# NPBSS: A new PacBio sequencing simulator for generating the continuous long reads with an empirical model

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## Section 1. NPBSS Methods

### Modeling the length distribution

Suppose that variable $x$ indicates the read length, its probability density function can be expressed as [1]:

$$p(x;\mu,\sigma)=\frac{1}{x\sigma\sqrt{2\pi}}\exp\left[-\frac{(In\,x-\mu)^2}{2\sigma^2}\right] \tag{1}$$

where $\mu$ and $\sigma$ are the mean value and standard deviation of the variable $x$ natural logarithm. If we use $E(x)$ and $Var(x)$ to respectively represent the mean value and variance of the read length derived from Fig.S1-S2, the parameters $\mu$ and $\sigma$ can be estimated by the following equations.

$$\begin{cases} E(x)=\exp\left(\mu+\dfrac{\sigma^2}{2}\right) \\ Var(x)=\left[\exp(\sigma^2)-1\right]\exp(2\mu+\sigma^2) \end{cases} \tag{2}$$

Thus, according to observed distributions of read length, the logarithmic normal distribution (Eq.1) can be used to model the length of CLR reads. Or, after the users provide the parameters of average read length and variance, NPBSS simulates the distribution of read length by using the Eq.1.

### Deletion, substitution and insertion errors assignment

After getting the overall error probability ($P_{error}$) of each base in read sequence, the deletion, substitution and insertion probabilities can be calculated by the following equations:

$$P_{del} = P_{error} \times \frac{R_{del}}{R_{del} + R_{ins} + R_{sub}} \quad (3)$$

$$P_{ins} = P_{error} \times \frac{R_{ins}}{R_{del} + R_{ins} + R_{sub}} \quad (4)$$

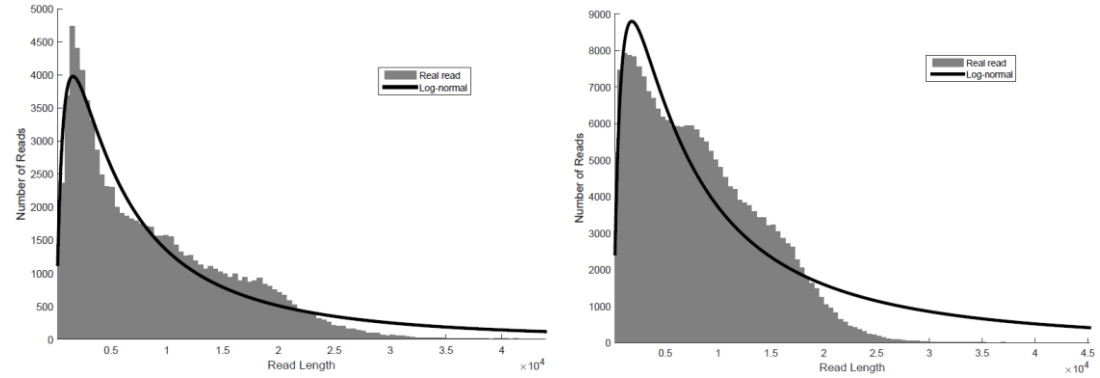
$$P_{sub} = P_{error} \times \frac{R_{sub}}{R_{del} + R_{ins} + R_{sub}} \quad (5)$$

where $P_{del}$, $P_{ins}$ and $P_{sub}$ are the probabilities of deletion, insertion and substitution of base in read sequence, respectively. $R_{del}$, $R_{ins}$ and $R_{sub}$ are the average accuracy over the length of each read, which can be taken from a normal distribution with parameters given by user, that is: $R_{del} \sim N(\mu_{del}, \sigma_{del})$, $R_{ins} \sim N(\mu_{ins}, \sigma_{ins})$ and $R_{sub} \sim N(\mu_{sub}, \sigma_{sub})$. The mean values and standard deviation also can be changed by users, which is more flexible to simulate.

**NPBSS for CCS generation**

For PacBio CCS reads simulation, NPBSS adopted a sampling-based simulation. In the CCS simulation, lengths and quality scores of reads are simulated by randomly sampling them in a real library of PacBio CCS reads (provided by the user). Subsequently, different errors of each base in read sequences are simulated by the same method with the CLR model-based simulation.

