.2	20	4.0	98
39-R (912000)	428.01	14.60	242.95	0.2	20	4.0	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
10F/10C/33C-F(911985)	450.73	13.87	236.13	0.3	20	6.0	248
10F/10C/33C-R (911986)	448.77	13.93	236.07	0.3	20	6.0	
5-F (914835)	485.51	12.87	237.13	0.3	20	6.0	362
5-R (914836)	353.73	17.67	232.33	0.3	20	6.0	
35F/47F-F (807848)	534.78	11.69	238.31	0.3	20	6.0	517
35F/47F-R (807849)	397.42	15.73	234.27	0.3	20	6.0	
17F-F (903755)	419.33	14.90	235.10	0.5	20	10.0	693
17F-R (903756)	360.93	17.32	232.68	0.5	20	10.0	
				3.4		68.0	



Primer Info		To get 25µM:		Primer Concentrations (µM)			bp
Reaction 7 (Primer ID)	Original Conc. (µM)	Primer (µL)	Water (µL)	Conc. (µM) l rxn	Per X Rxns (µM)	Amount to add (µL)	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
23B-F (914859)	509.56	12.27	237.73	0.2	20	4.0	199
23B-R (914860)	461.18	13.55	236.45	0.2	20	4.0	
35A/35C/42-F (911997)	503.85	12.40	237.60	0.3	20	6.0	280
35A/35C/42-R (911998)	488.57	12.79	237.21	0.3	20	6.0	
34-F (807844)	463.03	13.50	236.50	0.3	20	6.0	408
34-R (807845)	465.17	13.44	236.56	0.3	20	6.0	
9N/9L-F (906187)	424.63	14.72	235.28	0.5	20	10.0	516
9N/9L-R (906188)	490.97	12.73	237.27	0.5	20	10.0	
31-F (906193)	449.31	13.91	236.09	0.5	20	10.0	701
31-R (906194)	445.43	14.03	235.97	0.5	20	10.0	
				3.8		76.0	

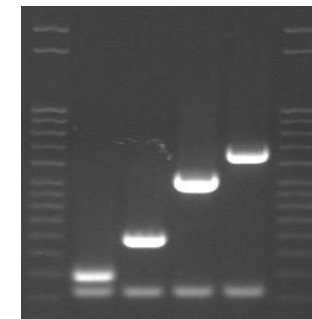
USA Reaction 7



23B 35A 34 9N 31

Primer Info		To get 25µM:		Primer Concentrations (µM)			bp
Reaction 8 (Primer ID)	Original Conc. (µM)	Primer (µL)	Water (µL)	Conc. (µM) l rxn	Per X Rxns (µM)	Amount to add (µL)	
CPSA-F (913424)	454.92	13.74	236.26	0.1	20	2.0	160
CPSA-R (913425)	484.20	12.91	237.09	0.1	20	2.0	
21-F (914857)	455.75	13.71	236.29	0.2	20	4.0	192
21-R (914858)	467.8	13.36	236.64	0.2	20	4.0	
2-F (914829)	432.37	14.46	235.54	0.3	20	6.0	290
2-R (914830)	374.93	16.67	233.33	0.3	20	6.0	
20-F (903761)	390.2	16.02	233.98	0.3	20	6.0	514
20-R (903762)	313.49	19.94	230.06	0.3	20	6.0	
13-F(911987)	397.93	15.71	234.29	0.4	20	8.0	655
13-R (911988)	458.52	13.63	236.37	0.4	20	8.0	
				2.6		52.0	

USA Reaction 8



21 2 20 13