**Supporting materials**

**Supplemental Table 1**

Bacterial composition for the Mock Community

**Supplemental Table 2**

Primer sequence used for this study.

**Supplemental Table 3**

PCR conditions used for this study.

**Supplemental Table 4**

Sequence statistics for the 20 bacteria mock community data. Metrichor classified the reads into fail and pass. We used both fail and pass reads for the analysis.

**Supplemental Table 5**

Sensitivity was calculated as % bacteria assigned to 20 reference bacteria to total bacteria reads. Deviation was calculated as % expected reads for certain bacteria to all the reads assigned to the bacteria. Sensitivity and deviation at the genus level are shown.

**Supplemental Table 6**

Eleven different bacteria species (strains) obtained from BEI resource used for determining PCR condition.

**Supplemental Table 7**

Sequence statistics for the pleural effusion data.

**Supplementary Figure 1**

The bacterial classification at the species level using the Metrichor base-caller and BLAST-based searches. Deviation from % expected reads for certain

bacteria to all the reads assigned to bacteria are shown (a-g). IonPGM sequencing for 16S rDNA (a). MinION Rapid 1D Sequencing of the almost full-length 16S rDNA amplicon for 5 min (b), 15 min (c), 30 min (d), 1 h (e), 4 h (f). MinION 2D sequencing for 16S rDNA (g). The percentage of reads assigned to any of the 20 bacteria among all reads classified as bacterial reads (h).

**Supplementary Figure 2**

The bacterial classification at the genus level using Metrichor basecaller and BLAST-based searches. Deviation from % expected reads for certain bacteria to all the reads assigned to bacteria are shown (a-g). IonPGM sequencing for 16S rDNA (a). MinION Rapid 1D Sequencing of the almost full-length 16S rDNA amplicon for 5 min (b), 15 min (c), 30 min (d), 1 h (e), 4 h (f). MinION 2D sequencing for 16S rDNA (g). The percentage of reads assigned to any of the 20 bacteria among all reads classified as bacterial reads (h).

**Supplementary Figure 3**

Eleven different bacteria species (strains) obtained from BEI resource were amplified either using S-D-Bact-0008-c-S-20 and S-D-Bact-1391-a-A-17 primer set (a) or six primers covering V2, V3, V4, V6-7, V8 and V9 (b), using KAPA HiFi HotStart ReadyMix (KAPA Biosystems, MA, USA). Amplified 11 bacteria species (strains) are shown in Supplemental Table 6.

**Supplementary Figure 4**

The bacterial classification at the species level using Nanonet base-caller and Centrifuge. Deviation from % expected reads for certain bacteria to all the reads assigned to bacteria are shown (a-g). IonPGM sequencing for 16S rDNA (a). MinION Rapid 1D Sequencing of the almost full-length of the 16S rDNA amplicon for 5 min (b), 15 min (c), 30 min (d), 1 h (e), 4 h (f). MinION 2D sequencing for 16S rDNA (g). The percentage of reads assigned to any of the 20 bacteria among all reads classified as bacterial reads (h).

**Supplementary Figure 5**

The bacterial classification at the genus level using Nanonet base-caller and *Centrifuge*. Deviation from % expected reads for certain bacteria to all the reads assigned to bacteria are shown (a-g). IonPGM sequencing for 16S rDNA (a). MinION Rapid 1D Sequencing of the almost full-length 16S rDNA amplicon for 5 min (b), 15 min (c), 30 min (d), 1 h (e), 4 h (f). MinION 2D sequencing for 16S rDNA (g). The percentage of reads assigned to any of the 20 bacteria among all reads classified as bacterial reads (h).

**Supplementary Figure 6**

The bacterial classification at the species level using Metrichor base-caller and *Centrifuge*. Deviation from % expected reads for certain bacteria to all the reads assigned to bacteria are shown (a-g). IonPGM sequencing for 16S rDNA (a). MinION Rapid 1D Sequencing of the almost full-length 16S rDNA amplicon for 5 min (b), 15 min (c), 30 min (d), 1 h (e), 4 h (f). MinION 2D sequencing for 16S rDNA (g). The percentage of reads assigned to any of the 20 bacteria among all reads classified as bacterial reads (h).

**Supplementary Figure 7**

The bacterial classification at the genus level using Metrichor base-caller and *Centrifuge*. Deviation from % expected reads for certain bacteria to all the reads assigned to bacteria are shown (a-g). IonPGM sequencing for 16S rDNA (a). MinION Rapid 1D Sequencing of the almost full-length 16S rDNA amplicon for 5 min (b), 15 min (c), 30 min (d), 1 h (e), 4 h (f). MinION 2D sequencing for 16S rDNA (g). The percentage of reads assigned to any of the 20 bacteria among all reads classified as bacterial reads (h).

| | Species | Strain | Genome size | 16S copy number | Taxonomy ID |
|---|---|---|---|---|---|
| 1 | *Acinetobacter baumannii* | 5377 | 3,976,747 | 6 | 470 |
| 2 | *Actinomyces odontolyticus* | 1A.21 | 2,391,230 | 3 | 1660 |
| 3 | *Bacillus cereus* | NRS 248 | 5,224,283 | 13 | 1396 |
| 4 | *Bacteroides vulgatus* | ATCC® 8482ä | 5,163,189 | 7 | 821 |
| 5 | *Clostridium beijerinckii* | NCIMB 8052 | 6,000,632 | 14 | 1520 |
| 6 | *Deinococcus radiodurans* | R1 (smooth) | 2,648,638 | 2 | 1299 |
| 7 | *Enterococcus faecalis* | OG1RF | 2,739,625 | 4 | 1351 |
| 8 | *Escherichia coli* | MG1655 | 4,641,652 | 7 | 562 |
| 9 | *Helicobacter pylori* | 26695 | 1,667,867 | 2 | 210 |
| 10 | *Lactobacillus gasseri* | 63 AM | 1,894,360 | 6 | 1596 |
| 11 | *Listeria monocytogenes* | EGDe | 2,944,528 | 6 | 1639 |
| 12 | *Neisseria meningitidis* | MC58 | 2,272,360 | 4 | 487 |
| 13 | *Propionibacterium acnes* | KPA171202 | 2,560,265 | 3 | 1747 |
| 14 | *Pseudomonas aeruginosa* | PAO1-LAC | 6,264,404 | 4 | 287 |
| 15 | *Rhodobacter sphaeroides* | ATH 2.4.1 | 4,131,542 | 4 | 1063 |
| 16 | *Staphylococcus aureus* | TCH959 | 2,830,017 | 5 | 1280 |
| 17 | *Staphylococcus epidermidis* | PCI 1200 | 2,499,279 | 6 | 1282 |
| 18 | *Streptococcus agalactiae* | 2603 V/R | 2,160,267 | 7 | 1311 |
| 19 | *Streptococcus mutans* | UA159 | 2,032,925 | 5 | 1309 |
| 20 | *Streptococcus pneumoniae* | TIGR4 | 2,160,842 | 4 | 1313 |

