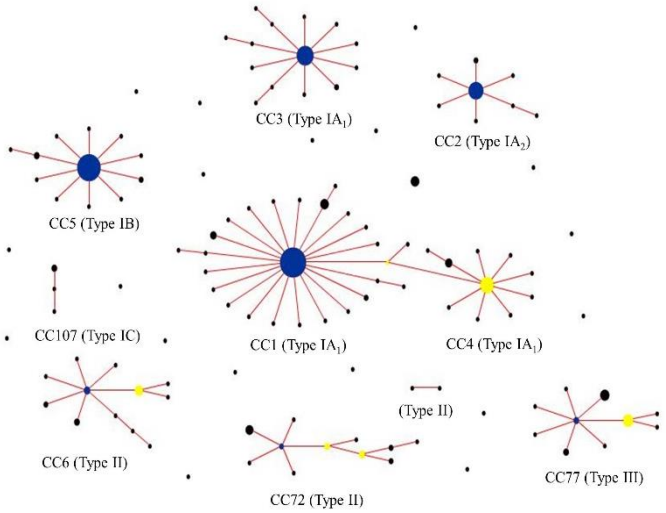
MLST of *Cutibacterium acnes*

The *Cutibacterium acnes* MLST scheme uses partial sequences from six core housekeeping genes and complete sequences from two putative virulence genes (4253bp). The scheme will resolve query isolates into distinct sequence types within the genetic groups IA_1, IA_2, IB, IC, II and III. Phylogenetic inferences using this scheme are highly concordant with results from phylogenomic analyses.

The eight genes used are:

Shikimate 5-dehydrogenase (*aroE*)
ATP synthase beta chain (*atpD*)
Guanylate kinase (*gmk*)
GMP synthase (*guaA*)
GTP-binding protein (*lepA*)
Superoxide dismutase (*sodA*)
Putative haemolysin (*tly*)*
Camp2 homologue (*camp2*)



PCR amplification and sequencing conditions:

For housekeeping genes, reaction conditions for all primer sequences are as follows: initial denaturation at 94°C for 1 min; 30 cycles of denaturation at 94°C for 1 min, primer annealing at 58 °C for 1 min and extension at 72°C for 2 min; followed by a final extension step at 72°C for 10 min. Each 50 µl amplification reaction mixture comprises ~10 ng DNA, 20 pmol forward and reverse primer and x1 PCR buffer (Qiagen) containing 1.5 mM MgCl2, 0.8 mM dNTPs (Qiagen),1xQ solution (Qiagen) and 1.25 U Taq (Qiagen). For *tly* and *camp2* genes, amplification conditions are as described in:

1. McDowell, Valanne S, Ramage G, Tunney MM, Glenn JV, et al. (2005) *Propionibacterium acnes* types I and II represent phylogenetically distinct groups. J. Clin. Microbiol. 43: 326-334.

2. Valanne S, McDowell A, Ramage G, Tunney MM, Einarsson GG, et al. (2005) CAMP factor homologues in Propionibacterium acnes: a new protein family differentially expressed by types I and II. Microbiology 151: 1369-1379.

The amplification products are analysed by electrophoresis on a 1.5% agarose gel and then purified using MinElute UF plates (Qiagen) following the manufacturer's protocol before being used in the sequencing reaction. Sequencing is carried out on each DNA strand with BigDye Terminator Ready Reaction Mix v3.1 (PE Biosystems, Foster City, US) under standard sequencing conditions according to the manufacturer's protocol. Unincorporated dye terminators are removed by precipitation with 95% alcohol. The reaction products are then separated and detected on an ABI PRISM genetic analyser 3100 (PE Biosystems).

***Note:** Tly protein may actually be an RNA-binding FtsJ-like methyltransferase involved in ribosomal biogenesis.