**PROPRIONIBACTERIUM ACNES PHYLOGENETIC TYPE III IS ASSOCIATED WITH PROGRESSIVE MACULAR HYPMELANOSIS**

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Progressive macular hypomelanosis (PMH) is a skin disorder that is characterized by hypopigmented macules and usually seen in young adults. The skin microbiota, in particular the bacterium *Propionibacterium acnes*, is suggested to play a role.

Here, we compared the *P. acnes* population of 24 PMH lesions from eight patients with corresponding nonlesional skin of the patients and matching control samples from eight healthy individuals using an unbiased, culture-independent next-generation sequencing approach. We also compared the *P. acnes* population before and after treatment with a combination of lymecycline and benzoylperoxide.

We found an association of one subtype of *P. acnes*, type III, with PMH. This type was predominant in all PMH lesions (73.9% of reads in average) but only detected as a minor proportion in matching control samples of healthy individuals (14.2% of reads in average). Strikingly, successful PMH treatment is able to alter the composition of the *P. acnes* population by substantially diminishing the proportion of *P. acnes* type III.

Our study suggests that *P. acnes* type III may play a role in the formation of PMH. Furthermore, it sheds light on substantial differences in the *P. acnes* phylotype distribution between the upper and lower back and abdomen in healthy individuals.

**Keywords:** progressive macular hypomelanosis, *Propionibacterium acnes*, *Cutibacterium acnes*, next-generation sequencing, subtype III, skin microbiota, single locus sequencing type, phylotype

**Abbreviations:** PMH, progressive macular hypomelanosis; SLST, single-locus sequencing typing; ST, sequence type; NGS, next-generation sequencing; BPO, benzoylperoxide

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**Introduction**

Progressive macular hypomelanosis (PMH) is characterized by symmetric non-scaly hypopigmented skin areas that are predominantly visible in the sebaceous areas of the trunk. In the lower back and abdomen, discrete lesions are distinguished while they are more confluent on the upper trunk. There is no inflammation, pain, or itching associated with PMH, but the disease can have a major psychosocial effect on patients. PMH appears to be more frequent in young women, and although the disorder has a worldwide distribution, it is most often identified in dark-skinned populations [1–3].

Several treatment modalities are used against PMH including topical benzoylperoxide 5% (BPO) and clindamycin 1% alone or in combination with ultraviolet A (UVA) or narrow band ultraviolet B (UVB) irradiation [4–7], oral lymecycline in combination with topical BPO 5% for 3 months [8], and low-dose isotretinoin for 1 month [9]; however, the ideal treatment is not yet defined.

The etiology of PMH is not known; however, several studies indicate that the Gram-positive anaerobic bacterium *Propionibacterium acnes* may play a pivotal role [10, 11]. Biopsy specimens from PMH lesions contained *P. acnes* in pilosebaceous ducts in contrast to biopsies

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from healthy skin [11], real-time PCR showed a significant predominance of *P. acnes* in lesional skin as compared to nonlesional skin [10], and red fluorescence was detected in lesions when subjected to Woods light [11]. Finally, antibacterial treatment effective against *P. acnes* leads to repigmentation [4, 8]. It is not known how *P. acnes* could induce hypopigmentation. Using microscopy, a decrease in melanin production and a change in the distribution of melanosomes with a resultant decrease in epidermal melanin was shown in PMH lesions [12, 13].

Based on multilocus sequence typing (MLST) and single-locus sequence typing (SLST) schemes and complete genome sequencing, the population of *P. acnes* has been shown to consist of several phylogenetic subtypes commonly designated IA1, IA2, IB, IC, II, and III [14–21]. A previous study based on bacterial cultivation has indicated that certain subtypes of *P. acnes* may be associated with PMH: an abundance of a specific type of *P. acnes* was observed in PMH lesions that is different from *P. acnes* types isolated from acne lesions [22]. Recently, we cultured *P. acnes* isolates belonging to the otherwise uncommon type III from lesions of PMH patients and sequenced their genomes [23], and a very recent study revealed an abundance of *P. acnes* type III in bacterial cultures from lesional skin in 14 of 34 PMH patients [24].

In the present study, the type distribution of the entire population of *P. acnes* in affected and unaffected skin areas of PMH patients, including samples after treatment, and matching control samples was determined using a culture-independent next-generation sequencing (NGS)-based SLST approach. Results show a strong association of *P. acnes* type III with disease.

**Materials and methods**

**Patient and control cohort, treatment regimen**

Eight patients with PMH were recruited by voluntary consent in a private dermatology practice in Aalborg, Denmark. The patients were all clinically examined by a specialist in dermatology (H.B. Lomholt), and PMH was diagnosed based on the finding of clinically characteristic lesions, patient history, and a lack of *Malassezia* in microscopic inspections. All patients were female between 18 and 31 years of age (mean, 23.5 years) (see Supplementary Table S1 online). Five of the patients were treated in a 3-month course with oral lymecycline (300 mg, daily) combined with a daily wash using 5% BPO washing gel, and one patient used only the 5% BPO wash (see Supplementary Table S2 online). As controls, eight healthy volunteers were recruited among students at the University of Aarhus, Denmark. They were all female between 24 and 31 years of age (mean, 26.5 years). Information regarding the study participants and treatment is summarized in Supplementary Tables S1 and S2 online.

**Sampling sites and procedure**

Samples were taken in all patients from three lesional areas including the lower, middle, or upper back (depending on the position of the PMH lesions) and the abdomen, and additional samples were taken from patients in unaffected adjacent skin areas. One patient had no lesions on the abdomen and was sampled from all three positions on the back. Six of the patients were additionally sampled after treatment from the same areas as the pretreatment sample. Samples from the lower and upper back, and abdomen were taken from the eight healthy controls. In addition, samples from the forehead and the buccal cavity were taken from patients (Fig. S1). Samples for NGS-based SLST were obtained by swabbing the skin firmly for 20 s with a sterile cotton swab moistened in sampling buffer (0.1% detergent [Triton X-100] in 0.075 M phosphate buffer, pH 7.9) [25]. DNA was extracted from all collected samples, and SLST fragment amplification was performed as described previously [20]. The amplicons were then subjected to next-generation sequencing (NGS) using the pyrosequencing technology. Subsequent bioinformatics analysis included quality control and sequence read assignment to the STs of the SLST scheme (http://medbac.dk/slst/pacnes), resulting in a high-resolution phylotype analysis of the *P. acnes* population in each sample.

Samples for bacterial cultivation were taken with a sterile charcoal swab moistened in sampling buffer firmly scrubbed on the skin for 20 s. From each patient, two swabs were obtained from lesional skin on the back and abdomen, respectively, and, in addition, two swabs from adjacent nonlesional skin. For a semiquantitative estimation of the number of bacteria, the primary charcoal cotton swabs were streaked on tryptone-yeast-glucose (TYG) agar plates and incubated for 120 h in an anaerobic chamber (Forma Scientific Anaerobic System model 1024).

