

emm Typing Protocol

Conventional PCR and Sequencing

DNA extraction

- Any manual or automated DNA extraction methods are acceptable.
- Boiling fresh growth from pure isolates can result in good DNA extracts.

Conventional PCR amplification

PCR primers (Frost et al., 2020)

CDC1 (Forward)	TATT(C/G)GCTTAGAAAATTA
CDC3 (Reverse)	TTCTTCAAGCTCTTTGTT

PCR master mix

PCR reaction mix	1X
Master mix (2X)	12.5 µl
Water	9.5 µL
Forward Primer (25 µM)	1.0 µL
Reverse Primer (25 µM)	1.0 µL
DNA	1 µL
Total Volume	25 µL

Steps

1. Vortex the reaction mix gently.
2. Centrifuge for few seconds to collect reaction mix at the bottom of the PCR tube/plate.
3. Place the PCR reaction tubes/plate on thermocycler under the conditions listed below.

Note: Place the PCR plate on thermocycler only after reaching the initial sample temperature (94°C).

94°C	1 min	1 cycle
94°C	15 sec	30 cycles
47°C	30 sec	
72°C	1 min 25 sec	
72°C	7 min	1 cycle

Sequencing

1. Purification of PCR reaction for sequencing

Prepare sequencing template from 2-11 μL aliquot of PCR product to be sequenced with [ExoSAP-IT](#) as follows:

PCR product	5 μL
ExoSAP-IT	2 μL
Total	7 μL

Mix and incubate in a thermocycler using the following conditions:

37°C	15 min	1 cycle
80°C	15 min	1 cycle

2. Sequence reaction

Sequencing primer: *emmseq2* – TATTCGCTTAGAAAATTAACAGG

Dilute BigDye V1.1 to 1:5 with the [buffer provided with the kit](#).

Primer <i>emm seq2</i> (3.2 pmole/ μL)	1 μL
Diluted BigDyeV1.1 (1:5)	6 μL
dH ₂ O	12 μL
Purified PCR product	1 μL
Total	20 μL

For sequencing reaction, use the cycling parameters in the following table.

96°C	1 min	1 Cycle
96°C	10 Sec	25 Cycles
55°C	5 sec	
60°C	4 min	
4°C	Hold	

Store the sequencing reactions at -20°C until use.

Reference:

Frost, H.R., M.R. Davies, S. Velusamy, V. Delforge, A. Erhart, S. Darboe, A. Steer, M. J. Walker, B. Beall, A. Botteaux, and P.R. Smeesters. 2020. Updated *emm*-typing protocol for *Streptococcus pyogenes*. Clin Microbiol Infect . 2020 Jul;26(7):946.e5-946.e8. doi: 10.1016/j.cmi.2020.02.026. Epub 2020 Feb 28.