

## PCR Determination of Pneumococcal Serotype 6C

Updated 1/14/09

### References:

1. In Ho Park et al. *Inf. Imm.* 2007(75): 4482.
2. M. da Gloria Carvalho et al. *J Clin Micro.* 2009

**Materials** (Comparable items from other manufacturers may be used.)

PCR Primers:

1<sup>st</sup> primer set: (6A/6C) (Reference: Park IH, et al. *Inf. Imm.* 2007)

6A/C-fwd (#5106)      5'-TAC CAT GCA GGG TGG AAT GT

6A/C-rev (#3101)      5'-CCA TCC TTC GAG TAT TGC

2<sup>nd</sup> primer set (6C) (M. da Gloria Carvalho et al. *J Clin Micro.* 2009)

6C-fwd (#5325)      5'-CAT TTT AGT GAA GTT GGC GGT GGA GTT

6C-rev (#3325)      5'-AGC TTC GAA GCC CAT ACT CTT CAA TTA

6C DNA control (a positive control for PCR, available from Nahm Lab.)

Place the filter paper impregnated with 6C DNA in 100 ul of water.

Discard the paper and recover the water with DNA as 6C DNA control.

Blood Agar Plate (Remel, Catalog# R01202)

Wizard Genomic DNA Purification Kit (Promega, Catalog# A1120)

LA Taq Polymerase (Takara Biomedical, Catalog# RR002M)

Agarose (Fisher Scientific, Catalog# BP1356)

PCR Tubes (Fisher Scientific, Catalog# 08-408-214)

Thermal Cycler (Eppendorf, Mastercycler gradient)

DNA 1Kb Ladder Plus (Invitrogen, Catalog# 10787-018)

Gel Electrophoresis Equipment (BioRad)

6X DNA Loading Dye (Fermentas, Catalog# R0611)

Ethidium Bromide (Invitrogen, Catalog#15585-011)

Running Buffer (1X TAE)

4.84 g Tris-Base (Sigma, Catalog# T1503)

1.09 g Glacial Acetic Acid (Fisher Scientific, Catalog# A38C)

0.292 g EDTA (Fisher Scientific, BP120)

Add distilled water up to 1 liter, pH should be 8.2-8.3

### Method

1. Streak test strain(s) onto blood agar plates, and culture overnight at 37°C in a reduced oxygen environment (e.g., candle jar).

2. Isolate genomic DNA with the Wizard Genomic DNA Purification Kit following the manufacturer's instructions. Dilute purified DNA to 10-30 micrograms/ml.

Alternatively, 2-3 colonies of pneumococci can be used directly for PCR (no need to purify genomic DNA).

3. Prepare the reaction mixture by mixing reagents as below:

Reagents	Stock Concentration	Volume per sample (ul)
Distilled Water		39.75
10X PCR Buffer		5
dNTP mix	2.5 mM each	2
Primer #5106	5 nanomole/ml	1
Primer #3101	5 nanomole/ml	1
LA Taq Polymerase	5000 u/ml	0.25

4. Add 49 ul of the reaction mixture to each PCR tube.

5. Add 1 ul of 6C DNA control or genomic DNA (10-30 ng), or 2-3 colonies of pneumococci to the reaction mixture.

6. Transfer PCR tube(s) to thermal cycler and begin PCR program:

Step 1: Initial denaturation at 95°C for 3 minutes

Step 2: Denaturation at 95°C for 30 seconds

Step 3: Annealing at 58°C for 30 seconds

Step 4: Extension at 72°C for 3 minutes

Repeat steps 2 through 4 for a total of 35 cycles

Step 5: Final extension at 72°C for 10 minutes

7. Prepare an agarose gel (1% agarose in 1X TAE containing 200 ng/ml of ethidium bromide).

8. Mix 5 microliters of PCR product (or 1Kb DNA ladder (diluted 1:10)) with 1 microliter of 6X DNA Loading Dye.

9. Load PCR product/DNA Loading Dye mixture onto the agarose gel

10. Perform electrophoresis at ~90V for 30 minutes.

11. Take a picture of the gel under UV light.

12. Interpret results:

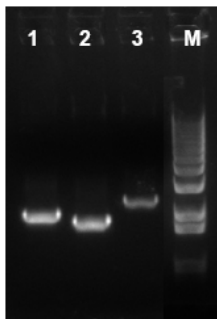
1) 1<sup>st</sup> primer set:

Serotype 6C isolates produce a ~1.8 kb product.

Serotype 6A and 6B isolates produce a ~2 kb product, and serotype 6A & 6B isolates containing the INDEL insert produce a ~2.3 kb product (Mavroidi A, et al. J.Bact. 2004(186): 8181)

2) 2<sup>nd</sup> primer set:

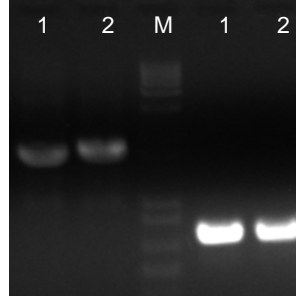
This 6C pair gives 727 bp product only for 6C (not for 6A or 6B)



1. 6A control  
2. 6C control  
3. 6A with INDEL  
M. 1Kb DNA ladder Marker

3.0 kb  
2.0 kb  
1.6 kb  
1.0 kb

6A/C PCR      6C PCR



Lane 1: 6C lysate  
Lane 2: 6C control  
M: Marker

2000bp  
1000bp  
850bp  
650bp