**Next-generation sequencing-based single-locus sequence typing (NGS-based SLST)**

Cotton swab samples from both patients and healthy controls were transferred to 1.5-ml Eppendorf tubes, and bacterial DNA was isolated and purified using PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO, Carlsbad, CA, United States) according to the manufacturer’s instruction. The isolated DNA was then subjected to amplification by PCR as described previously [20]. All primer sequences are given in Supplementary Table S3 online. All PCR reactions were made by mixture of 5 μl DNA sample, 2.5 μl AccuPrime PCR Buffer II (Invitrogen), 1.5 μl of forward primer, 1.5 μl of reverse primer (10 μM), 0.15 μl AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen), and 14.35 μl PCR-grade water into a total volume of 25 μl. All samples were amplified by an initial denaturation for 2 min at 94 °C, followed by 35 cycles of 20 s of denaturation at 94 °C, 30 s at 55 °C for annealing, and 60 s of extension at 68 °C, ending with a 5-minute step of ex-
tension at 68 °C. Three PCRs per sample were performed and verified on an agarose gel, pooled together, and purified using a NucleoSpin Extract Kit (Macherey-Nagel). The concentration of purified DNA samples was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific). The amplicons were pooled in batches of 20 and sequenced unidirectionally from the forward primer using Roche GS FLX+ pyrosequencing technology either at Institute of Microbiology and Genetics, Georg-August University Göttingen, Göttingen, Germany or at Eurofins Genomics, Ebersberg, Germany. The data were then processed using the PyroNoise implementation in Mothur v. 1.36.1 [26]. All sequences have been deposited at NCBI with the project number PRJNA347641. The sequence reads were aligned to all the known STs in the SLST database (http://medbac.dk/slst/pacnes) using BLASTn [27] and assigned an individual ST based on a best-hit model with a cut-off value of 99.5%; anything below this threshold or reads with two or more identical best hits were discarded as unassigned reads.

**Sanger sequencing-based SLST of bacterial isolates**

SLST typing of *P. acnes* isolates has been described previously [20]. In brief, the sampled charcoal cotton swabs were streaked on tryptone-yeast-glucose (TYG) agar plates and incubated for 72 h in an anaerobic chamber (Forma Scientific Anaerobic System model 1024). This primary culture was then examined; up to 10 random single colonies resembling *P. acnes* were selected and individually subcultivated on new TYG agar plates for 72 h under anaerobic conditions. After growth, the *P. acnes* isolates were harvested and subjected to DNA extraction by a boiling procedure at 100 °C for 10 min in 0.5 ml Eppendorf PCR tubes containing 300 μl PCR-grade water. A PCR was carried out with the SLST primers (see Supplementary Table S3 online) as follows: 2 μl of the crude DNA extract was mixed with 1 μl of each forward and reverse primer (10 μM), 10 μl 5’-PRIME Hotmastermix (5 PRIME, Hamburg, Germany), and 11 μl of PCR-grade water. PCR conditions were as follows: initial denaturation of 40 s at 96 °C followed by 35 cycles of 35 s of denaturation at 96 °C, 40 s of annealing at 55 °C, and 40 s of extension at 72 °C, followed by a final 7-minute extension step at 72 °C. The resulting PCR products were run on a 1% agarose gel to ensure quality and were then sequenced at GATC Biotech AG (Konstanz, Germany) with the forward and reverse SLST primers. The sequences were assembled and trimmed to the known SLST fragment size (484 bp) using MEGA v.6.06 [28]. Finally, the resulting sequences were assigned to STs using the SLST database (http://medbac.dk/slst/pacnes).

**Statistical analysis**

The proportions of *P. acnes* type III were compared between mean values of the three lesional sites in patients.
and corresponding sites in controls using the unpaired Wilcoxon rank sum test/Mann–Whitney U test. Lesional sites in patients before and after treatment were compared using the paired Wilcoxon signed rank test.

Ethics statement

The study protocol was approved by the Ethics Committee of Region North, Denmark (document N-20120050), and the study was conducted according to the principles of the declaration of Helsinki. Written informed consent was obtained from all study participants.

Results

Type III P. acnes predominates in PMH samples

SLST amplicons from 96 samples were selected for pyrosequencing. In average, 7911 sequence reads per sample were obtained and 97% of the reads could be assigned to a P. acnes sequence type (ST) (see Supplementary Table S4 online). In the whole data set, the SLST scheme distinguished 90 different STs of P. acnes.

The results of the P. acnes ST distribution in PMH lesions from eight patients and eight matching healthy controls are shown in (Fig. 1a,b). A clear distinction was found when comparing PMH lesional samples to matching controls: P. acnes type III (the corresponding ST is designated “L”) was the dominating phylotype in PMH lesions (Fig. 1a); on average, 73.9% of the reads belonged to this type. In contrast, in the matching control samples, only 14.2% of the reads could be assigned to P. acnes type III (p value 7.8 × 10⁻⁵). Instead, control samples contained a higher proportion of type IA1 (STs “A” to “E”) strains (56.8%) and type II (ST “K”) strains (22.3%), while these were found in much lower proportions in PMH lesions: type IA1 (12.8%) and type II (5.6%). Individual variation among the patients and controls was observed: the proportion of type III in the total P. acnes population varied between 50% and 91% in PMH lesions and between 0% and 53% in control samples (Fig. 1b).

We wanted to confirm these findings with a culture-dependent technique: swab samples taken from PMH lesions and healthy controls were cultivated anaerobically. Up to 10 P. acnes colonies per sample were randomly selected from the agar plates; for each isolate, the classical SLST assignment by Sanger sequencing of the PCR-amplified SLST fragment was carried out. In average, 39.6% of the P. acnes colonies were type III in lesion samples, in contrast to only 12.5% in controls (see Supplementary Fig. S2 online).

P. acnes type III in PMH patients is enriched in PMH regions and adjacent skin areas but is rarely found at other body sites

A recent study [29] suggested that the phylotype distribution of P. acnes is relatively uniform and stable throughout the body. Thus, we wanted to investigate if PMH patients are also predominantly colonized by P. acnes type III at different body sites other than the PMH lesions. Samples from different locations including the lower and upper back, the forehead and the buccal mucosa were analyzed from patients and controls.

Type III was rarely detected on the forehead or in the buccal mucosa of patients (Fig. 2a). Most patients had a mixture of different P. acnes types on the forehead, in particular, strains of the phylotypes IA2, IC, and II. Only one

![Fig. 2. Average P. acnes ST distribution shown for different body sites in patients and controls. a) P. acnes ST distribution in patient PMH lesions on lower back, upper back, and abdomen, and unaffected skin on the forehead and buccal mucosa. b) ST distribution in controls at sites corresponding to patient lesions on lower back, upper back, and abdomen. Each ST (A to L) is given a color as indicated, and the corresponding P. acnes subtype is given in brackets](image-url)
patient was found to have a significant proportion of type III \textit{P. acnes} on the forehead. On the buccal mucosa, type IA1 was predominant in most patients.