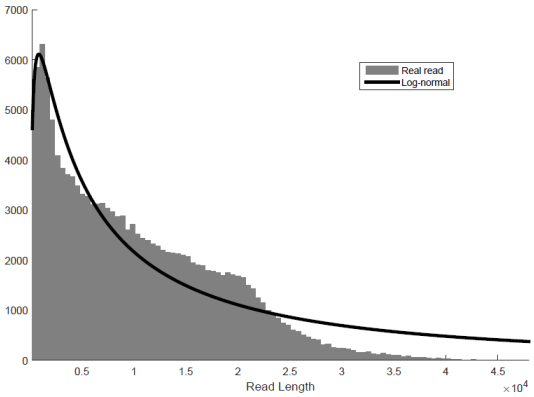
# Section 2. Figures and Tables



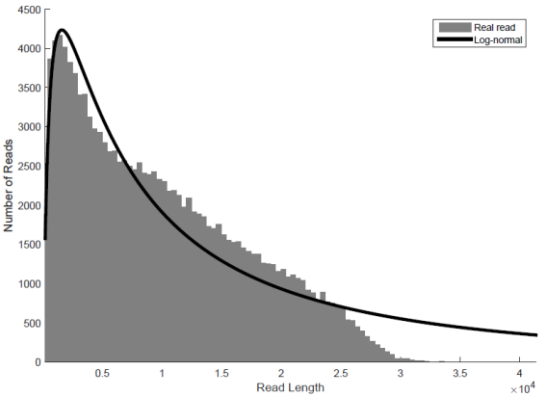

(a) *E. coli K12*



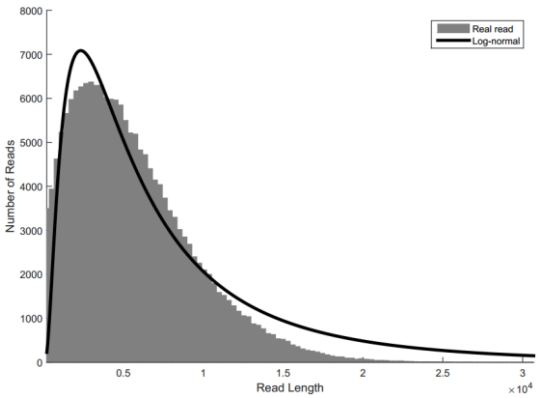

(b) *A. thaliana*




(c) *C. elegans*

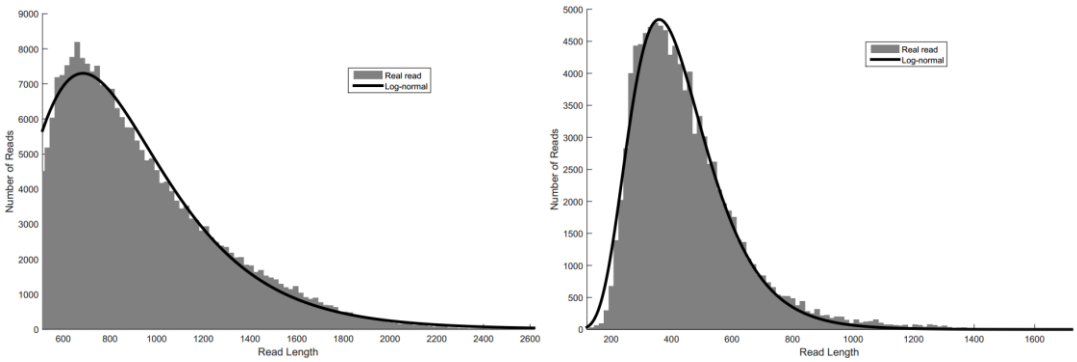



(d) *D. melanogaster*



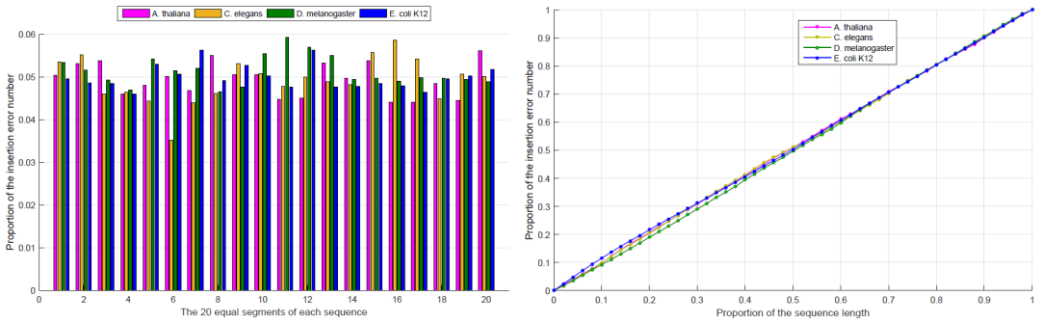

(e) *N. crassa*

**Fig. S1** Distributions of CLR lengths of four datasets generated by PacBio RS platform. The column diagram and black lines indicate the distribution of lengths of real reads and a log-normal distribution, respectively.



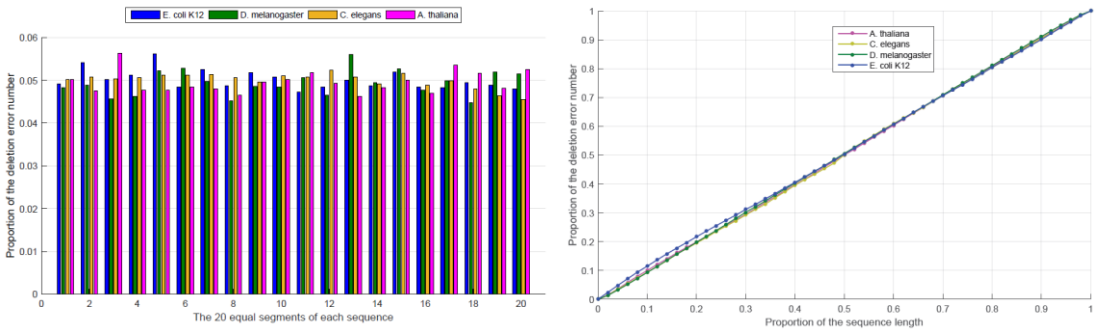

(a) *E. coli* K12 MG1655 (b) *E. coli* C227-11

**Fig. S2** Distributions of CCS lengths of two datasets generated by PacBio platform. The column diagram and black lines indicate the distribution of lengths of real reads and a log-normal distribution, respectively.



