

Supplementary materials

# Rapid DNA sequencing technology based on the Sanger method for bacterial identification

Shunsuke Furutani <sup>1</sup>, Nozomi Furutani <sup>1</sup>, Yasuyuki Kawai <sup>2</sup>, Akifumi Nakayama <sup>3</sup> and Hidenori Nagai <sup>1,\*</sup>

<sup>1</sup> Advanced Photonics and Biosensing Open Innovation Laboratory (Photo-BIO OIL), National Institute of Advanced Industrial Science and Technology (AIST), 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

<sup>2</sup> Department of Emergency and Critical Care Medicine, Nara Medical University, 840 Shijo, Kashihara, Nara 634-8522, Japan

<sup>3</sup> Department of Medical Technology, School of Health Sciences, Gifu University of Medical Science, 795-1 Ichihiraga, Seki, Gifu 501-3892, Japan

\* Correspondence: hide.nagai@aist.go.jp

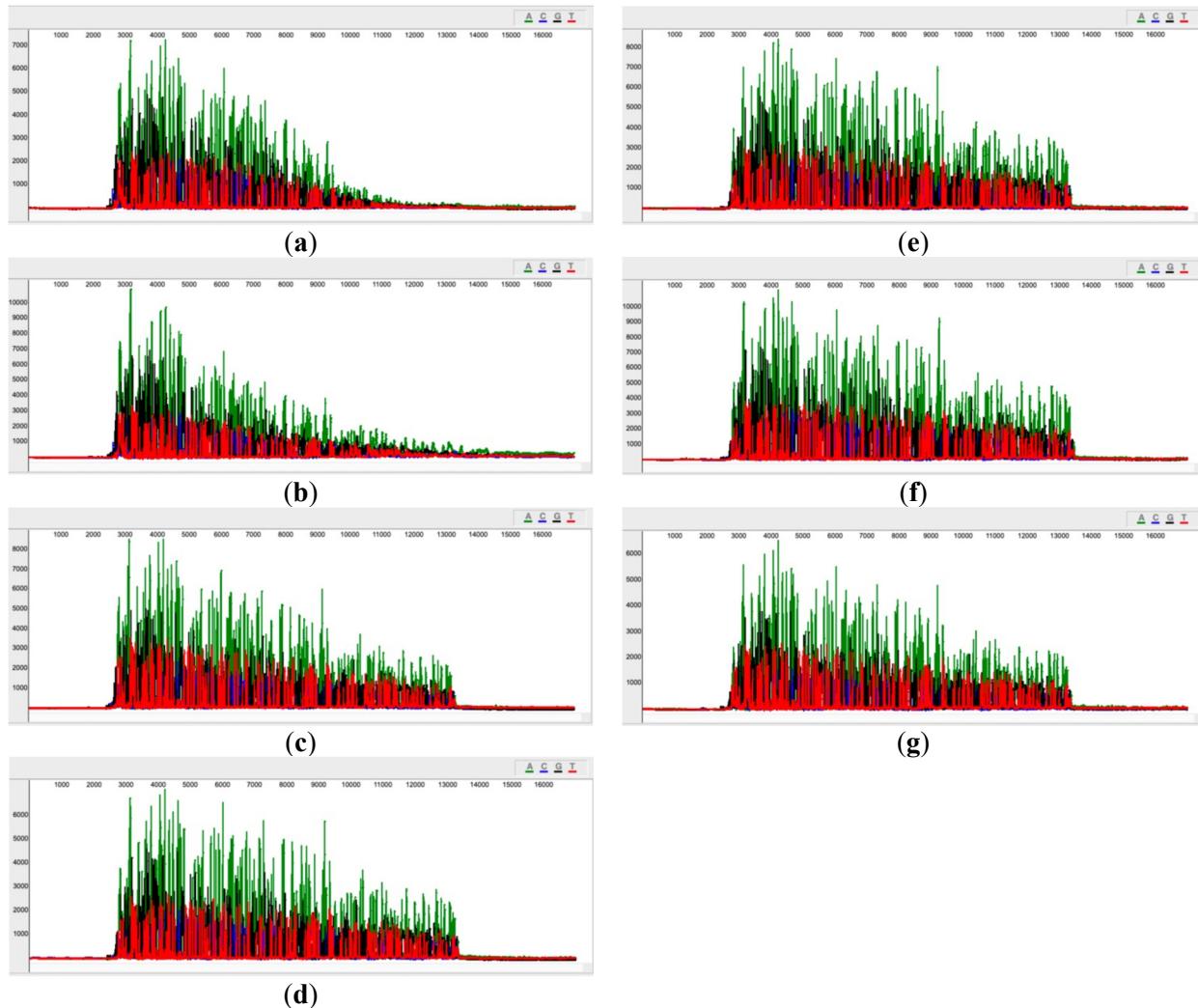


Figure S1. Raw data from a capillary DNA sequencer for different cycle sequencing reaction durations. (a) 20 s. (b) 30 s. (c) 40 s. (d) 50 s. (e) 60 s. (f) 90 s. (g) 120 s. Blue shaded portions indicate the fluorescence intensity of the end portion of the amplification product.

| Escherichia coli strain LD26-1 chromosome, complete genome |  |                             |                                      |                          |         |
|--|--|-----------------------------|--------------------------------------|--------------------------|---------|
| Sequence ID: <a href="#">CP047665.1</a>                    |  |                             | Length: 4774827 Number of Matches: 7 |                          |         |
| Range 1: 2686504 to 2686163                                |  |                             | <a href="#">GenBank</a>              | <a href="#">Graphics</a> |         |