**Supplemental Table 1. BEI Resourse bacterial mock community**

| Primers | Primer sequence | Product size |
|---|---|---|
| V2_Foward | AGNGGCGNACGGGTGAGTAAC | |
| V3_Foward | ACTGAGACACGGTCCANACTCCTACGGG | 230 |
| V4_Foward | CCAGAGCCGCGGTAATACGNAG | |
| V6-7_Foward | ACAAGCGGNGGANCATGTGGTTTAATTCGA | 280 |
| V8_Foward | TGTCGTCAGCTCGTGTCG | |
| V9_Foward | GAGGAAGGTGGGGATGACGTC | 270 |
| V2_Reverse | CTGCCTCCCGTAGGAGTCTG | |
| V3_Reverse | GTATTACCGCGGCTGCTGGCAC | 270 |
| V4_Reverse | GGACTACCAGGGTATCTAATCCTGTT | |
| V6-7_Reverse | TTGACGTCATCCCCACCTTCCTCC | 290 |
| V8_Reverse | CGATTACTAGCGANTCCNNCTTC | |
| V9_Reverse | GTTACGACTTCACCCCANTC | 330 |
| S-D-bact-0008-c-S20 | AGRGTTYGATYMTGGCTCAG | |
| S-D-bact-1391-a-A-17 | GACGGGCGGTGWGTRCA | 1399 |

**Supplemental Table 2. Primer sequences used for this study**

**PCR condition for V2, V4, V8, V9**

| | | |
|---|---|---|
| 95℃ | 5min | |
| 98℃ | 20sec | 25 cycle |
| 59.5℃ | 15sec | |
| 72℃ | 5sec | |
| 72℃ | 5min | |

**PCR condition for V3, V6-7**

| | | |
|---|---|---|
| 95℃ | 5min | |
| 98℃ | 20sec | 25 cycle |
| 62℃ | 15sec | |
| 72℃ | 5sec | |
| 72℃ | 5min | |

**PCR condition for full length 16S**

| | | |
|---|---|---|
| 95℃ | 5min | |
| 98℃ | 20sec | 25 cycle |
| 60℃ | 15sec | |
| 72℃ | 15sec | |
| 72℃ | 5min | |

**Supplemental Table 3. PCR conditions used in this study**

| | | | | | read length | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| basecaller | method | fail/pass | total reads | total base pairs | mean | median | min | max | N25 | N50 | N75 |
| Nanonet | Rapid 1D (5min) | NA | 1167 | 975842 | 836.20 | 804 | 9 | 6489 | | | |
| Nanonet | Rapid 1D (15min) | NA | 3170 | 2652458 | 836.74 | 801 | 5 | 6489 | | | |
| Nanonet | Rapid 1D (30min) | NA | 5733 | 4904350 | 855.46 | 810 | 5 | 9473 | | | |
| Nanonet | Rapid 1D (1hr) | NA | 9510 | 8144141 | 856.38 | 812.5 | 5 | 9473 | | | |
| Nanonet | Rapid 1D (4hr) | NA | 20541 | 17965589 | 874.62 | 813 | 5 | 9473 | | | |
| Nanonet | 2D | NA | 125382 | 64189793 | 511.95 | 446 | 7 | 9455 | | | |
| Metrichor | Rapid 1D (5min) | fail | 263 | 208046 | 791.05 | 751 | 111 | 1507 | 1085 | 884 | 662 |
| | | pass | 923 | 798052 | 864.63 | 830 | 133 | 2160 | 1186 | 959 | 734 |
| Metrichor | Rapid 1D (15min) | fail | 668 | 532385 | 796.98 | 747 | 111 | 8196 | 1099 | 894 | 668 |
| | | pass | 2478 | 2157453 | 870.64 | 834 | 133 | 2241 | 1183 | 984 | 728 |
| Metrichor | Rapid 1D (30min) | fail | 1174 | 954386 | 812.94 | 767 | 111 | 8196 | 1108 | 904 | 689 |
| | | pass | 4456 | 3930651 | 882.1 | 847 | 133 | 8089 | 1189 | 995 | 738 |
| Metrichor | Rapid 1D (1hr) | fail | 2003 | 1621719 | 809.65 | 782 | 111 | 8196 | 1104 | 904 | 693 |
| | | pass | 7189 | 6355257 | 884.03 | 850 | 133 | 8767 | 1187 | 993 | 740 |
| Metrichor | Rapid 1D (4hr) | fail | 4873 | 4017117 | 824.36 | 787 | 111 | 26840 | 1121 | 917 | 695 |
| | | pass | 14502 | 12802088 | 882.78 | 840 | 105 | 64458 | 1188 | 988 | 739 |
| Metrichor | 2D | fail | 520645 | 228194084 | 438.29 | 297 | 5 | 382528 | 1134 | 572 | 325 |
| | | pass | 205725 | 86904188 | 422.43 | 329 | 103 | 5827 | 649 | 487 | 308 |

