

*FANSe2*: a robust and cost-efficient alignment tool for quantitative next-generation sequencing applications

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## Supplementary Materials

**Table S1:** Six datasets downloaded from DDBJ Sequence Read Archive to analyze the error distribution.

| Accession Number | Sample type                | Sequencer                         | Errors allowed when mapped with FANSe |
|------------------|----------------------------|-----------------------------------|---------------------------------------|
| ERR039477        | <i>E. coli</i> genomic DNA | Life Technologies Ion Torrent PGM | 9                                     |
| SRR360459        | <i>E. coli</i> genomic DNA | Illumina GAIIx                    | 9                                     |
| SRR057661        | <i>E. coli</i> genomic DNA | Roche 454 GS FLX                  | 8                                     |
| SRR028774        | Yeast miRNA                | Illumina GAIIx                    | 3                                     |
| SRR363968        | Yeast mRNA                 | Illumina GAIIx                    | 4                                     |
| SRR034447        | Yeast mRNA                 | Helicos Heliscope                 | 4                                     |

**Table S2:** The gene-specific PCR primers for validation of gene identifications. The genes identified solely by Bowtie2 are shaded as gray, and the genes identified solely by FANSe2 are not shaded.

| Gene name      | Whole Transcriptome qPCR Primers database ID | Forward primer (5'-3')  | Reverse primer (5'-3')   | Expected product size (nt) |
|----------------|--|-------------------------|--------------------------|----------------------------|
| RPL36A-HNRNPH2 | RPL36A-HNRNPH2_uc022cag.1_3_1_2              | AGCATTTTGAAGTGGGAGGAG   | ATCATGGTGATTGTTGGGCT     | 93                         |
| RPS10-NUDT3    | PB *   | ATGCCTAAGAAGAACCGGATTGC | GCTGCCACCACATGCCTTATT    | 993                        |
| BCL2L2-PABPN1  | BCL2L2-PABPN1_uc001wjh.4_2_2_1               | GGACAAGTGCAGGAGTGGAT    | TCTCCCTGACTCGAGCTTTG     | 109                        |
| HSPE1-MOB4     | HSPE1-MOB4_uc021vum.1_1_1_2                  | ATTGCAAGCAACAGTAGTCGC   | CAAAGGATTCATCAGGCCAA     | 77                         |
| COMMD3-BMI1    | COMMD3-BMI1_uc009xkg.3_4_2_2                 | GTTGCAGCATGGAACAATTACA  | ACACACATCAGGTGGGGATT     | 98                         |
| DNAJC25-GNG10  | PB *   | CGCGGGACTGCTACGAG       | ACGATGGGGCAGCCTAATG      | 979                        |
| URGCP-MRPS24   | PB *   | TTGGGAGAAGTAGCCCCAGAA   | GTGGAGTCGCACAGGACATT     | 574                        |
| RBM14-RBM4     | PB *   | GGCTGCGGCGACAAAATGAA    | ACACACATCCCACCTCAAGC     | 1056                       |
| RGPD6          | PB *   | TCCTGCATAGATCTCGTCCTG   | TGTATCCTCGGCGACGTC       | 315                        |
| C7orf55-LUC7L2 | PB *   | AGTCGGTAGTCTGTCCGAC     | CTTGCGTTACGTGACATG       | 1169                       |
| SYNJ2BP-COX16  | SYNJ2BP-COX16_uc021rv2m.1_2_1_1              | TCGTAATGCAGGCTATGCTGT   | CTCTTCACAGCATCATATCGGA   | 111                        |
| PCDHGB3        | PCDHGB3_uc003ljw.2_1_2_2                     | ACGGACTGGCGTTTCTCTC     | GGCTTGCAGCATCTCTGTGT     | 111                        |
| SPIN2A         | PB *   | GCAGAAAAAGGGCAGAGACTAC  | CTCGCTGGCCGTCTACCTC      | 103                        |
| LOC647859      | OCLN_uc011cru.1_2_1_2                        | ACAACTGGTGGCGAGTCCT     | TTGTTGATCTGAAGTGATAGGTGG | 78                         |
| PNMA6A         | PB *   | CGAGTCCCAGGCGACC        | CGAAAGCTGAGAGACGCTC      | 74                         |
| PPIAL4F        | PB *   | GGTGACTTCACACGCCCTAA    | TGCCATCCAACCACTCAGTC     | 181                        |

\* PB: primer pair not available in whole transcriptome qPCR primer database. The primers are automatically designed using NCBI-PrimerBLAST.

**Table S3:** Mapping programs tested in this study.

| Mapping tool | Type * | Version     | Operating system            | Citation   |
|--------------|--------|-------------|-----------------------------|------------|
| FANSe2       | Seed   | 2.0         | Windows 7 64-bit            |            |
| FANSe        | Seed   | 1.7.2       | Windows 7 64-bit            | [1]        |
| Bowtie       | BWT    | 0.12.8      | Windows 7 64-bit            | [2]        |
| Bowtie2      | BWT    | 2.0.0-beta7 | Ubuntu 12.04 64-bit (Linux) | [3]        |
| BWA          | BWT    | 0.6.2       | Ubuntu 12.04 64-bit (Linux) | [4]        |
| SHRiMP2      | Seed   | 2.2.3       | Ubuntu 12.04 64-bit (Linux) | [5]        |
| Novoalign    | Seed   | 3.02.02     | Ubuntu 12.04 64-bit (Linux) | Commercial |

\* Seed = seed-based algorithm; BWT = Burrows-Wheeler Transform-based algorithm.

**Table S4:** Test parameters for Figure 4.

| Mapping tool | Parameter        | Meaning  |
|--------------|------------------|--|
| Bowtie2      | --very-sensitive | Recommended most sensitive parameter set   |
| Bowtie       | -n 3             | The largest mismatch allowed in the mapping to maximize the sensitivity                                  |
| BWA          | -n 5 and -n 7    | Allowing 5 or 7 mismatches, comparable to FANSe2 setting.  |
| SHRiMP2      | -h 91%           | For 75-nt reads, 91% homology is approximately equivalent to 7 mismatches, comparable to FANSe2 setting. |
| Novoalign    | default          |  |
| FANSe2       | -E5, -E6 and -E7 | Allowing 5~7 mismatches  |

### Supplementary References

1. Zhang G, Fedyunin I, Kirchner S, Xiao C, Valleriani A, et al. (2012) FANSe: an accurate algorithm for quantitative mapping of large scale sequencing reads. *Nucleic Acids Res* 40: e83.
2. Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10: R25.
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4. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25: 1754-1760.
5. David M, Dzamba M, Lister D, Ilie L, Brudno M (2011) SHRiMP2: sensitive yet practical SHort Read Mapping. *Bioinformatics* 27: 1011-1012.