| Score<br>905 bits(490)                                     | Expect<br>0.0  | Identities<br>542/546 (96%) | Gaps<br>15/564(2%)                   | Strand<br>Plus/Minus     |         |
| Query 1  | AGC-TGCTGCTTCCGCTGACAGTGCGGACGGGTGACTAATGCTTGAAACTGC-TGA     |                             |                                      |                          | 58      |
| Sbjct  | AGCTTGCTT-GTCCTGACGAGTGCGGACGGGTGACTAATGCTTGAAACTGC-TGA      |                             |                                      |                          | 2686105 |
| Query 59   | TGGGAGGGGGATAACTACTGAAACG-TAGCTTAATCCGCATAACGTGCGA-GAACAAAG  |                             |                                      |                          | 116     |
| Sbjct  | T-GGAGGGGGATAACTACTGAAACGGTACTAATCCGCATAACGTGCGAACGAAAC      |                             |                                      |                          | 2686046 |
| Query 117  | AGGGGGACCTTCGGGCTGCTTG-CATCGGATGTCGCCAGATGGAT-AGCTAGTAGGTG   |                             |                                      |                          | 174     |
| Sbjct  | AGGGGGACCTTCGGGCTT-CGGCATCGGATGTCGCCAGATGGATAGCTAGTAGGTG     |                             |                                      |                          | 2685987 |
| Query 175  | GGGGTAAACGGCTCACCTAGGGCGACGATCCTCGCTGTTGAGAGGATGCAACGAC      |                             |                                      |                          | 234     |
| Sbjct  | GGGGTAAACGGCTCACCTAGGGCGACGATCCTCGCTGTTGAGAGGATGCAACGAC      |                             |                                      |                          | 2685927 |
| Query 235  | TGGAACCTGAGAACCGCTCACAGCTCTTACGGGAGGCGACAGTGGGAATTATGCAACATG |                             |                                      |                          | 294     |
| Sbjct  | TGGAACCTGAGAACCGCTCACAGCTCTTACGGGAGGCGACAGTGGGAATTATGCAACATG |                             |                                      |                          | 2685867 |