**Supplemental Table 4. Sequencing statistics**

| | | | Centrifuge | | | | BLAST/GenomeSync | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sequencer | Basecaller | Method | Total reads | Total Bacterial Reads | Sensitivity (%) | Average % deviation from expected (=0) | Total reads | Total Bacterial Reads | Sensitivity (%) | Average % deviation from expected (=0) |
| IonPGM | NA | Ion 16S™ Metagenomics Kit | 378324 | 377582 | 86.2 | -18.0 | 330123 | 322213 | 92.2 | -11.2 |
| MinION | Nanonet | Rapid 1D (5min) | 1257 | 1108 | 62.6 | -34.9 | 1067 | 1167 | 97.0 | -0.7 |
| MinION | Nanonet | Rapid 1D (15min) | 3247 | 2885 | 60.9 | -36.1 | 2895 | 3170 | 97.3 | -1.1 |
| MinION | Nanonet | Rapid 1D (30min) | 5751 | 5132 | 60.2 | -36.9 | 5259 | 5733 | 96.9 | -1.8 |
| MinION | Nanonet | Rapid 1D (1h) | 9445 | 8469 | 58.9 | -38.3 | 8642 | 9510 | 96.5 | -2.1 |
| MinION | Nanonet | Rapid 1D (4h) | 17268 | 19430 | 51.8 | -45.5 | 18665 | 20541 | 95.1 | -3.3 |
| MinION | Nanonet | 2D (6 primer set) | 85779 | 84432 | 68.0 | -29.5 | 125382 | 91475 | 89.5 | -9.0 |
| MinION | Metrichor | Rapid 1D (5min) | 1236 | 1185 | 63.2 | -34.5 | 1149 | 1186 | 94.0 | -3.7 |
| MinION | Metrichor | Rapid 1D (15min) | 3333 | 3190 | 65.0 | -33.2 | 3066 | 3146 | 94.0 | -4.1 |
| MinION | Metrichor | Rapid 1D (30min) | 5731 | 6014 | 61.2 | -37.2 | 5500 | 5630 | 93.9 | -4.8 |
| MinION | Metrichor | Rapid 1D (1h) | 9237 | 9694 | 60.8 | -37.2 | 8969 | 9192 | 93.6 | -4.7 |
| MinION | Metrichor | Rapid 1D (4h) | 19031 | 19903 | 59.3 | -38.4 | 18908 | 19376 | 93.1 | -5.2 |
| MinION | Metrichor | 2D (6 primer set) | 93417 | 92289 | 68.0 | -30.1 | 139784 | 121282 | 89.4 | 10.7 |

**Supplemental Table 5. Sensitivity to detect 20 mock bacteria and deviation from expected read number.**

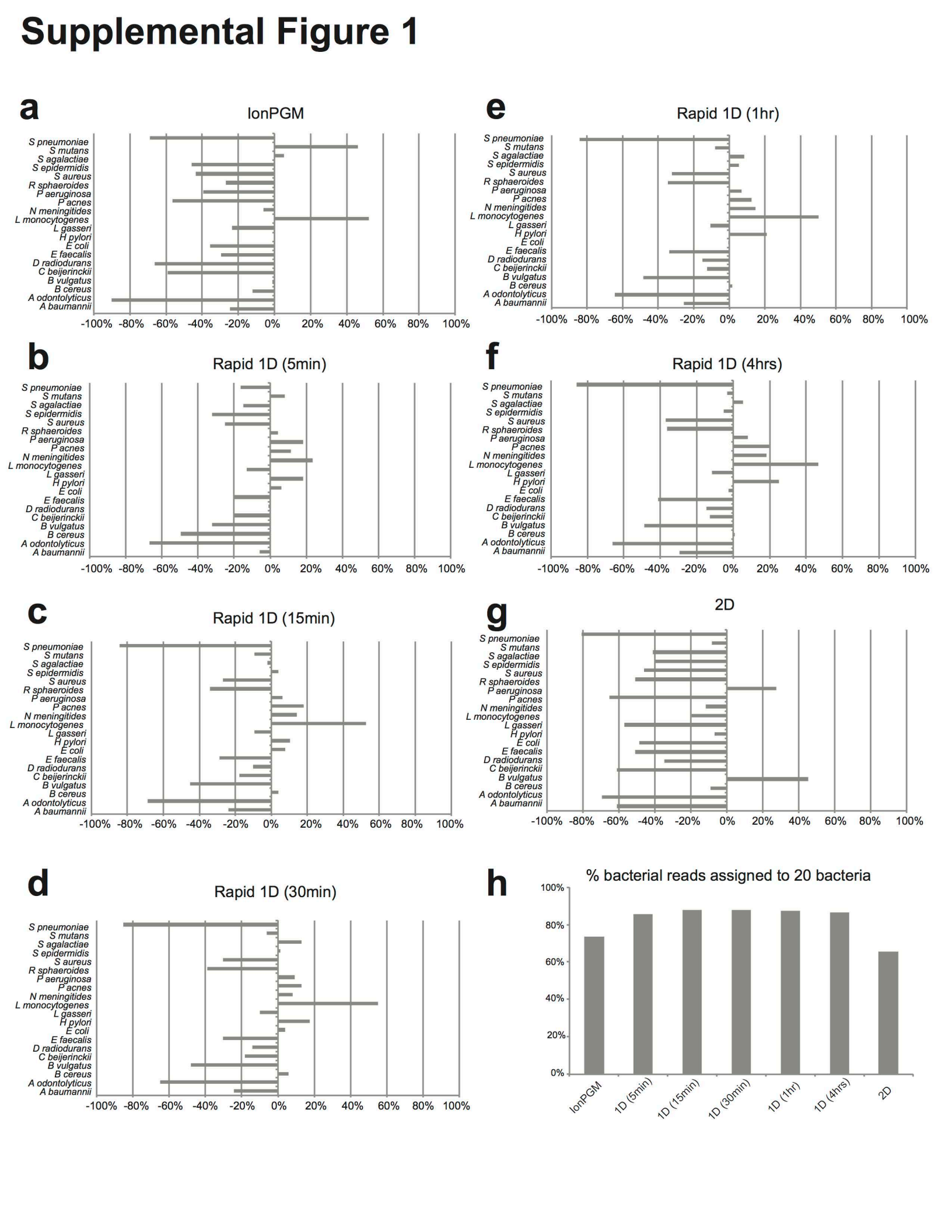
| | Species | strain |
|---|---|---|
| 1 | *Staphylococcus aureus* | MRSA131 |
| 2 | *Staphylococcus aureus* | HFH-29568 |
| 3 | *Staphylococcus aureus* | HFH-30106 |
| 4 | *Escherichia coli* | BAA-1161 |
| 5 | *Streptococcus pneumoniae* | TCH8431 |
| 6 | *Campylobacter jejuni* | UA466 |
| 7 | *Bacillus cereus* | G9241 |
| 8 | *Bacillus samanii* | C1 |
| 9 | *Enterococcus faecalis* | TX0104 |
| 10 | *Enterococcus faecalis* | TUSoD11 |
| 11 | *Listeria monocytogenes* | 10403S |