Looking at the \textit{P. acnes} phylotype distribution of patients on nonlesional skin sites, a dominance of type III strains was detected, albeit at a lesser extent than on lesional skin, 53\% versus 74\%, respectively (Fig. 3).

Healthy individuals were analyzed as well. Interestingly, in average, we could detect a larger proportion of type III strains (21.7\%) on the lower back skin compared to the upper back skin (5.0\%), indicating that the lower back is the preferred habitat for type III strains (Fig. 2b). The dominant \textit{P. acnes} type on the lower back skin of healthy controls was type II (33.5\%), a type that was rarely detected on the upper back (4.0\%). The dominating type of the upper back and the abdomen was type IA1 (44.7\% and 40.6\%, respectively).

\textit{PMH treatment alters the \textit{P. acnes} population and diminishes the proportion of type III strains}

Next, we wanted to investigate how the treatment of PMH with a combination of lymecycline and BPO might alter

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\textbf{Fig. 3.} Comparison of average \textit{P. acnes} ST distribution in PMH patients in lesional versus nonlesional skin based on next-generation sequencing data. Each ST (A to L) is given a color as indicated, and the corresponding \textit{P. acnes} phylotype is given in brackets.

\textbf{Fig. 4.} Clinical PMH treatment responses. Lesional skin on the back before and after treatment with lymecycline 300 mg and BPO daily washes for 3 months shown for patient P2 (A and B) and patient P4 (C and D).
the *P. acnes* type distribution. Six patients were treated for 3 months, and samples were taken before and after treatment from the same skin location. Information about the treatment regimen and the response for each patient is

**Fig. 5.** Comparison of *P. acnes* ST distribution in PMH patients before and after treatment based on next-generation sequencing data. a) Average *P. acnes* ST proportions in patient samples before and after treatment. b) Each column represents ST proportions as an average of the three sampling sites (upper back, lower back, and abdomen). Data are given for six patients before and after treatment. Each ST type A to L is given a color as indicated, and the corresponding *P. acnes* subtype is given in brackets

**Fig. 6.** Comparison of average ST distribution among *P. acnes* subtype III strains in PMH patients versus controls based on next-generation sequencing data. Each type III ST (L1 to L6) is given a color as indicated
given in Supplementary Table S2 online. Representative images of the back skin of two patients who responded well to the treatment are shown (Fig. 4).

We could detect a striking reduction of the proportion of type III \textit{P. acnes} from an average of 80\% before treatment to 22\% after treatment ($p = 0.015$) (Fig. 5a). In all six patients, the type III proportion was diminished after treatment in PMH-affected skin sites. In three patients (P2, P4, and P8), the type III population was almost completely eradicated after treatment (Fig. 5b). Interestingly, these three patients showed a particular good response to the treatment with almost no remaining PMH lesions. The type distribution after treatment resembled in average the one detected in controls samples (Fig. 1b). In contrast, in patients with a less good treatment response, a substantial type III population could be still detected (Fig. 5b).

Existence of a specific type III lineage associated with PMH lesions?

Since type III strains were also detected, albeit fewer, in healthy samples, in particular on the lower back skin, we wanted to investigate if PMH-associated type III strains belong to a specific lineage that is different from health-associated type III strains. Our SLST scheme can differentiate six STs within the type III lineage. The analyses showed that PMH-associated type III \textit{P. acnes} belong predominantly to the STs L1 (56\%) and L6 (25\%); the latter ST was detected at lower rates among health-associated type III \textit{P. acnes} (9\%) (Fig. 6). Overall, our data do not reveal a clear-cut difference between the type III populations of healthy and PMH-affected individuals.

Discussion

We report the hitherto most detailed data on the distribution of \textit{P. acnes} subtypes on multiple skin sites on eight PMH patients and eight healthy controls, using a highly discriminative NGS-based SLST approach. The study revealed an association of one particular type of \textit{P. acnes}, the phyotype III, with the skin disorder progressive macular hypomelanosis. Moreover, an indication that subtype III is involved in the PMH disease pathogenesis was revealed by the comparison of patient samples before and after treatment: therapy with lymecycline and BPO, both highly active against \textit{P. acnes}, led to a diminished proportion of type III, which was paralleled by the disappearance of clinical PMH lesions.

The applied NGS-based SLST approach gave an average of 7911 reads per sample with 97\% of reads assigned to known \textit{P. acnes} STs. This provided a robust basis for a high-resolution estimate of the type distribution in each sample. Though this technique is regarded as less biased than culture-dependent techniques, a PCR bias due to disproportional amplification of certain sequences cannot be ruled out. Therefore, up to 10 randomly selected colonies were cultured from samples and analyzed by traditional Sanger sequencing for comparison. Importantly, the dominance of type III strains in PMH lesions as compared to controls was consistent in both techniques.

\textit{P. acnes} subtype III was first reported as a new phylogenetic type in 2008 based on four strains isolated from spinal intervertebral disc material [17]. In addition, subtype III isolates were recently detected in surgically excised lumbar disc herniations from 5 of 24 patients [30]. Subtype III bacterial cells differ from other \textit{P. acnes} types in showing a long filamentous morphology reminiscent of \textit{Propionibacterium propionicum}. Subtype III differs also in biochemical tests, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS) spectra, and genetic markers; thus, it was recently proposed as a new subspecies with the name \textit{P. acnes} subsp. \textit{elongatum} [31]. This subtype has rarely been cultured from healthy controls, opportunistic infections, or acne patients [14, 16, 18, 32]. In contrast, the present study showed a striking predominance of subtype III in PMH lesional samples from the lower, middle, and upper back, and abdomen. This corroborates the previous finding of a unique, unidentified \textit{P. acnes} type in PMH patients [22], and the recent report that type III strains were cultured from lesions of 14 of 34 PMH patients [24].

The NGS-based SLST approach provided no data on the presence of other bacterial species or the total bacterial numbers but only on the proportional distribution of \textit{P. acnes} subtypes. Previous studies have found a highly significant increase in \textit{P. acnes} numbers in PMH lesions [11, 24]. In accordance, we could also confirm that \textit{P. acnes} is more abundant in lesions compared to nonlesional skin: a semiquantitative analysis revealed that all PMH samples from patients had a higher colony-forming unit count as compared to adjacent nonlesional skin (data not shown). As in other studies, no \textit{Malassezia} fungus was detected and only few bacteria of other species, mainly \textit{Staphylococcus epidermidis}, were found.