| Query 295  | GGCGAACGCTGTAGCAGGCGATCGGCGTGTATGAAAGGCTTCCGGTTAAAGTAC       |                             |                                      |                          | 354     |
| Sbjct  | GGCGAACGCTGTAGCAGGCGATCGGCGTGTATGAAAGGCTTCCGGTTAAAGTAC       |                             |                                      |                          | 2685807 |
| Query 355  | TTTACGGGGAGGGAGGGAGTAAAGTTAACCTTTCGCTATTGAGCTTACCCGAGA       |                             |                                      |                          | 414     |
| Sbjct  | TTTACGGGGAGGGAGGGAGTAAAGTTAACCTTTCGCTATTGAGCTTACCCGAGA       |                             |                                      |                          | 2685748 |
| Query 415  | AAGAACGCCGGCTAACCTCGCTGGCCGGCGG-GGTAAATCCGGGAGGTTGAAACGGTTAA |                             |                                      |                          | 473     |
| Sbjct  | AAGAACGCCGGCTAACCTCGCTGGCCGGCGG-GGTAAATCCGGGAGGTTGAAACGGTTAA |                             |                                      |                          | 2685688 |
| Query 474  | CGGAAAT-ACTGGGGCTAAAGGG-ACGGCGCCGGTTGT-AAGTCAGATGGAAATCCC-G  |                             |                                      |                          | 529     |
| Sbjct  | CGGAAAT-ACTGGGGCTAAAGGG-ACGGCGCCGGTTGT-AAGTCAGATGGAAATCCC-G  |                             |                                      |                          | 2685628 |
| Query 530  | GGCTAACCTGGGAACCTGATTG 553                                   |                             |                                      |                          |         |
| Sbjct  | GGCTAACCTGGGAACCTGATTG 2685604                               |                             |                                      |                          |         |