**Supplemental Table 6. Eleven bacterial species used for PCR condition determination.**

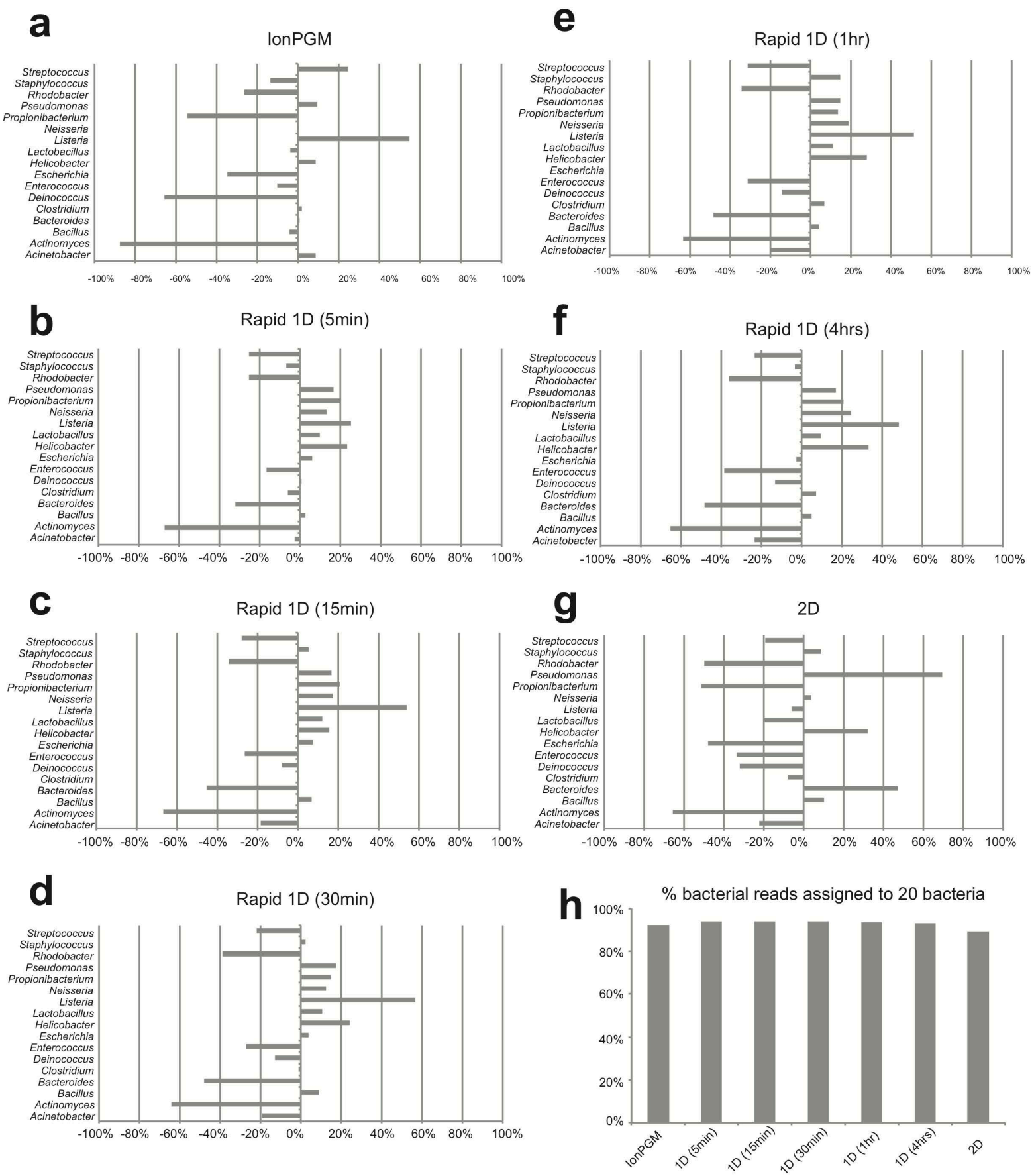
| | | | | | read length | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| sequencer | method | basecaller | total reads | total base pairs | mean | median | min | max | N25 | N50 | N75 |
| MinION | Rapid 1D (5min) | MinKNOW | 670 | 421945 | 629.77 | 545 | 127 | 9002 | 1032 | 718 | 507 |
| MinION | Rapid 1D (15min) | MinKNOW | 2179 | 1447755 | 664.41 | 562 | 39 | 15219 | 1128 | 770 | 538 |
| MinION | Rapid 1D (4h) | MinKNOW | 38976 | 31298407 | 803.02 | 614 | 39 | 170904 | 1291 | 861 | 603 |