Our study revealed a high proportion (74\%) of \textit{P. acnes} type III in lesional skin and also a relatively high proportion (53\%) of type III isolates in adjacent nonlesional skin of the patients. In contrast, Barnard et al. cultured \textit{P. acnes} from nonlesional skin in only one of 34 patient samples, indicative of low \textit{P. acnes} numbers in nonlesional skin. Therefore, the type distribution at normal skin sites could not be assessed in their study [24]. In addition, the difference may reflect different sampling and sample processing approaches in the two studies. Barnard et al. cultivated bacteria from a homogenized 4-mm punch skin biopsy sample, whereas we used surface skin swabs from a circular area of approximately 1.5 cm in diameter. We noticed that it is difficult to completely separate lesions from adjacent nonlesional skin sites as lesions may be small and plenty and not well defined. This may explain the relatively high proportion of type III strains in nonlesional skin of patients in the present study. Normal controls harbored only a minor proportion of \textit{P. acnes} type III on corresponding skin sites.
The SLST scheme can distinguish six different STs among type III strains and four of these were detected in PMH lesions with no clear differences in their relative proportions in patients and controls. Interestingly, a regional difference in prevalent type III clones was suggested, based on comparisons of PMH isolates from Europe and Brazil [24].

The mechanism leading to macular hypomelanosis is not known, but ultrastructural studies have shown less melanized and aggregated melanosomes instead of single mature melanosomes transferred from melanocytes to keratinocytes [12]. The association with P. acnes type III suggests that a type III-specific factor could be involved. Comprehensive comparison of type III genomes to P. acnes genomes of other subtypes has identified several genomic regions specific to type III genomes encoding functions such as type II secretion system, ABC transporter, inositol transport/modification, gyrase, integrase, transposase, oligopeptidase transport, and processing of sugars/amino acids [24, 33, 34]. In addition, some genes are absent from type III genomes but present in all other types including hyaluronic lyase, magnesium-chelatase, iron transporter, bacteriocin, 3-isopropylmalate dehydrogenase, maltose transporter, and periplasmic binding protein. It has to be investigated in the future if and which factors of P. acnes type III are important in PMH pathogenesis.

At present, there is a major interest in defining the normal human skin microbiome as it is considered important for maintaining healthy skin. Several metagenomic studies have described the skin microbiome on species level, but it has become increasingly clear that subtypes within species may play important roles. Most previous P. acnes studies have shown a predominance of type IA1 followed by type II and IB among skin isolates derived from the face or upper back [14, 16, 18]. The present study is the most detailed study on P. acnes subtype distribution on different body sites. It was shown that subtype III is not normally dominant in skin areas of healthy skin; it was rarely found on the face and in the oral cavity. On healthy skin, type III is more frequent at the lower back and abdomen, together with type II, relative to other body sites.

The present study has some limitations. The number of patients and controls is low, even though many samples were analyzed from each person. In general, nonlesional skin was sampled lateral to the lesional area and, therefore, not on exactly corresponding skin areas. After treatment, faint residual lesions in three patients were detectable, even though the clinical response was satisfactory. We do not know if the bacteria most important in the disease process reside on the skin or in the follicles and the surface swab technique employed may have missed bacteria residing in follicles. Furthermore, detailed data on the total number of bacteria at each site and the presence and proportions of other microorganisms besides P. acnes were not obtained in this study.

In conclusion, the present study showed that P. acnes phylotype III is associated with PMH lesions and, therefore, may play a role in the disease pathogenesis. In the normal human microbiome, P. acnes subtype III appears to constitute only a minor portion, mainly residing on the lower back and abdomen, among the P. acnes population. The findings open for future studies of specific type III traits to further define the role of the bacterium and increase our understanding of the PMH disease pathogenesis.

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**Authors’ contributions**

H.B.L. conceived the study idea, obtained ethical approval, included and treated the patients, and collected all bacterial samples from patients and controls. R.L.W. prepared samples for Sanger and next-generation sequencing. R.L.W., C.F.P.S., and A.J. analyzed the sequencing data. R.L.W., H.B.L., and H.B. interpreted the data and prepared the draft article. All authors read, corrected, and approved the article.

**Competing interests**

The authors state no conflict of interest.

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## Supplementary material

### Table S1. Data on eight patients (P) and eight controls (C) included in the study

|   | Sex | Age |   | Sex | Age |
|---|-----|-----|---|-----|-----|
| P1 | Female | 19 | C1 | Female | 28 |
| P2 | Female | 24 | C2 | Female | 31 |
| P3 | Female | 18 | C3 | Female | 27 |
| P4 | Female | 22 | C4 | Female | 26 |
| P5 | Female | 24 | C5 | Female | 25 |
| P6 | Female | 31 | C6 | Female | 25 |
| P7 | Female | 29 | C7 | Female | 26 |
| P8 | Female | 21 | C8 | Female | 24 |

### Table S2. Treatment and treatment responses in six PMH patients treated with benzoyl peroxide (BPO) daily washes and/or peroral lymecycline (LC) 300 mg once daily for three months

| Patient no. | Treatment | Outcome |
|------------|-----------|---------|
| P1         | BPO, LC   | Good response with a few residual lesions |
| P2         | BPO, LC   | Good response |
| P3         | Irregular BPO, no LC | Improved with residual lesions |
| P4         | BPO, LC   | Good response |
| P7         | Irregular BPO, irregular LC | Good response with a few residual lesions |
| P8         | BPO, LC   | Good response |

### Table S3. Primers used in this study

**Primers for NGS-based SLST**

| SLST_PA_REV | 5´-CCTATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID1 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID2 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID3 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID4 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID5 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID6 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID7 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID8 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID9 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID10 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID11 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID12 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID13 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID14 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID15 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID16 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID17 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID18 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID19 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID20 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID21 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |
| SLST_PA_MID22 | 5´-CCATCTCATCCCTGTCGTTGCGCTGCAAATGAGGCAT-3´ |

**Primers for Sanger-sequencing-based SLST**

| Sanger_SLST_for | 5´-CGCCATCAAGGCAACCAACA-3´ |
| Sanger_SLST_rev | 5´-ATATCGGGCCGTTATGGCC-3´ |

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Table S4. Next-generation sequencing data: numbers of sequence reads and ST assignment in 96 samples

| Patient | P1  | P1  | P1  | P1  | P1  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P2  | P3  | P3  | P3  | P3  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Sampling spot | LB | LB | LB | UB | UB | UB | UB | UB | UB | UB | AB | F  | M  | 2-LB | 2-UB | 2-UB | 2-ABD | LB | LB | MB | ABD | F  | 2-LB | 2-MB | 2-ABD | 2-A |
| Number of reads | 6661 | 10951 | 3840 | 5336 | 10036 | 4974 | 4383 | 9758 | 3189 | 7728 | 3760 | 3046 | 2485 | 185 | 10144 | 7440 | 2064 | 5196 |
| Number of assigned reads | 6341 | 10762 | 3175 | 5250 | 9992 | 14715 | 14883 | 781 | 3938 | 5059 | 690 | 6379 | 4974 | 4383 | 9758 | 3189 | 7728 | 3760 | 3046 | 2485 | 185 | 10144 | 7440 | 2064 | 5196 |
| % unassigned reads | 4.80 | 1.73 | 17.32 | 1.13 | 2.69 | 1.68 | 1.13 | 0.72 | 2.10 | 0.92 | 1.21 | 0.63 | 1.94 | 1.13 | 2.53 | 2.43 | 1.41 | 0.54 | 1.19 | 1.68 | 3.15 | 1.44 |
| Unassigned | 320 | 169 | 665 | 86 | 44 | 168 | 21 | 66 | 57 | 5 | 134 | 46 | 53 | 61 | 62 | 87 | 95 | 74 | 36 | 1 | 121 | 125 | 65 | 75 |