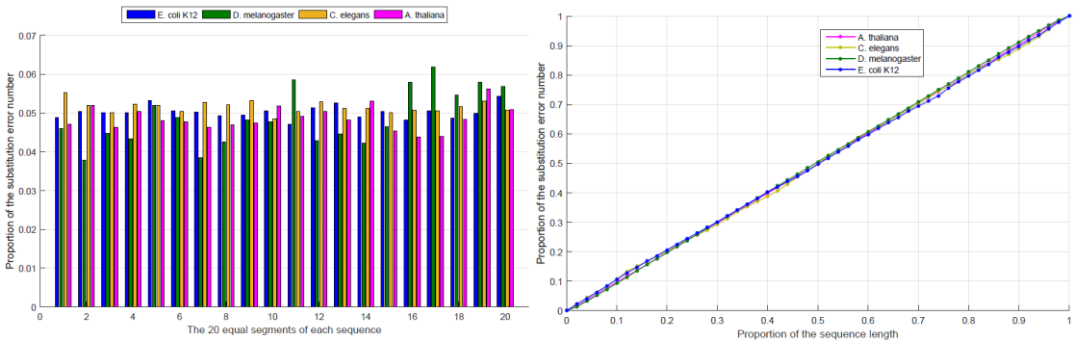

(a) (b)

**Fig. S3** Insertion error distribution. (a) The distribution of insertion errors in the equally divided sequence segments intervals. (b) The corresponding cumulative curves between the sequence length and number of errors.




(a) (b)

**Fig. S4** Deletion error distribution. (a) The average distribution of deletion errors in the equal divided sequence segments intervals. (b) The corresponding cumulative curves between the sequence length and number of errors.




(a) (b)

**Fig. S5** Substitution error distribution. (a) The average distribution of substitution errors in the equal divided sequence segments intervals. (b) The corresponding cumulative curves between the sequence length and number of errors.

**Remarks for Fig. S3-S5**

- Because the length of PacBio reads varies greatly (from hundreds bp to thousands bp), we equally divided one aligned sequence into 20 fragments, then separately counted the number of errors of insertion, deletion and substitution in each fragment. Thus, the proportion of error number can be defined as that the error number in each segment is divided by the total number (the sum number of errors in all fragments) of the whole sequence.
- It is obvious that the error distribution of insertion, deletion and substitution present us a nice uniform layout, therefore, the errors of PacBio differ with the second-generation sequencing systems that are concentrated around certain segments (e.g., errors increase in distal segment and do not occur randomly) [2]. The PacBio sequencing errors occur randomly in one sequence of the PacBio sequencing system.