**Supplemental Table 7. Sequence statistics for Pleural effusion sample.**

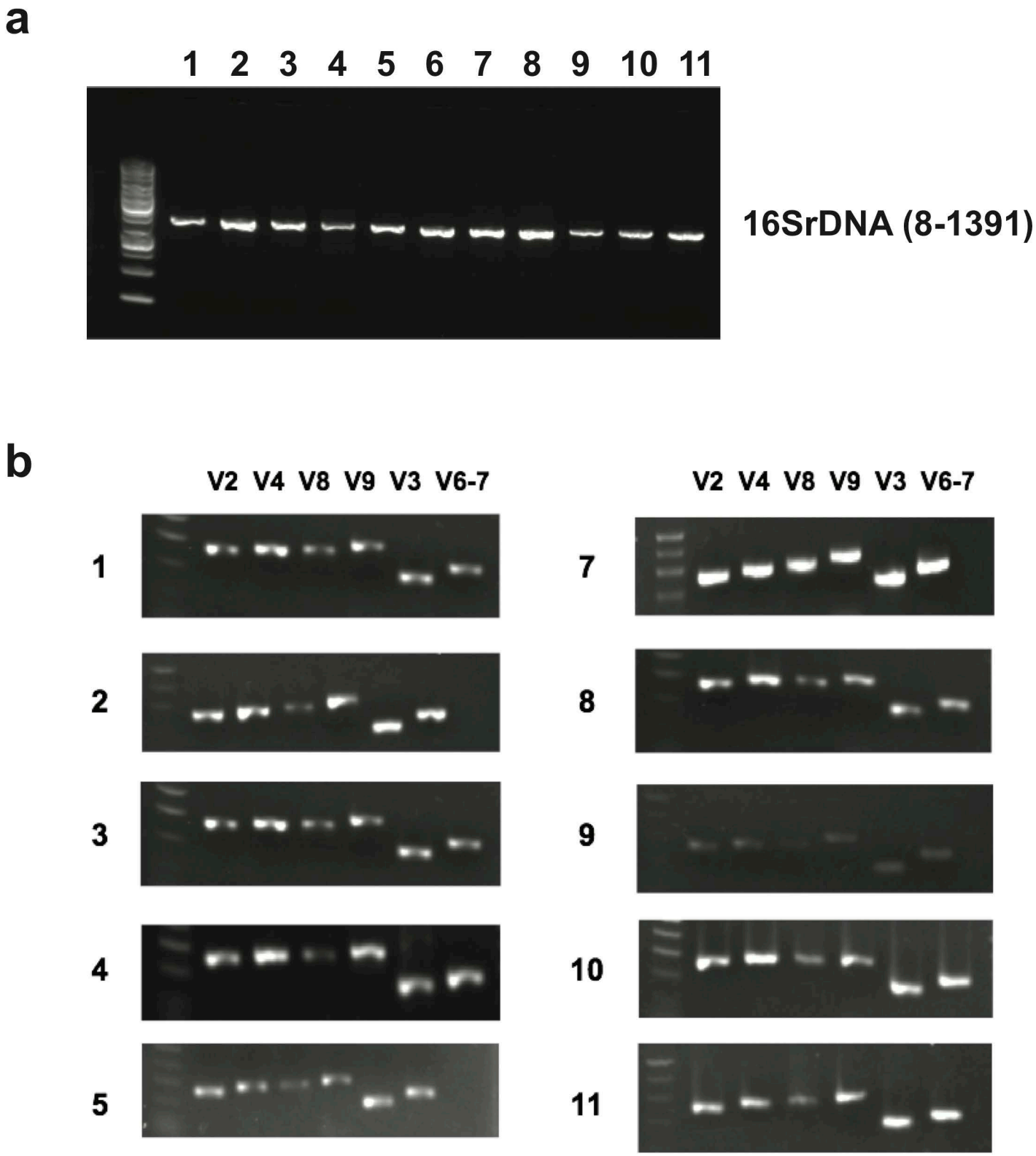
# Supplemental Figure 1



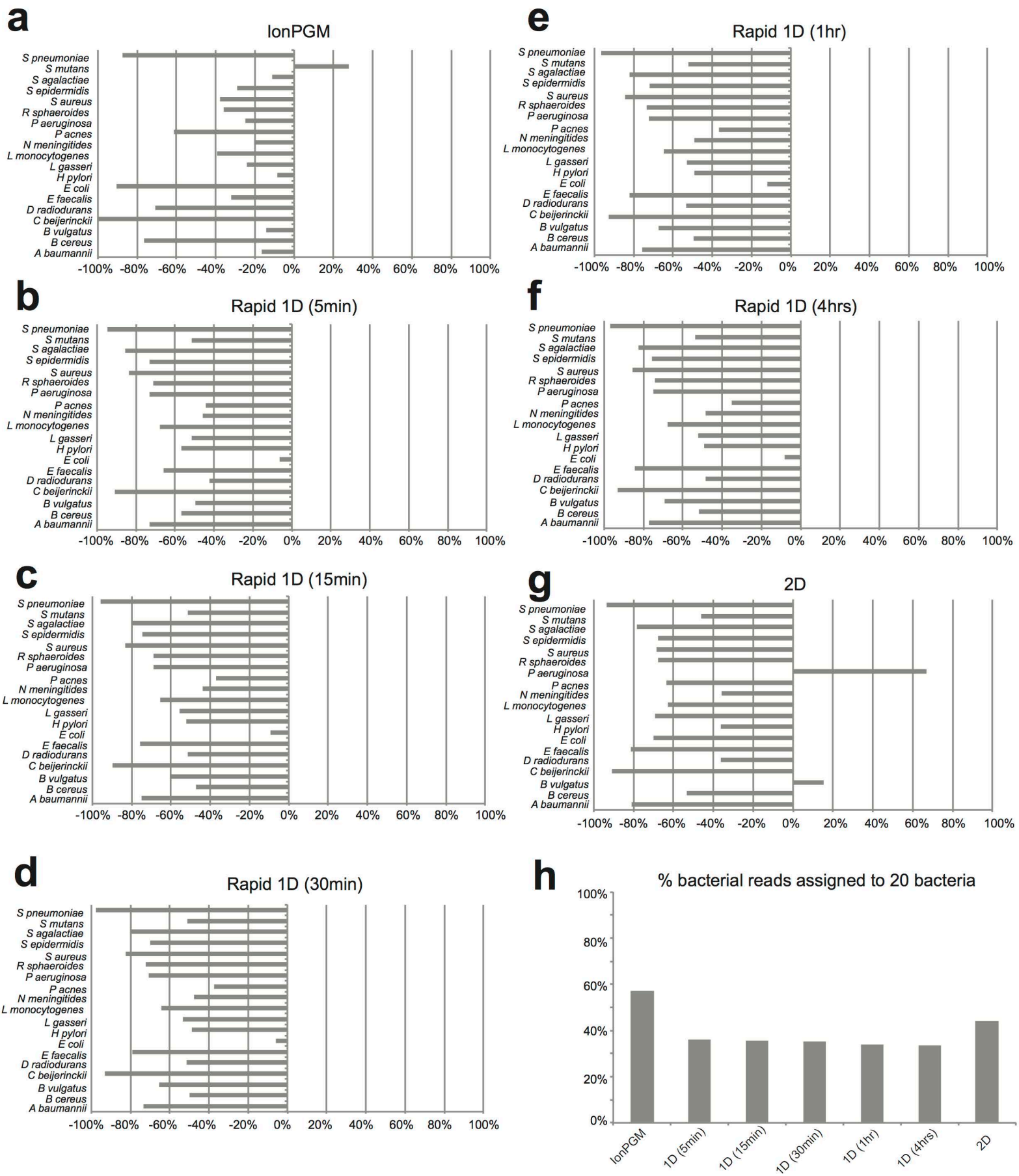