(a)

Escherichia coli strain 2014C-3307 chromosome, complete genome

Sequence ID: [CP027368.1](#) Length: 4965987 Number of Matches: 7

| Range 1: 2606877 to 2607626 | GenBank   | Graphics     | ▼ Next Match | ▲ Previous Match |
|-----------------------------|---|--------------|--------------|------------------|
| Score                       | Expect  | Identities   | Gaps         | Strand           |
| 1349 bits(730)              | 0.0   | 740/750(99%) | 0/750(0%)    | Plus/Minus       |
| Query 3                     | AGGCCCTAACMCTCGAACCTGGAAGYGGTAAACAGGAAARMGGCTTCGCTKTTYYGCTGAGGAGT |              |              | 62               |
| Sbjct 2607626               | AGGCCCTAACATCGAACCTGGAAGCCTAACAGGAAACAGTGTGCTTCGCTGAGGAGT         |              |              | 2607567          |
| Query 63                    | GCGGCAGCCGGTGAGTTAATGCTCTGGAAACCTGCTGTAGTGGGGGGATAACTACTGGAAAC    |              |              | 122              |
| Sbjct 2607566               | GCGGCAGCCGGTGAGTTAATGCTCTGGAAACCTGCTGTAGTGGGGGGATAACTACTGGAAAC    |              |              | 2607587          |
| Query 123                   | GTTGACTGTAATACCGCCATAACGTCGCAAGAACAAAAGGGGGGACCTTCGGGCTCTTGCCT    |              |              | 182              |
| Sbjct 2607506               | GTTGACTGTAATACCGCCATAACGTCGCAAGAACAAAAGGGGGGACCTTCGGGCTCTTGCCT    |              |              | 2607447          |
| Query 183                   | TGGCATGTGCCCGAGATTGGGATTAGTCTGTWTGGTGTGGGTAACGGCTTACCCWAGGGCAGAT  |              |              | 242              |
| Sbjct 2607446               | TGGCATGTGCCCGAGATTGGGATTAGTCTGTWTGGTGTGGGTAACGGCTTACCCWAGGGCAGAT  |              |              | 2607387          |
| Query 243                   | CCCTAGCTGGCTGAGAGGATGACCGACCAACTGGAACTGAGACACGGCTCAGACTCT         |              |              | 302              |
| Sbjct 2607386               | CCCTAGCTGGCTGAGAGGATGACCGACCAACTGGAACTGAGACACGGCTCAGACTCT         |              |              | 2607327          |
| Query 303                   | ACGGGGAGCCGAGCTGGGATTATTCACAATGGGGCGGACCTGGCTGAGCACCTGGC          |              |              | 362              |
| Sbjct 2607326               | ACGGGGAGCCGAGCTGGGATTATTCACAATGGGGCGGACCTGGCTGAGCACCTGGC          |              |              | 2607267          |
| Query 363                   | GTGATAGAAGAAGGGCTTCGGGGTTGTAAGACTTTCAGCGGGGAGGAAGGGAGTAAAGT       |              |              | 422              |
| Sbjct 2607266               | GTGATAGAAGAAGGGCTTCGGGGTTGTAAGACTTTCAGCGGGGAGGAAGGGAGTAAAGT       |              |              | 2607287          |
| Query 423                   | TAATACCTTTGCTATTGGCTATCCCGAGTACCCCGAGAAGAACGCCGCTAACCTGGTGCAGCA   |              |              | 482              |
| Sbjct 2607206               | TAATACCTTTGCTATTGGCTATCCCGAGTACCCCGAGAAGAACGCCGCTAACCTGGTGCAGCA   |              |              | 2607147          |
| Query 483                   | GCCCCGGTAAATCGGGAGGTGCAACGGTTAACCGGTTACTGGGCTAAAGCGCAAGCA         |              |              | 542              |
| Sbjct 2607146               | GCCCCGGTAAATCGGGAGGTGCAACGGTTAACCGGTTACTGGGCTAAAGCGCAAGCA         |              |              | 2607087          |
| Query 543                   | GCGCGTTGTTAAGCTGAGTGTAACTGGCTTACACTGGGAACTGCTGATAC                |              |              | 602              |
| Sbjct 2607086               | GCGCGTTGTTAAGCTGAGTGTAACTGGCTTACACTGGGAACTGCTGATAC                |              |              | 2607027          |
| Query 603                   | TGGCAAGCTTGGAGTCTCGTAGAGGGGGGTTAGATTCCAGGTTGAGGGTAAATGGTAG        |              |              | 662              |
| Sbjct 2607026               | TGGCAAGCTTGGAGTCTCGTAGAGGGGGTTAGATTCCAGGTTGAGGGTAAATGGTAG         |              |              | 2606967          |
| Query 663                   | AGATCTGGAGGAATACCGTGGCAAGGCGGCCCTTGAGCAGAACGACTGAGCTCAGGTG        |              |              | 722              |
| Sbjct 2606966               | AGATCTGGAGGAATACCGTGGCAAGGCGGCCCTTGAGCAGAACGACTGAGCTCAGGTG        |              |              | 2606907          |
| Query 723                   | CAGAACGGCTGGGAGAACCAAGGGATTAGATA                                  | 752          |              |                  |
| Sbjct 2606906               | CAGAACGGCTGGGAGAACCAAGGGATTAGATA                                  | 752          |              | 2606877          |

(b)