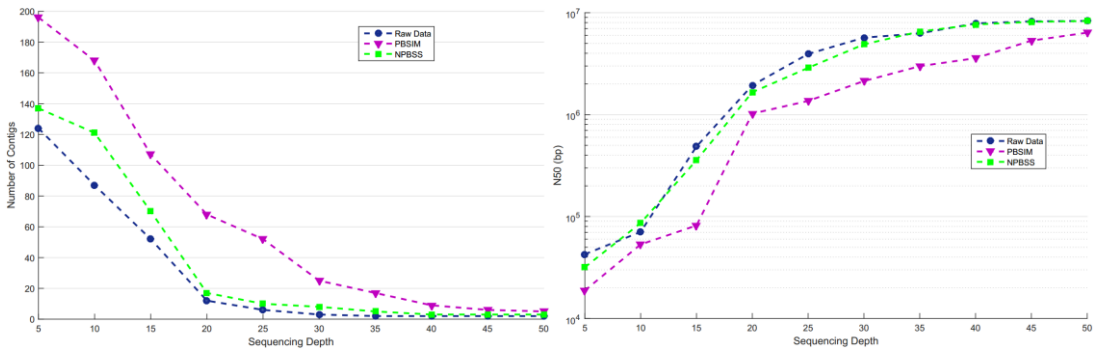



**Fig. S6** The numbers (left figure) and N50 (right figure) of contigs in the assembly test for *A. thaliana* data.

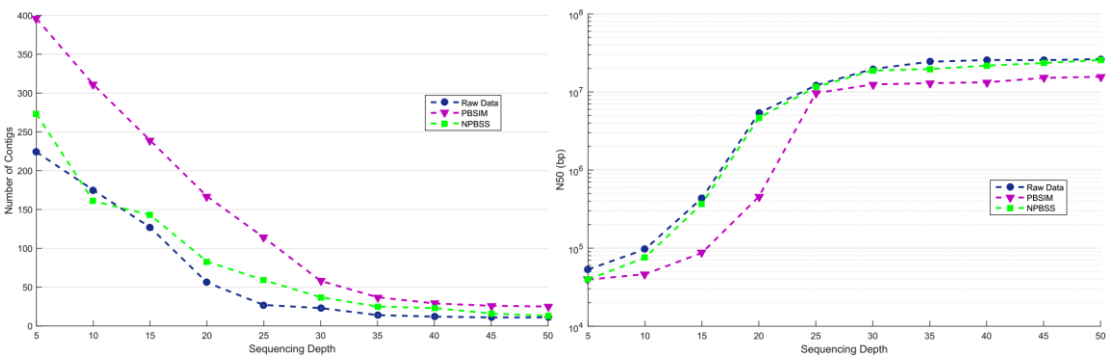



**Fig. S7** The numbers (left figure) and N50 (right figure) of contigs in the assembly test for *D. melanogaster* data.

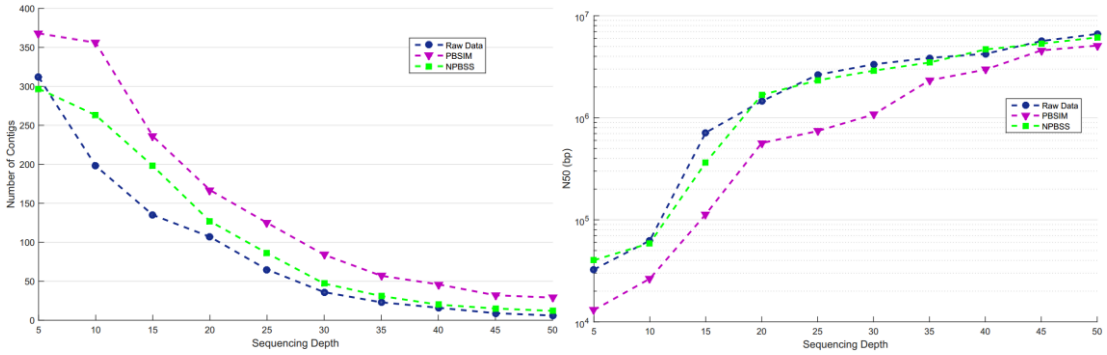



**Fig. S8** The numbers (left figure) and N50 (right figure) of contigs in the assembly test for *C. elegans* data.

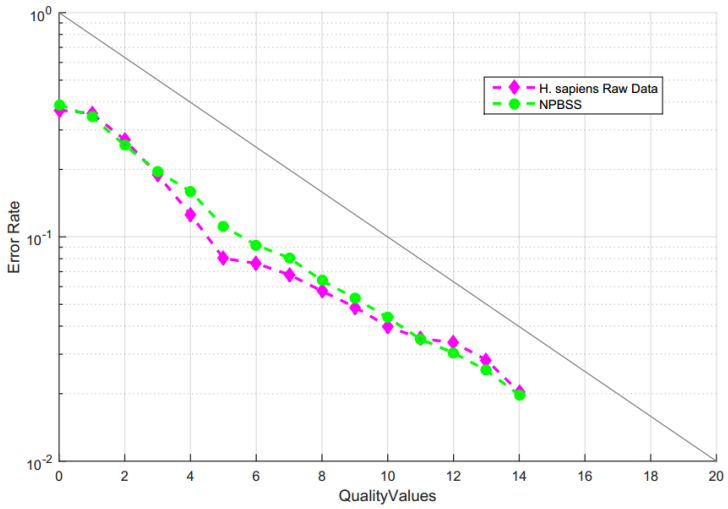
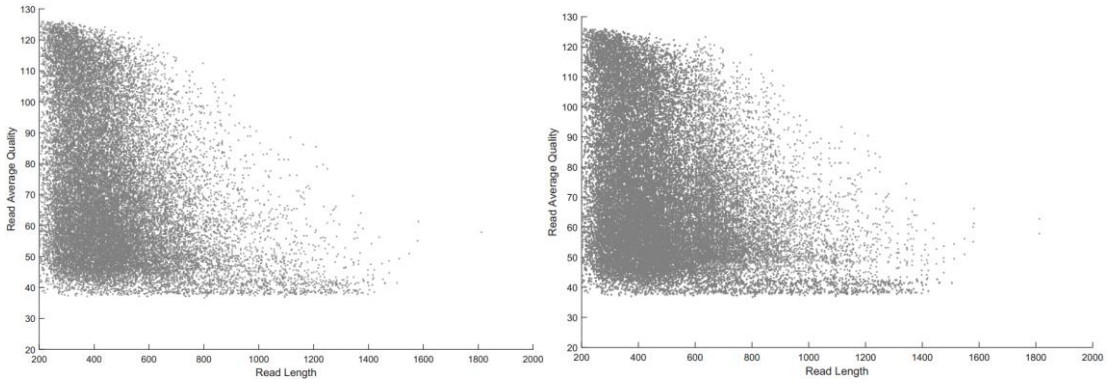
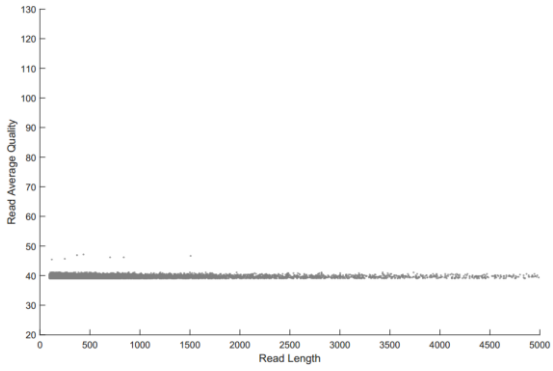