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| Patient | Sampling spot | LB | UB | AB | MB | AD | F | M | 2-LB | 2-UB | 2-AB | 2-MB | 2-AD | 2-A |
|---------|---------------|----|----|----|----|----|---|---|------|------|------|------|------|------|-----|
| D1      |               | 0  | 0  | 0  | 0  | 0  | 1  | 12 | 0    | 0    | 12   | 0    | 64   | 8    | 13   |
| D2      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| D3      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E1      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E2      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E3      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E4      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E5      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E6      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E7      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 10   |
| E8      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| E9      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 3    | 0    | 3    | 0    |
| F1      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F2      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F3      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F4      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F5      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F6      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F7      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F8      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F9      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| F10     |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| G1      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| H1      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| H2      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| H3      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| H4      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| H5      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| K1      |               | 37 | 7  | 38 | 3  | 0  | 0  | 2  | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| K2      |               | 30 | 5  | 72 | 20 | 10 | 0  | 2  | 0    | 14   | 1    | 7    | 0    | 28   | 1    |
| K3      |               | 0  | 0  | 19 | 0  | 0  | 0  | 0  | 0    | 0    | 0    | 0    | 3    | 0    | 0    |
| K4      |               | 0  | 0  | 0  | 0  | 0  | 0  | 63 | 0    | 0    | 26   | 0    | 0    | 0    | 17   |
Table S4. (cont’d)

| Patient | Sampling spot | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P1 | P2 | P2 | P2 | P2 | P2 | P2 | P2 | P2 | P2 | P2 | P2 | P3 | P3 | P3 | P3 | P3 |
|---------|---------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|         |               | LB | LB | UB | UB | ABD | F | M | 2-LB | 2-UB | 2-UB | 2-ABD | LB | MB | ABD | F | 2-LB | 2-MB | 2-ABD | 2-A | LB | LB | UB | UB | LB | LB | UB | UB |
| K5      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K6      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K7      |               | 0  | 0  | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K8      |               | 800| 300| 620| 2758| 54 | 3  | 17 | 121| 4503| 472| 61 | 0  | 117| 19 | 490| 623| 442| 109| 167| 57 | 0  | 9  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K9      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K10     |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K11     |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K12     |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K13     |               | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K14     |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| L1      |               | 5288| 10375| 2191| 2002| 9822| 7  | 33 | 11  | 4  | 31 | 3177| 0  | 2715| 9503| 57 | 0  | 5  | 23 | 0  | 0  | 0  | 0  | 0  | 25 | 3  |
| L2      |               | 149 | 38 | 145 | 136 | 56 | 0  | 0  | 3587| 9  | 0  | 38 | 0  | 2  | 1  | 14 | 0  | 20 | 7  | 0  | 0  | 0  | 0  | 1  | 0  |
| L3      |               | 0  | 0  | 14  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| L4      |               | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| L5      |               | 25 | 2  | 71  | 6  | 4  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4907| 6  | 5  | 0  | 0  | 21 | 0  | 0  | 0  | 0  | 0  | 1  | 21 |
| L6      |               | 0  | 1  | 14  | 1  | 1  | 7  | 0  | 0  | 0  | 5  | 1  | 4  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 9623| 3839| 1349| 2245|
| Patient | P3 | P3 | P3 | P3 | P3 | P4 | P4 | P4 | P4 | P4 | P4 | P4 | P4-2 | P5 | P5 | P5 | P5 | P6 | P6 | P6 | P6 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| ABD     | F  | 2-LB | 2-UB | 2-ABD | LB | MB | UB | F  | 2-LB | 2-UB | 2-UB | 2-LB | 2-ABD | CH | F  | LB | LB | LB | ABD | F  |
| Number of reads | 11989 | 7710 | 11831 | 16240 | 11453 | 5424 | 16143 | 4381 | 3373 | 4886 | 5056 | 4288 | 3316 | 4418 | 8036 | 643 | 1816 | 12527 | 6613 | 1176 | 1111 | 4158 | 12345 | 12196 |
| Number of assigned reads | 11816 | 7493 | 11443 | 14467 | 13916 | 5236 | 15702 | 9234 | 5856 | 4288 | 3316 | 4418 | 8036 | 643 | 1816 | 12527 | 6613 | 1176 | 1111 | 4158 | 12345 | 12196 |
| % unassigned reads | 1.44 | 2.81 | 3.28 | 10.92 | 2.64 | 1.57 | 2.36 | 4.99 | 1.26 | 2.12 | 1.69 | 1.54 | 0.89 | 3.74 | 2.26 | 1.42 | 2.86 | 5.76 | 2.05 | 3.61 | 2.09 | 4.63 |
| Unassigned | 173 | 217 | 388 | 173 | 378 | 180 | 128 | 441 | 485 | 75 | 93 | 57 | 69 | 72 | 25 | 42 | 181 | 195 | 1078 | 128 | 67 | 282 | 283 | 592 |
| A1 | 286 | 908 | 5007 | 626 | 2686 | 37 | 1287 | 28 | 7 | 559 | 2601 | 29 | 1802 | 104 | 6 | 184 | 29 | 1262 | 324 | 397 | 967 | 3463 | 908 | 1883 |
| A2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| A3 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| A4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| A5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A6 | 1 | 2 | 16 | 0 | 7 | 0 | 0 | 0 | 0 | 3 | 0 | 11 | 0 | 0 | 1 | 0 | 3 | 0 | 1 | 3 | 19 | 3 | 0 |
| A7 | 0 | 1 | 2 | 1 | 1 | 0 | 9 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 4 | 0 | 4 | 0 |
| A8 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 |
| A9 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A11 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| A12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A15 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A16 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A18 | 1 | 9 | 3 | 0 | 3 | 0 | 7 | 0 | 0 | 0 | 1 | 1 | 2 | 3 | 0 | 0 | 0 | 12 | 0 | 1 | 1 | 2 | 2 | 3 |
| A19 | 2 | 1 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| A20 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 12 | 1 | 0 | 0 | 0 |
| A21 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| A22 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| A23 | 0 | 0 | 2 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| B1 | 7 | 41 | 187 | 1019 | 65 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 4 | 0 | 0 | 1 | 20 | 89 | 2 |
| C1 | 19 | 35 | 174 | 47 | 29 | 0 | 13 | 1 | 1 | 10 | 22 | 5 | 10 | 1 | 1 | 0 | 2 | 42 | 154 | 0 | 5 | 25 | 330 | 244 |
| C2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 313 | 0 | 216 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C3 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 |
### Table S4. (cont’d)

