

Protocol for Multiplex PCR - *S. pneumoniae* SEROTYPING - clinical specimens-African set
 Purpose: *S. pneumoniae* serotyping directly from clinical specimens - African set

RXN I

Date _____, 2011

| Reagent | Final Conc. | Volume (µL) | Total Rxns | Final Volume* |
|--|-------------|-------------|------------|---------------|
| PCR H ₂ O | vary | 3.5 | 20 | 70 |
| 2X PCR Buffer - QIAGEN Multiplex PCR Kit(cat#206143) | | 12.5 | 20 | 250 |
| Fwd Primers | vary | see below | | |
| Rev Primers | vary | see below | | |
| Clinical sample DNA [QIAamp DNA Mini Kit (cat# 51306)] (see extraction protocol) | n/a | 5.0 | 20 | |
| Total | | 25.0 | 20 | 500.0 |

| | |
|------------------------|-----------|
| Number of Rxns: | 20 |
|------------------------|-----------|

| Thermal Cycling Parameters | | Post-Reaction Electrophoresis | |
|----------------------------|-------------------|--|-------|
| 1X | 95°C for 15min | 2% Nusieve Agarose (cat# 50090) | 1.6g |
| 35X | 94°C for 30sec | 1X TAE | 80mL |
| | 54°C for 90sec | 0.5µg/µL GelRed- Biotium (cat# 41003) | 5.0µL |
| | | 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 | Run at 100V for 1:30 h | |

| Primer Info | | To get 25µM: | | Primer Concentrations (µM) | | |
|---------------------------------|---------------------|--------------|------------|----------------------------|-----------------|---------------|
| Reaction I(Primer ID) | Original Conc. (µM) | Primer (µL) | Water (µL) | Conc. (µM) I rxn | Per X Rxns (µM) | Amount to add |
| CPSA-F (913424) | 454.92 | 13.74 | 236.26 | 0.1 | 20 | 2.0 |
| 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 |
| 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 |
| 1-R (914828) | 496.11 | 12.60 | 237.40 | 0.3 | 20 | 6.0 |
| 5-F (914835) | 485.51 | 12.87 | 237.13 | 0.3 | 20 | 6.0 |
| 5-R (914836) | 353.73 | 17.67 | 232.33 | 0.3 | 20 | 6.0 |
| 4-F (914833) | 462.37 | 13.52 | 236.48 | 0.3 | 20 | 6.0 |
| 4-R (914834) | 478.61 | 13.06 | 236.94 | 0.3 | 20 | 6.0 |
| 18/(18A/18B/18C/18F)-F (914853) | 449.23 | 13.91 | 236.09 | 0.3 | 20 | 6.0 |
| 18/(18A/18B/18C/18F)-R (914854) | 419.56 | 14.90 | 235.10 | 0.3 | 20 | 6.0 |
| | | | | 3.2 | | 64.0 |