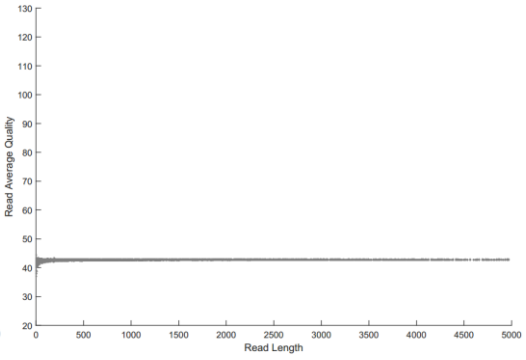

**Fig. S9** The relationship between error rate and QVs in simulated CLR reads generated by NPBSS for *H. sapiens* genome.



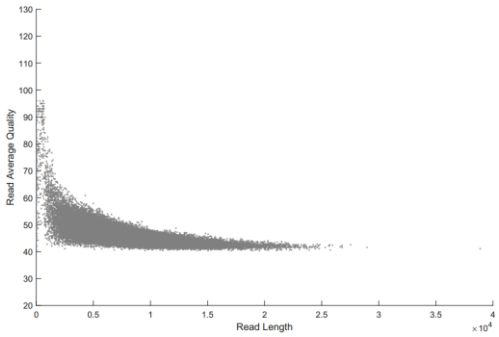

(a) Raw CCS data (b) CCS read dataset generated by NPBSS




(c) CCS read dataset generated by PBSIM

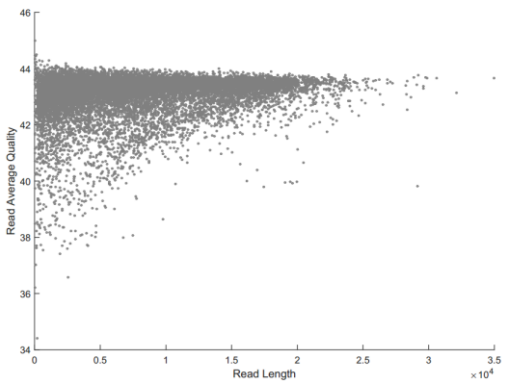



(d) CCS read dataset generated by FASTQSim

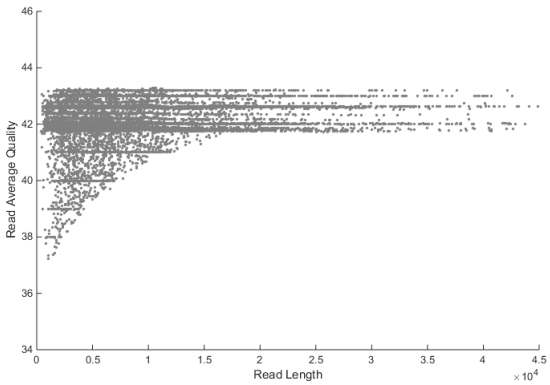



(e) CCS read dataset generated by SimLoRD

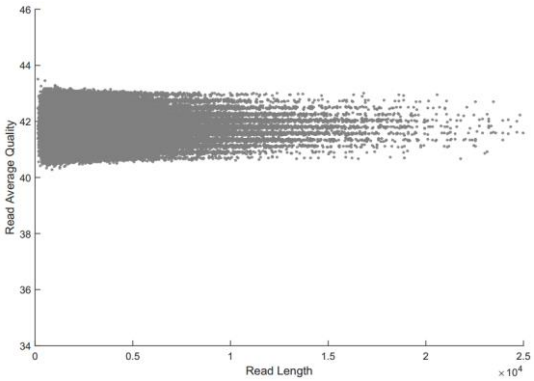
**Fig. S10** The scatter plot of read length and average base quality per read for CCS reads: (a) the raw CCS data of *E. coli* C227-11; (b) NPBSS simulation; (c) PBSIM simulation; (d) FASTQSim simulation; and (e) SimLoRD simulation.