| Mycoplasma pneumoniae strain 16-734 chromosome, complete genome |  |                            |                         |                      |                          |
|---|--|----------------------------|-------------------------|----------------------|--------------------------|
| Sequence ID: CP039761.1   |  | Length: 818445             |                         | Number of Matches: 1 |                          |
| Range 1: 118272 to 118814                                       |  |                            | <a href="#">GenBank</a> |                      | <a href="#">Graphics</a> |
| Score<br>761 bits(412)  | Expect<br>0.0  | Identities<br>513/555(92%) | Gaps<br>34/555(6%)      | Strand<br>Plus/Plus  |                          |
| Query 1   | ATACATTCTGGGAGGAACCGGTGGAGTAAACAGTTATCCCAACTTACCTTATAATGGG     |                            |                         |                      | 60                       |
| Sbjct 118272  | ATAC-TTATAG-GCG-AACGGT-GGTAACAGC-TAT-CCAACTAACCTTATAATGGG      |                            |                         |                      | 118325                   |
| Query 61  | GGGATACTAG-TGAAAAGACTAGCTAATACCGCATAGAACCTTTGGTCGCATGAACTAT    |                            |                         |                      | 119                      |
| Sbjct 118326  | GG-ATTAATCAGTTGAAAGACTAGCTAATACCGCATAGAACCTTTGGTCGCATGAACTA-   |                            |                         |                      | 118383                   |
| Query 120   | AAGTTGAAAGGACTCTGGAAAGGGTTCTGTTATTTGATGAGGGTGGCCATATCAGCTAGTTG |                            |                         |                      | 179                      |
| Sbjct 118384  | AAGT-GAAAGGACTCTGGAAAGGGTTCGTTATTTGATGAGGGTGGCCATATCAGCTAGTTG  |                            |                         |                      | 118443                   |
| Query 180   | GTGGGGGTAACGCCCTACCAAGGCAATGAGCTGTAGCTATGCTGAGAAGTAGAATAGCCA   |                            |                         |                      | 239                      |
| Sbjct 118444  | GT-GGGGTAAACGCCCTACCAAGGCAATGAGCTGTAGCTATGCTGAGAAGTAGAATAGCCA  |                            |                         |                      | 118502                   |
| Query 240   | CAATGGGACTGAGACACGG-CCATACTCTACGGGGAGGCCAGCACT-GGGAAATTTCACA   |                            |                         |                      | 297                      |
| Sbjct 118503  | CAATGGGACTGAGACACGG-CCATACTCTACGGGGAGGCCAGCACT-GGGAAATTTCACA   |                            |                         |                      | 118562                   |
| Query 298   | ATAGCGGAAAGCTTATGGAGCAATGGCGGCTGAAAGCATGTTGCTTAAAGGTG-AA       |                            |                         |                      | 356                      |
| Sbjct 118563  | ATAGCGGAAAGCTTATGGAGCAATGGCGGCTGAAAGCATGTTGCTTAAAGGTG-AA       |                            |                         |                      | 118622                   |
| Query 357   | GT-C-TTTATTGCGGAAAGATGCTTACCGGGTAAATGCGTAGGGTTGACTGTCCC        |                            |                         |                      | 414                      |
| Sbjct 118623  | GTCTTATTGCGGAAAGATGCTTACCGGGTAAATGCGTAGGGTTGACTGTCCC           |                            |                         |                      | 118679                   |
| Query 415   | ATTTTGAAATA-TGCACTAACTATGGGCAGCAGCTGGGGTAA-CATAG-TCG-AAG       |                            |                         |                      | 469                      |
| Sbjct 118680  | ATTTTGAAATAAGTGCACTAACTATGGGCAGCAGCTGGGGTAAATACATAGGTGCAAAG    |                            |                         |                      | 118739                   |
| Query 470   | C-T-ATCCGG-TTATGGCGGCTAACGCA-GC-CAG-CGG-TTGAAG-GTCTGG-GTTAA    |                            |                         |                      | 520                      |
| Sbjct 118740  | CTTATGCGGATTTATGGCGGCTAACGCA-GC-CAG-CGG-TTGAAG-GTCTGG-GTTAA    |                            |                         |                      | 118799                   |
| Query 521   | --GCAGCTTCTTAAAC 533   |                            |                         |                      |                          |
| Sbjct 118800  | AGGCAGCTCTTAAAC 118814   |                            |                         |                      |                          |

(c)

Streptococcus pneumoniae strain 4559 genome assembly, chromosome: 1

Sequence ID: [LR595848.1](#) Length: 2150981 Number of Matches: 4

(d)

Figure S2. Alignment results of an NCBI BLAST search. (a) *E. coli* by microchip electrophoresis. (b) *E. coli* by capillary DNA sequencer. (c) *M. pneumoniae* by microchip electrophoresis. (d) *S. pneumoniae* by microchip electrophoresis.