# Supplemental Figure 2



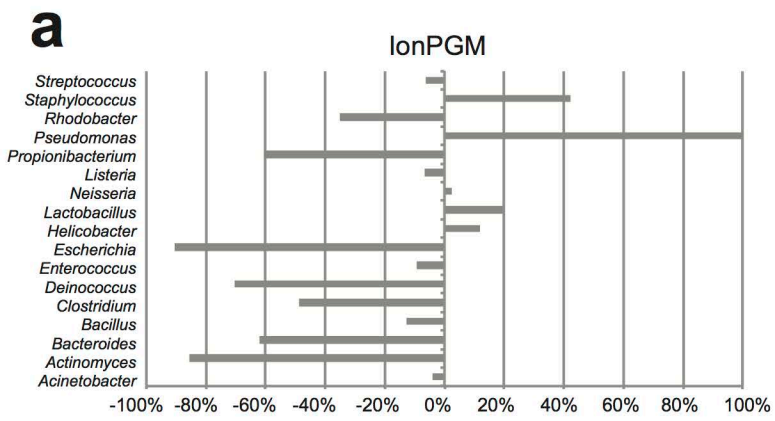
# Supplemental Figure 3

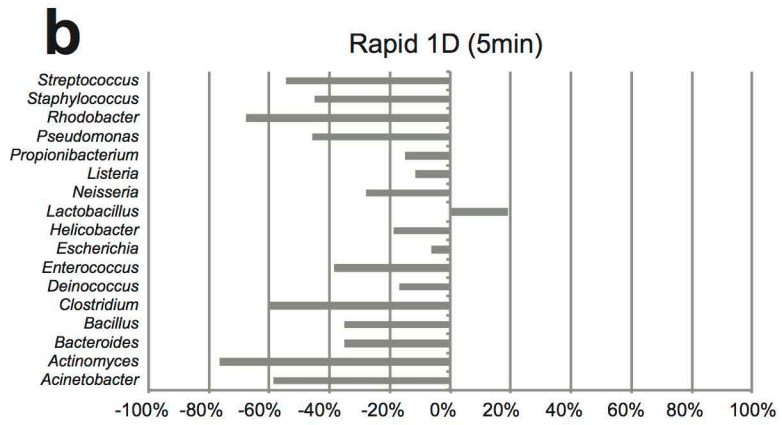


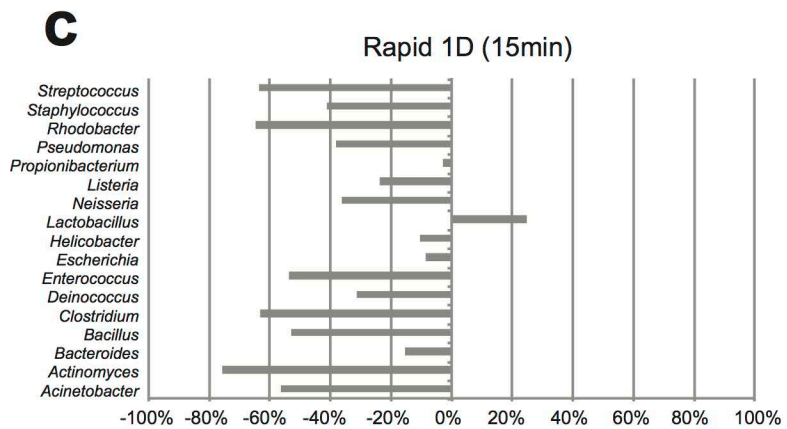