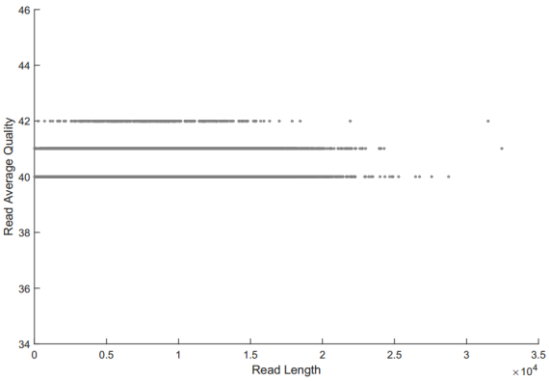



(a) CLR raw data

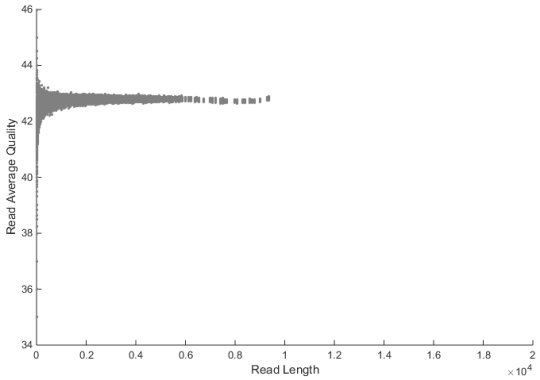



(b) CLR read dataset generated by NPBSS



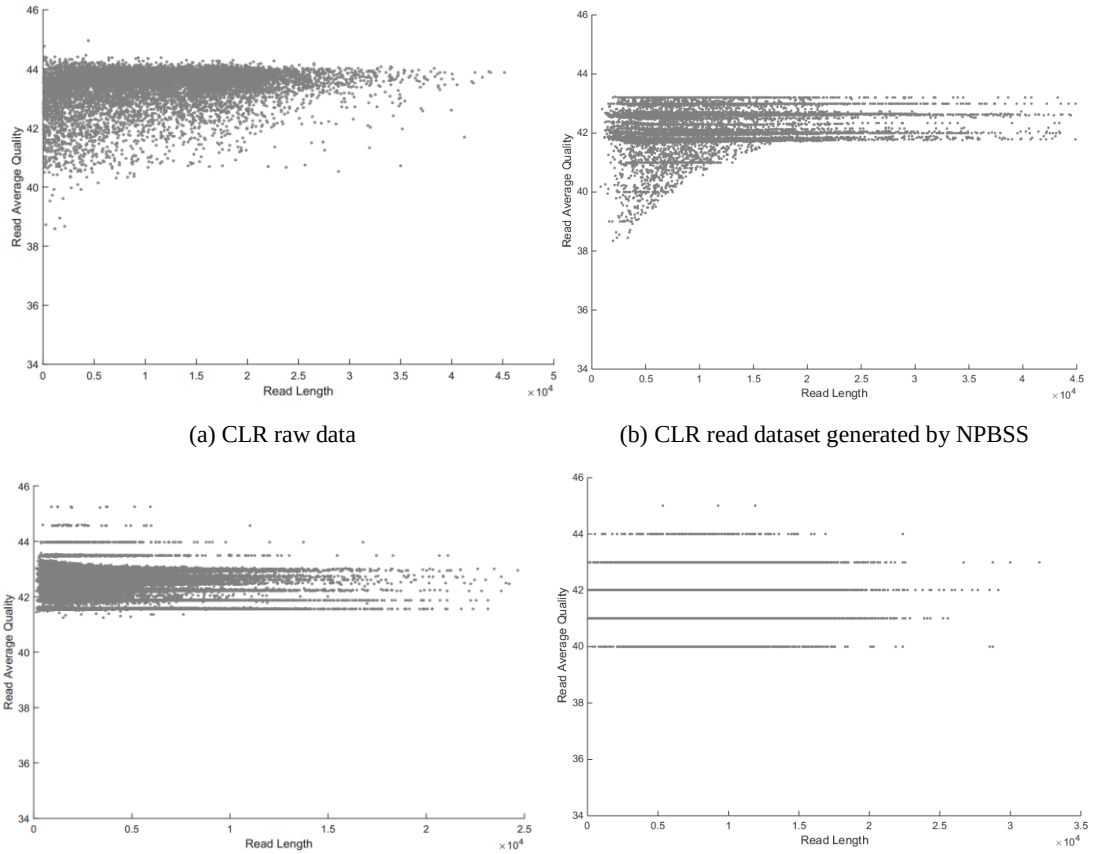



(c) CLR read dataset generated by PBSIM (d) CLR read dataset generated by SimLoRD



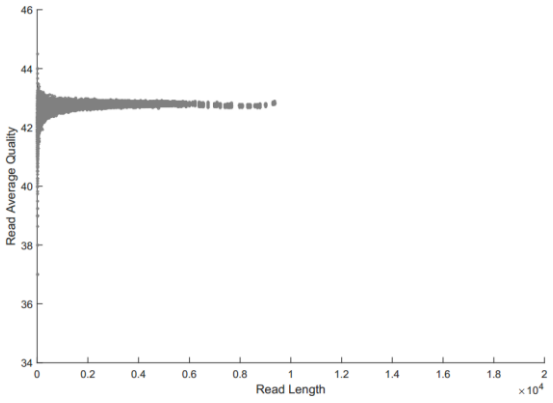

(e) CLR read dataset generated by FASTQSim

**Fig. S11** The scatter plot of read length and average base quality per read for CLR reads of *A. thaliana*: (a) the raw CLR data of *A. thaliana*; (b) NPBSS simulation; (c) PBSIM simulation; (d) SimLoRD simulation; and (e) FASTQSim simulation.