bp

160

189

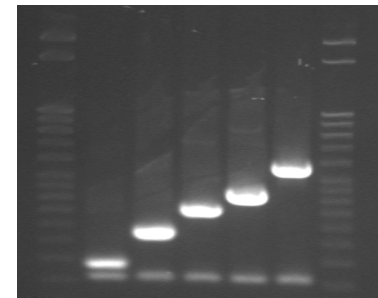
280

362

430

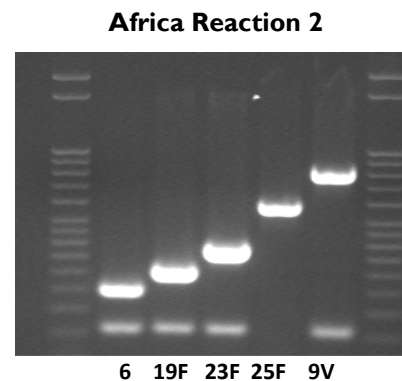
573

Africa Reaction I

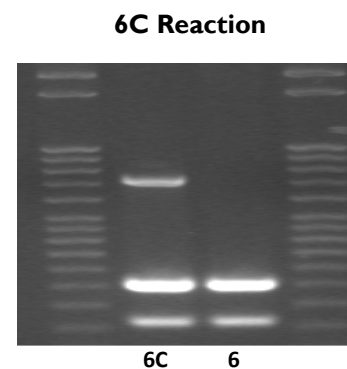


14 1 5 4 18

| Primer Info | | To get 25 μ M: | | Primer Concentrations (μ M) | | | bp |
|------------------------|---------------------------|--------------------|------------------|----------------------------------|-----------------------|--------------------------|-----|
| Reaction 2 (Primer ID) | Original Conc. (μ M) | Primer (μ L) | Water (μ L) | Conc. (μ M) rxn | Per X Rxns (μ M) | Amount to add (μ 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) | 479.31 | 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 | |
| 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 | |
| 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 | |
| 25F/25A/38-F (807850) | 453.44 | 13.78 | 236.22 | 0.3 | 20 | 6.0 | 574 |
| 25F/25A/38-R (804851) | 412.76 | 15.14 | 234.86 | 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 | |
| | | | | 4.4 | | 88.0 | |

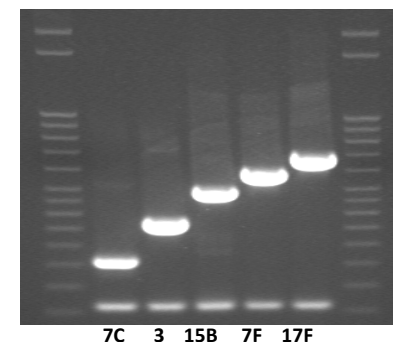


| Primer Info | | To get 25 μ M: | | Primer Concentrations (μ M) | | | bp |
|------------------------|---------------------------|--------------------|------------------|----------------------------------|-----------------------|---------------|-----|
| Reaction 6C(Primer ID) | Original Conc. (μ M) | Primer (μ L) | Water (μ L) | Conc. (μ M) rxn | Per X Rxns (μ M) | Amount to add | |
| 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 | |
| | | | | 1.8 | | 36.0 | |



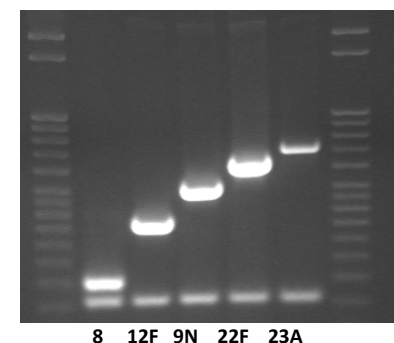
| Primer Info | | To get 25 μ M: | | Primer Concentrations (μ M) | | | bp |
|------------------------|---------------------------|--------------------|------------------|----------------------------------|-----------------------|--------------------------|-----|
| Reaction 3 (Primer ID) | Original Conc. (μ M) | Primer (μ L) | Water (μ L) | Conc. (μ M) rxn | Per X Rxns (μ M) | Amount to add (μ 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 | |
| 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 | |
| 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 | |
| 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 | |
| 7F/7A-F (918151) | 381.91 | 16.37 | 233.63 | 0.4 | 20 | 8.0 | 599 |
| 7F/7A-R (918152) | 472.92 | 13.22 | 236.78 | 0.4 | 20 | 8.0 | |
| 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 | |
| | | | | 3.8 | | 76.0 | |

Africa Reaction 3



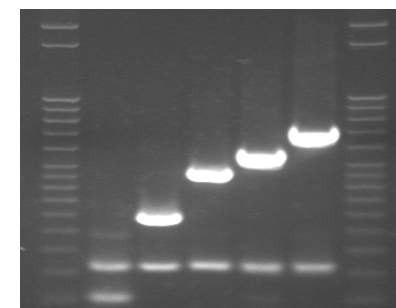
| Primer Info | | To get 25 μ M: | | Primer Concentrations (μ M) | | | bp |
|----------------------------|---------------------------|--------------------|------------------|----------------------------------|-----------------------|--------------------------|-----|
| Reaction 4 (Primer ID) | Original Conc. (μ M) | Primer (μ L) | Water (μ L) | Conc. (μ M) rxn | Per X Rxns (μ M) | Amount to add (μ 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 | |
| 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 | |
| 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 | |
| 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 | |
| 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 | |
| | | | | 4.6 | | 92.0 | |