| Patient | P3 | P3 | P3 | P4 | P4 | P4 | P4 | P4 | P4 | P4 | P4-2 | P5 | P5 | P6 | P6 | P6 | P6 |
|---------|----|----|----|----|----|----|----|----|----|----|------|----|----|----|----|----|----|
| C4      | ABD| F  | 2-LB| 2-UB| 2-ABD| LB  | MB  | UB  | F  | 2-LB| 2-UB| 2-UB-2| LB  | ABD | CH | F  | LB  | LB  |
| D1      | 40 | 32 | 1148| 1711| 177 | 28  | 269 | 99  | 1432| 121 | 88   | 464  | 416 | 818 | 105| 618 | 9   | 1583|
| D2      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 1  | 0   | 0   | 0   |
| D3      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 2  | 0   | 0   | 0   |
| E1      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 1   | 0   | 0   |
| E2      | 0  | 0  | 0   | 0   | 2   | 1   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| E3      | 0  | 0  | 2   | 27  | 2   | 3   | 1   | 0   | 3   | 1   | 13   | 103  | 42  | 177 | 0  | 0   | 0   | 1   |
| E4      | 0  | 0  | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| E5      | 0  | 0  | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| E6      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| E7      | 38 | 38 | 115 | 849 | 65  | 93  | 544 | 221 | 1617| 128 | 308  | 2705 | 1137| 6920| 0  | 0   | 0   | 0   |
| E8      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| E9      | 16 | 1359| 1785| 5413| 772 | 7   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| F1      | 0  | 0  | 1   | 0   | 1   | 0   | 2   | 22  | 0   | 0   | 0    | 0    | 1   | 0   | 11 | 0   | 0   | 0   |
| F2      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| F3      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| F4      | 134| 790 | 337 | 882 | 1032 | 28 | 1532 | 0  | 2 | 1122 | 163 | 3 | 426 | 2 | 121 | 408 | 20 |
| F5      | 0  | 0  | 0   | 0   | 0   | 0   | 2   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| F6      | 0  | 0  | 0   | 0   | 0   | 1   | 0   | 0   | 1   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| F7      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| F8      | 10 | 40 | 2   | 0   | 0   | 3   | 10  | 0   | 6   | 0   | 0    | 0    | 0   | 0   | 1  | 0   | 47  | 0   |
| F9      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 0  | 0   | 0   | 0   |
| F10     | 0  | 1  | 1   | 86  | 0   | 8   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 1  | 0   | 0   | 0   |
| G1      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0   | 1  | 0   | 1   | 0   |
| H1      | 10 | 14 | 12  | 2   | 213 | 0   | 0   | 0   | 3861| 415 | 2   | 105  | 0   | 272 | 123 | 576 | 659 | 9462|
| H2      | 0  | 0  | 0   | 0   | 0   | 6   | 1   | 0   | 0   | 0   | 0    | 0    | 0   | 13 | 0   | 0   | 0   | 0   |
| H3      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 3  | 0   | 0   | 0   | 0   |
| H4      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 2   | 0   | 1  | 1   | 1   | 0   |
| H5      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 4   | 0   | 0  | 0   | 0   | 0   |
| K1      | 9  | 3  | 20  | 0   | 75  | 0   | 4   | 0   | 4   | 16  | 0   | 11   | 0   | 15  | 126 | 51  | 72  | 389  |
| K2      | 0  | 788| 20  | 7   | 87  | 0   | 0   | 0   | 12  | 58  | 0   | 23   | 0   | 1   | 2  | 631 | 0   | 0   |
| K3      | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0   | 0  | 0  | 0   | 0   | 0   |
**Table S4. (cont’d)**

| Patient | P3 | P3 | P3 | P3 | P4 | P4 | P4 | P4 | P4 | P4-2 | P5 | P5 | P5 | P5 | P6 | P6 | P6 | P6 | P6 |
|---------|----|----|----|----|----|----|----|----|----|------|----|----|----|----|----|----|----|----|----|
| Sampling spot | ABD | F | 2-LB | 2-UB | 2-ABD | LB | MB | UB | F | 2-LB | 2-UB | 2-UB | 2-UB-2 | LB | ABD | CH | F | LB | LB | UB | ABD | F |
| K4      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  |
| K5      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K6      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  |
| K7      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 3  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K8      | 4  | 97 | 6  | 0  | 41  | 8  | 0  | 0  | 0  | 0    | 100 | 0  | 11 | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K9      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K10     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K11     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K12     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K13     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| K14     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| L1      | 17 | 2  | 31  | 393 | 68  | 4  | 19 | 16 | 36  | 0    | 57  | 0  | 38  | 0  | 116 | 348 | 1172 | 2628 | 1  | 5217 | 2372 | 1661 | 10929 | 23 |
| L2      | 0  | 0  | 0  | 1  | 0   | 0  | 0  | 0  | 81   | 0    | 71  | 0  | 0  | 0  | 8  | 0  | 0  | 0  | 3  | 2  | 0  | 1  | 0  |
| L3      | 0  | 0  | 0  | 0  | 0   | 0  | 0  | 0  | 0    | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| L4      | 0  | 0  | 0  | 0  | 0   | 0  | 0  | 0  | 0    | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| L5      | 2  | 0  | 7  | 3  | 2   | 1  | 2  | 6  | 4    | 0    | 0  | 1  | 0  | 0  | 1  | 2  | 107 | 9  | 0  | 7  | 13 | 260 | 6  |
| L6      | 11240 | 3330 | 2554 | 3459 | 8492 | 11051 | 1552 | 15028 | 6109 | 23  | 5  | 2  | 55  | 0  | 1  | 1  | 3  | 1  | 1  | 2  | 0  | 4  | 12 | 0  |
| Patient | P6 | P7 | P7 | P7 | P7 | P7 | P7-2 | P7-2 | P8 | P8 | P8-2 | P8-2 | P8-2 | C1 | C1 | C1 | C1 | C2 | C2 |
|---------|----|----|----|----|----|----|------|------|----|----|------|------|------|----|----|----|----|----|----|
| M       | LB | LB | MB | ABD | M  | 2-LB | 2-LB | 2-MB | 2-ABD | LB | ABD | F   | M  | 2-LB | 2-LB | 2-ABD | LB | UB | ABD | LB | LB |
| Number of reads | 2655 | 4321 | 3223 | 3179 | 1811 | 5627 | 934 | 2667 | 5667 | 1914 | 8167 | 1348 | 3600 | 10240 | 2069 | 10891 | 13265 | 8920 | 13378 | 9225 | 9033 |
| Number of assigned reads | 2612 | 3943 | 285 | 10068 | 8766 | 3223 | 1864 | 5989 | 7437 | 10330 | 8430 | 3912 | 10423 | 2636 | 11079 | 14191 | 9044 | 13576 | 9422 | 9230 |
| % unassigned reads | 1.62 | 8.75 | 1.33 | 1.37 | 1.37 | 1.40 | 0.96 | 0.98 | 1.56 | 1.32 | 2.88 | 1.33 | 1.76 | 1.02 | 1.70 | 1.87 | 1.46 | 2.09 | 2.13 |