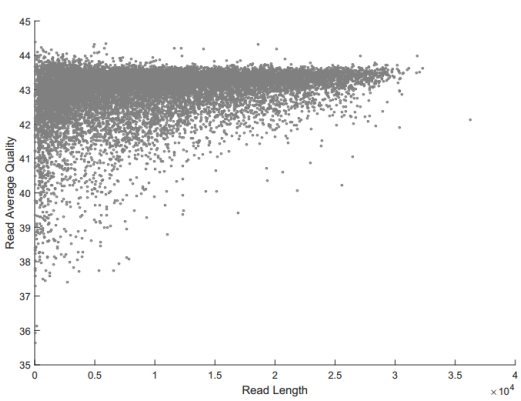

(a) CLR raw data (b) CLR read dataset generated by NPBSS

(c) CLR read dataset generated by PBSIM (d) CLR read dataset generated by SimLoRD

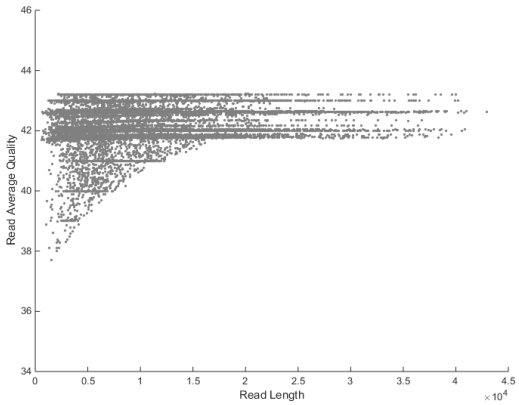



(e) CLR read dataset generated by FASTQSim

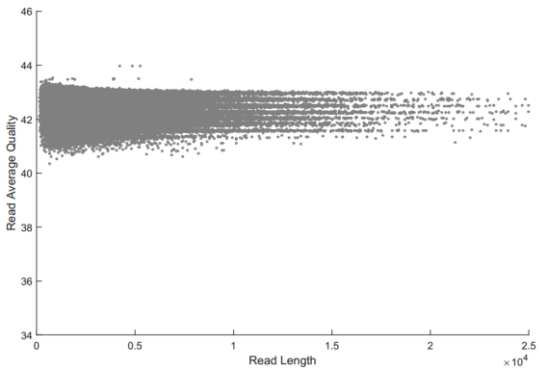
**Fig. S12** The scatter plot of read length and average base quality per read for CLR reads of *C. elegans*: (a) the raw CLR data of *C. elegans*; (b) NPBSS simulation; (c) PBSIM simulation; (d) SimLoRD simulation; and (e) FASTQSim simulation.



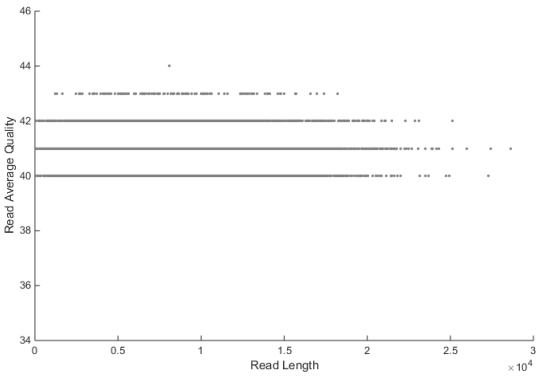

(a) CLR raw data



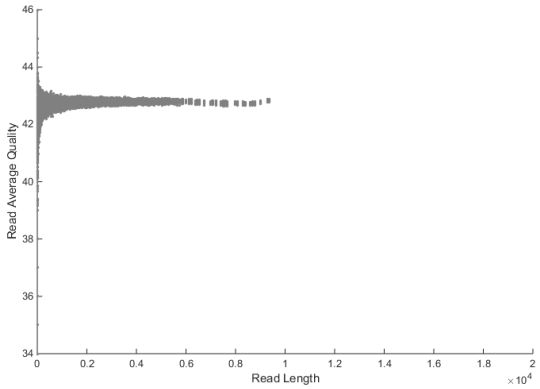

(b) CLR read dataset generated by NPBSS




(c) CLR read dataset generated by PBSIM




(d) CLR read dataset generated by SimLoRD




(e) CLR read dataset generated by FASTQSim

**Fig. S13** The scatter plot of read length and average base quality per read for CLR reads of *D. melanogaster*: (a) the raw CLR data of *D. melanogaster*; (b) NPBSS simulation; (c) PBSIM simulation; (d) SimLoRD simulation; and (e) FASTQSim simulation.