# Supplemental Figure 4

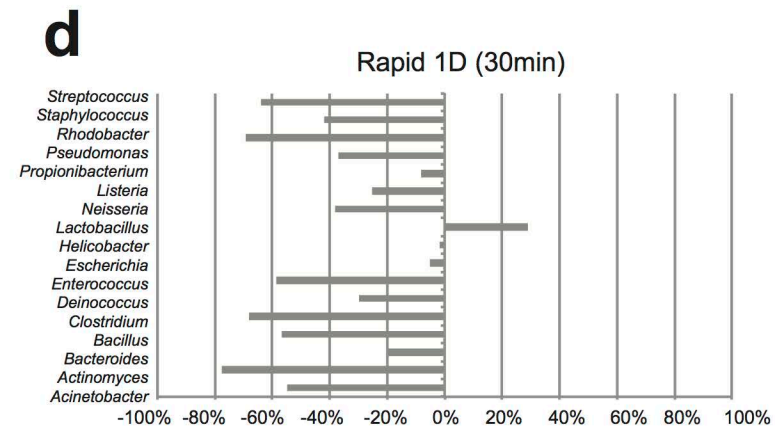


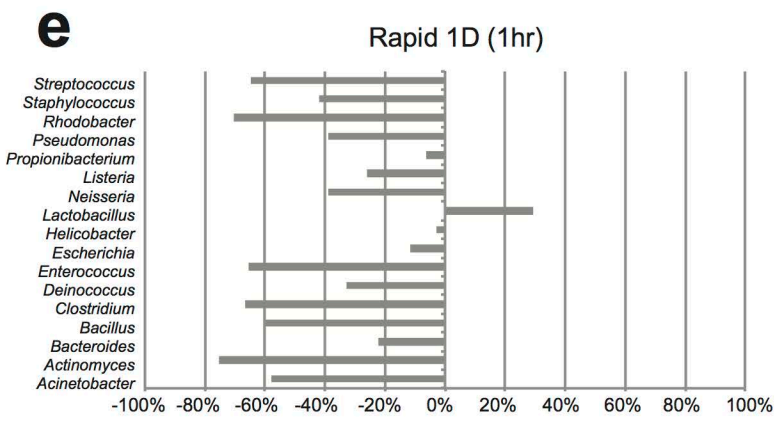
# Supplemental Figure 5

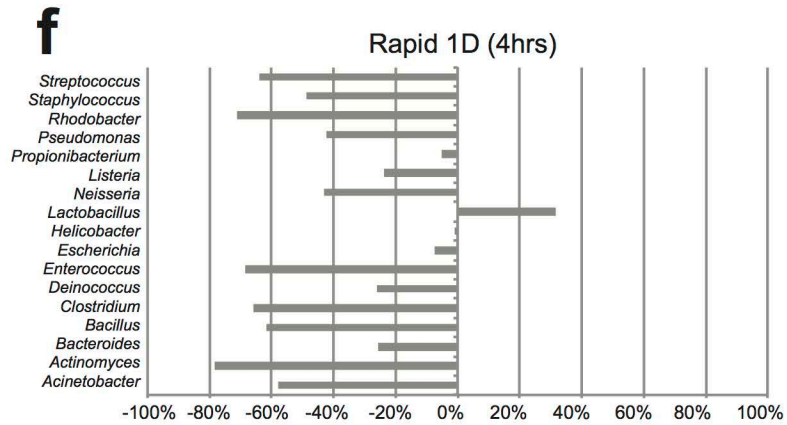


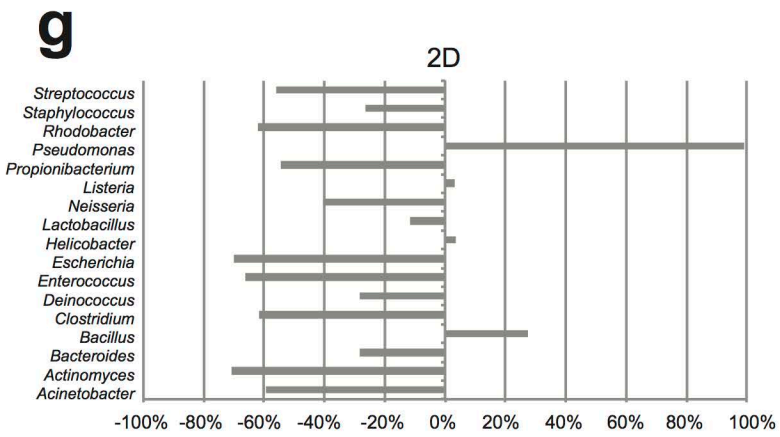