Table S4. (cont’d)
| Patient | P6 | P7 | P7 | P7 | P7 | P7-2 | P7-2 | P8 | P8 | P8 | P8 | P8-2 | P8-2 | C1 | C1 | C1 | C2 | C2 |
|---------|----|----|----|----|----|------|------|----|----|----|----|------|------|----|----|----|----|----|
| Sampling spot | M | LB | LB | MB | ABD | M | 2-LB | 2-LB | 2-MB | 2-ABD | LB | ABD | F | M | 2-LB | 2-UB | 2-ABD | LB | UB | ABD | LB | LB |
| D1 | 77 | 355 | 1 | 1 | 31 | 171 | 131 | 963 | 492 | 212 | 3 | 418 | 118 | 1324 | 0 | 486 | 665 | 251 | 1644 | 1057 | 8501 | 1332 | 5384 | 3034 |
| D2 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 2 | 2 |
| D3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 4 | 0 | 29 | 1 |
| E1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| E3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 |
| E4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E7 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 8 | 45 | 0 | 1 | 7 | 0 | 10 | 10 | 38 |
| F1 | 0 | 0 | 0 | 1 | 0 | 14 | 4 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 120 | 45 | 0 | 82 | 304 | 109 |
| F2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F4 | 111 | 11 | 0 | 3 | 32 | 384 | 34 | 522 | 51 | 197 | 0 | 3 | 1 | 0 | 13 | 3 | 6 | 0 | 7 | 5 | 0 | 1 | 261 | 380 |
| F5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| F7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F8 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 1 |
| G1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 190 | 486 |
| H1 | 192 | 300 | 3 | 39 | 158 | 1765 | 391 | 2263 | 6553 | 901 | 0 | 65 | 32 | 772 | 54 | 61 | 102 | 17 | 0 | 4 | 0 | 0 | 23 | 8 |
| H2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 66 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H5 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 9 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K1 | 60 | 2342 | 0 | 33 | 2 | 49 | 10 | 212 | 2156 | 9 | 4 | 14 | 34 | 35 | 38 | 178 | 396 | 163 | 148 | 270 | 12 | 352 | 8 | 7 |
| K2 | 0 | 124 | 0 | 0 | 7 | 2 | 2 | 2 | 0 | 21 | 452 | 242 | 30 | 3099 | 21 | 89 | 319 | 11 | 1 | 1 | 0 | 18 | 8 | 0 |
| K3 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
Table S4. (cont’d)

| Patient | P6 | P7 | P7 | P7 | P7 | P7 | P7-2 | P7-2 | P8 | P8 | P8 | P8-2 | P8-2 | P8-2 | C1 | C1 | C1 | C1 | C2 | C2 |
|---------|----|----|----|----|----|----|------|------|----|----|----|------|------|------|----|----|----|----|----|----|
| Sampling spot | M | LB | LB | MB | ABD | M | 2-LB | 2-LB | 2-MB | 2-ABD | LB | ABD | F | M | 2-LB | 2-UB | 2-ABD | LB | UB | ABD | LB | LB |
| K5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 1 | 0 | 344 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K7 | 0 | 1 | 0 | 6 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 13 |
| K8 | 348 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 11 | 0 | 7 | 47 | 1 | 6 | 1 | 10 | 3 | 0 | 9 | 3 | 35 |
| K9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K11 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 0 | 8 | 0 | 1 | 2 | 0 | 0 |
| K13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L1 | 24 | 273 | 9760 | 8360 | 53 | 1146 | 26 | 7 | 11578 | 0 | 0 | 1 | 208 | 0 | 0 | 0 | 11 | 2513 | 3 | 0 | 5 | 0 | 1 |
| L2 | 1 | 0 | 1 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L5 | 0 | 25 | 4 | 1 | 1 | 0 | 5 | 0 | 0 | 5 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| L6 | 0 | 0 | 1 | 28 | 28 | 1 | 3 | 0 | 1 | 10 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 52 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
### Table S4. (cont’d)