Africa Reaction 4



| Primer Info | | To get 25 μ M: | | Primer Concentrations (μ M) | | | |
|----------------------------|---------------------------|--------------------|------------------|----------------------------------|-----------------------|--------------------------|-----|
| Reaction 5 (Primer ID) | Original Conc. (μ M) | Primer (μ L) | Water (μ L) | Conc. (μ M) rxn | Per X Rxns (μ M) | Amount to add (μ L) | bp |
| 24(24A/24B/24F)-F (911995) | 458.05 | 13.64 | 236.36 | 0.2 | 20 | 4.0 | 99 |
| 24(24A/24B/24F)-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 | |
| 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 | |
| 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 | |
| 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 | |
| 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 | |
| | | | | 3.2 | | 64.0 | |

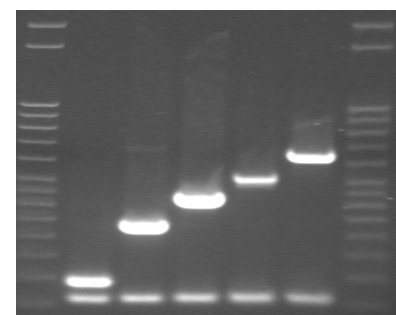
Africa Reaction 5



24 2 11A 19A 16F

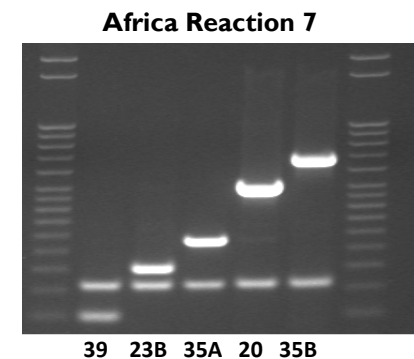
| Primer Info | | To get 25 μ M: | | Primer Concentrations (μ M) | | | |
|------------------------|---------------------------|--------------------|------------------|----------------------------------|-----------------------|--------------------------|-----|
| Reaction 6 (Primer ID) | Original Conc. (μ M) | Primer (μ L) | Water (μ L) | Conc. (μ M) rxn | Per X Rxns (μ M) | Amount to add (μ L) | bp |
| 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 (914829) | 432.37 | 14.46 | 235.54 | 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 | |
| 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 | |
| 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 | |
| | | | | 3.2 | | 64.0 | |

Africa Reaction 6



21 33F 15A 35F 13

| Primer Info | | To get 25µM: | | Primer Concentrations (µM) | | | |
|-----------------------|---------------------|--------------|------------|----------------------------|-----------------|--------------------|-----|
| Reaction 7(Primer ID) | Original Conc. (µM) | Primer (µL) | Water (µL) | Conc. (µM) rxn | Per X Rxns (µM) | Amount to add (µL) | bp |
| 39-F (911999) | 416.04 | 15.02 | 234.98 | 0.2 | 20 | 4.0 | 98 |
| 39-R (912000) | 428.01 | 14.60 | 235.40 | 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 | |
| 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 | |
| 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 | |
| 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 | |
| | | | | 3.2 | | 56.0 | |



| Primer Info | | To get 25µM: | | Primer Concentrations (µM) | | | |
|------------------------|---------------------|--------------|------------|----------------------------|-----------------|--------------------|-----|
| Reaction 8 (Primer ID) | Original Conc. (µM) | Primer (µL) | Water (µL) | Conc. (µM) rxn | Per X Rxns (µM) | Amount to add (µL) | bp |
| 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 | |
| 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 | |
| 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 | |
| 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 | 0.0 | |
| | | | | 3.4 | | 58.0 | |