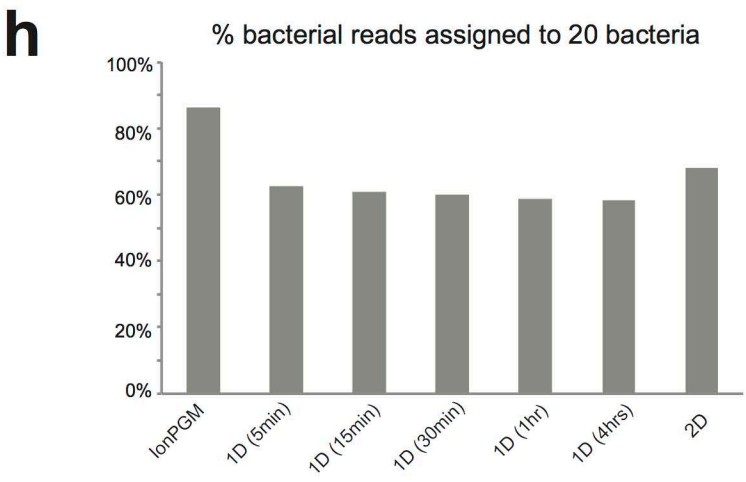



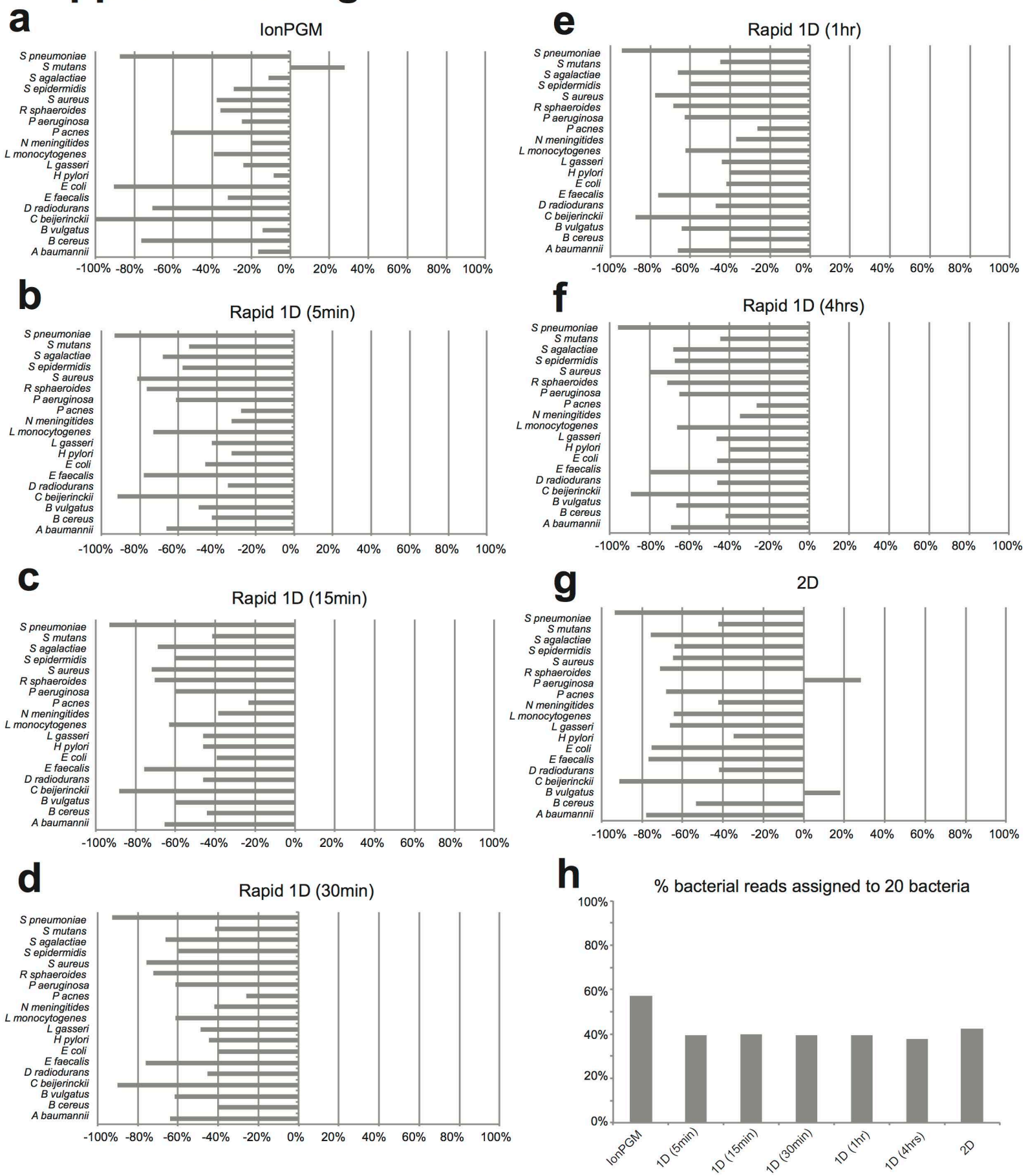
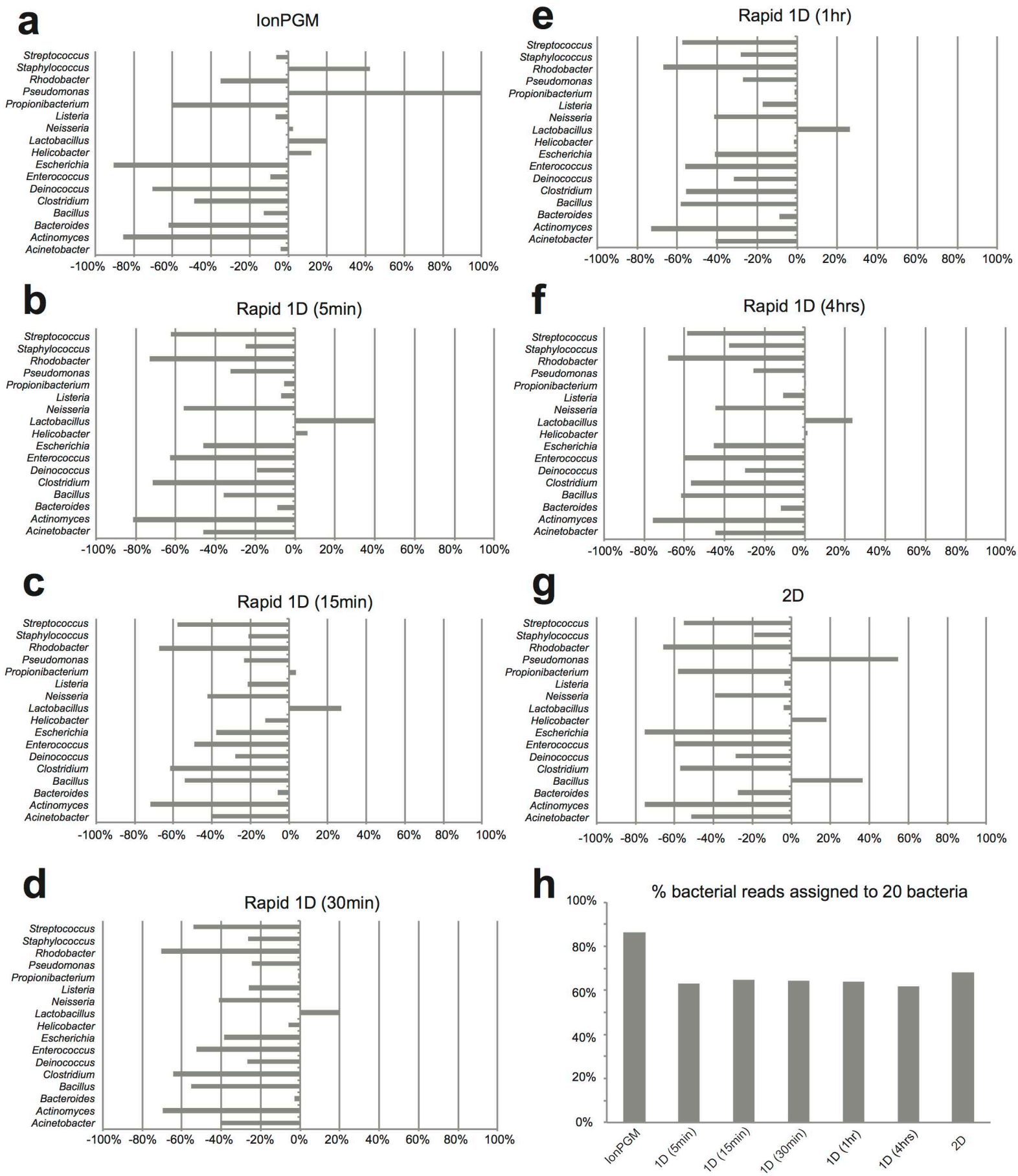



# Supplemental Figure 6



# Supplemental Figure 7