| Patient | C2  | C2  | C3  | C3  | C3  | C4  | C4  | C4  | C5  | C5  | C6  | C6  | C7  | C7  | C8  | C8  | C8  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|         | UB  | ABD | LB  | LB  | UB  | ABD | LB  | LB  | UB  | ABD | LB  | LB  | UB  | ABD | LB  | LB  | ABD |
| Number of reads | 10716 | 11233 | 13394 | 8265 | 19242 | 12935 | 6033 | 137 | 4298 | 7483 | 4560 | 14184 | 3374 | 12825 | 6636 | 9334 | 4230 |
| Number of assigned reads | 10559 | 10936 | 12988 | 8265 | 17996 | 12825 | 6033 | 71 | 4298 | 7483 | 4560 | 14184 | 3374 | 12825 | 6636 | 9334 | 4230 |
| % unassigned reads | 1.47 | 2.64 | 3.03 | 3.82 | 6.48 | 1.71 | 1.00 | 1.13 | 2.96 | 1.12 | 2.27 | 1.45 | 3.56 | 1.01 | 2.10 | 3.27 | 1.84 | 2.94 |
| Unassigned | 157 | 297 | 406 | 316 | 1246 | 277 | 129 | 67 | 414 | 48 | 170 | 66 | 503 | 137 | 71 | 419 | 126 | 274 |
|            | 147 | 147 | 171 | 137 | 515 | 278 | 10816 | 10816 | 10816 | 10816 | 10816 | 10816 | 10816 | 10816 | 10816 | 10816 | 10816 |
| A1        | 9314 | 5265 | 2052 | 2611 | 8947 | 14187 | 282 | 1235 | 5191 | 1353 | 2035 | 8822 | 1101 | 446 | 805 | 690 | 8054 |
| A2        | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A3        | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A4        | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A5        | 4 | 0 | 3 | 0 | 1 | 2 | 0 | 15 | 7 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 |
| A6        | 12 | 13 | 6 | 6 | 17 | 10 | 1 | 5 | 8 | 3 | 16 | 3 | 0 | 2 | 1 | 9 | 19 |
| A7        | 4 | 25 | 2 | 1 | 6 | 30 | 0 | 2 | 5 | 0 | 0 | 0 | 1 | 0 | 1 | 6 | 3 |
| A8        | 9 | 5 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 2 | 3 | 1 | 0 | 0 | 0 | 6 | 0 |
| A9        | 1 | 1 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A10       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A11       | 1 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| A12       | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 1 |
| A13       | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A14       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A15       | 1 | 10 | 0 | 1 | 1 | 4 | 0 | 6 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| A16       | 5 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| A17       | 1 | 1 | 1 | 2 | 2 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| A18       | 42 | 8 | 7 | 15 | 4 | 20 | 1 | 1 | 9 | 2 | 1 | 5 | 0 | 0 | 0 | 4 | 1 |
| A19       | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| A20       | 3 | 1 | 1 | 0 | 0 | 5 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 2 |
| A21       | 0 | 0 | 0 | 1 | 0 | 22 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 4 |
| A22       | 0 | 4 | 0 | 6 | 15 | 0 | 0 | 1 | 2 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 1 |
| A23       | 14 | 6 | 0 | 4 | 5 | 6 | 0 | 0 | 1 | 7 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| B1        | 7 | 2 | 7 | 0 | 0 | 1 | 2 | 9 | 10 | 3 | 9 | 1 | 0 | 23 | 0 | 0 | 0 |
| C1        | 55 | 35 | 12 | 73 | 1425 | 377 | 3 | 16 | 9 | 5 | 20 | 42 | 29 | 0 | 7 | 6 | 51 |
| C2        | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C3        | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 2 | 0 | 0 | 0 |
| C4        | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Patient | C2 | C2 | C3 | C3 | C4 | C4 | C4 | C4 | C5 | C5 | C5 | C6 | C6 | C6 | C7 | C7 | C7 | C8 | C8 | C8 | C8 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| UB | ABD | LB | LB | UB | ABD | LB | LB | UB | ABD | LB | LB | UB | ABD | LB | LB | UB | ABD | LB | LB | UB | ABD |
| D1 | 813 | 4237 | 668 | 2640 | 6895 | 505 | 10 | 2 | 0 | 60 | 1761 | 1571 | 3364 | 95 | 447 | 6424 | 914 | 1101 | 553 | 69 | 596 | 785 | 55 | 1567 |
| D2 | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| D3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 2 |
| E1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E3 | 0 | 0 | 1 | 4 | 67 | 1 | 3 | 36 | 36 | 38 | 4 | 1 | 6 | 2 | 16 | 29 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| E4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E7 | 16 | 21 | 1 | 45 | 440 | 108 | 197 | 1324 | 1896 | 1175 | 119 | 363 | 44 | 639 | 2697 | 356 | 26 | 30 | 23 | 0 | 0 | 1 | 3 |
| E8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F1 | 33 | 106 | 48 | 135 | 1 | 3 | 3 | 20 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 1 | 0 | 0 | 0 |
| F2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F4 | 30 | 609 | 1 | 3 | 160 | 3 | 0 | 82 | 2086 | 0 | 0 | 0 | 0 | 0 | 15 | 4 | 31 | 0 | 0 | 0 | 0 | 1 | 0 | 15 |
| F5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F10 | 0 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 244 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G1 | 145 | 388 | 6 | 2 | 0 | 12 | 0 | 9 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 2 | 0 |
| H1 | 0 | 0 | 8 | 13 | 0 | 48 | 1 | 17 | 2 | 16 | 6 | 40 | 22 | 20 | 14 | 12 | 3 | 207 | 1490 | 300 | 622 | 158 | 292 | 20 | 923 |
| H2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H4 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 13 | 53 | 0 | 0 | 0 | 0 |
| H5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 1 |
| K1 | 0 | 2 | 3 | 10 | 1 | 204 | 1 | 10 | 0 | 0 | 416 | 300 | 56 | 9 | 17 | 12 | 649 | 106 | 1075 | 1920 | 1800 | 2450 | 259 | 3862 |
| K2 | 0 | 0 | 4620 | 172 | 2 | 77 | 0 | 0 | 0 | 0 | 683 | 393 | 3790 | 12671 | 1184 | 2497 | 1646 | 4063 | 995 | 1060 | 34 | 40 | 8 | 64 |
| K3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 26 | 0 | 0 | 0 | 52 | 53 | 431 | 30 |
| Patient | C2 | C2 | C3 | C3 | C3 | C4 | C4 | C4 | C5 | C5 | C5 | C6 | C6 | C6 | C6 | C7 | C7 | C7 | C8 | C8 | C8 | C8 | C8 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Sampling spot | UB | ABD | LB | LB | UB | ABD | LB | LB | UB | ABD | LB | LB | ABD | LB | LB | UB | ABD | LB | LB | UB | ABD | LB |
| K5 | 0 0 1 2 0 0 0 26 0 6 0 3 0 0 0 1 1 12 2 0 1 0 0 |
| K6 | 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 1 0 0 0 |
| K7 | 0 0 0 0 0 0 8 8 0 2 0 0 0 0 0 0 0 0 0 0 0 2 2 0 9 |
| K8 | 2 20 5527 2164 2 225 0 16 0 6 10 1 3 32 29 12 296 19 22 35 48 84 13 874 |
| K9 | 0 0 2 0 0 1 0 0 0 0 0 0 2 1 0 0 0 1 0 0 0 0 0 |
| K10 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| K11 | 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 1 0 0 1 1 0 0 0 |
| K12 | 0 5 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 1 7 8 0 0 0 |
| K13 | 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 6 0 0 0 0 0 |
| K14 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1 1 0 0 0 0 |
| L1 | 24 1 3 31 2 5 12275 2902 3830 1572 357 188 875 71 116 0 126 35 32 105 0 0 0 0 |
| L2 | 19 109 0 1 0 1 3 1 0 1 27 26 11 0 0 0 20 89 158 48 17 1 26 |
| L3 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 576 208 128 0 0 0 0 |
| L4 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| L5 | 0 0 0 0 0 0 0 0 0 2 3 9 0 108 87 88 0 1 0 283 1 0 0 737 1235 251 1182 |
| L6 | 0 0 2 1 0 0 13 2 6 4 1 0 48 1 4 0 5 371 4 5 0 2 0 1 |
Fig. S1. Sampling sites in patients and controls. Patients were sampled from lesional skin on lower back, upper back, and abdomen and adjacent non-affected skin. In addition, unaffected skin of the forehead and buccal mucosa were sampled. After treatment and in healthy controls samples were obtained from areas corresponding to lesions.

Fig. S2. Comparison of two methods of *P. acnes* ST distribution analysis: cultivation and subsequent SLST of up to 10 colonies per lesion versus next-generation sequencing (NGS)-based SLST. a) Average ST proportions in patient and control samples analysed by culture versus NGS. b) Culture versus NGS determination of ST proportions shown for individual patients and controls. Each ST type A to L is given a color as indicated, and the corresponding *P. acnes* subtype is given in brackets.