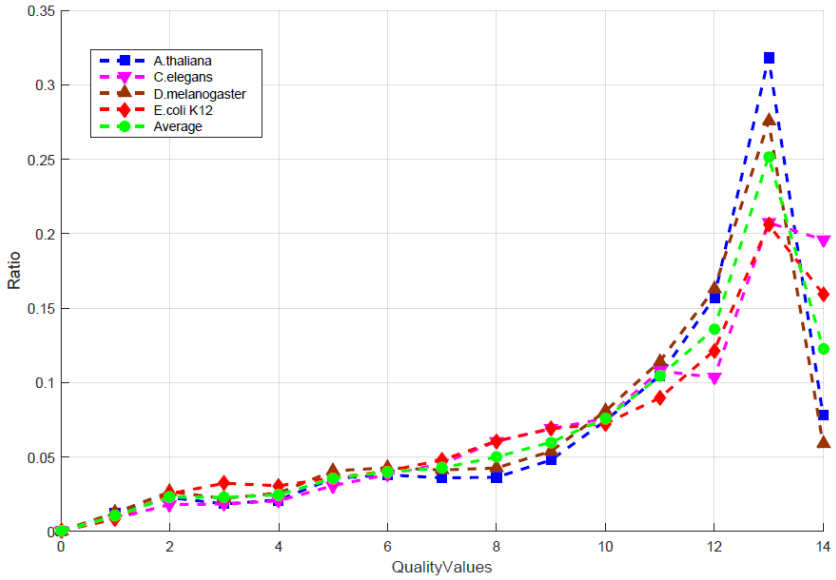



**Fig. S14** The proportion of different QVs in the four CLR datasets of *E. coli K12*, *C. elegans*, *A. thaliana* and *D. melanogaster*. We can see that the proportion curves present a rising trend with the QV growth from 0 to 13, and all of them have a peak value at QV=13 for each dataset, then drop down when QV=14. NPBSS will select different QVs according to the average proportion (green line).

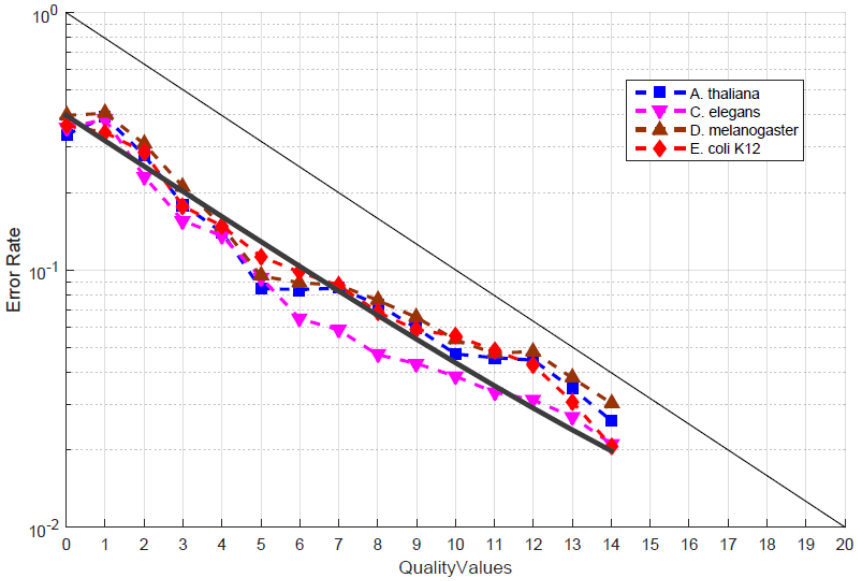



**Fig. S15** The curve between actual error rate and QVs in the four CLR datasets of *E. coli K12*, *C. elegans*, *A. thaliana* and *D. melanogaster*. We can see that the error rate curves are lower than the diagonal and present a downtrend with the increase of QV, meaning that the larger the QV, the lower the probability of error. These results show that the QV has a strong relation to the average error rate for each PacBio sequence, which should be considered when simulating PacBio sequences. The thick dark-gray line is the fitting curve produced by using the least square method.

**Table S1**. PacBio datasets website links

| Datasets | | Website links |
|---|---|---|
| CLR | *E. coli K12* | https://github.com/PacificBiosciences/DevNet/wiki/E.-coli-Bacterial-Assembly |
| | *C. elegans* | https://github.com/PacificBiosciences/DevNet/wiki/C.-elegans-data-set |
| | *A. thaliana* | https://github.com/PacificBiosciences/DevNet/wiki/Arabidopsis-P5C3 |
| | *D. melanogaster* | https://github.com/PacificBiosciences/DevNet/wiki/Datasets |
| CCS | *E. coli* K12 MG1655 | https://github.com/PacificBiosciences/DevNet/wiki/ |
| | *E. coli* C227-11 | https://www.ncbi.nlm.nih.gov/sra/SRX081567 |

**Table S2**. Assembly genome website links

| Genome | Website links |
|---|---|
| *E. coli K12* | https://s3.amazonaws.com/files.pacb.com/datasets/secondary-analysis/e-coli-k12-P6C4/polished_assembly.fastq.gz |
| *C. elegans* | http://datasets.pacb.com.s3.amazonaws.com/2014/c_elegans/40X/polished_assembly/polished_assembly.fasta.gz |
| *A. thaliana* | http://datasets.pacb.com.s3.amazonaws.com/2014/Arabidopsis/reads/polished_assembly.fasta |
| *D. melanogaster* | https://s3.amazonaws.com/datasets.pacb.com/2014/Drosophila/reads/dmel_FALCON_diploid_assembly.tgz |

**Table S3**. Statistics of four real CLR datasets

| **Datasets** | ***E. coli K12*** | ***C. elegans*** | ***A. thaliana*** | ***D. melanogaster*** |
|---|---|---|---|---|
| Enzyme-chemistry | P6-C4 | P6-C4 | P5-C3 | P5-C3 |
| Total number bases | 748,508,257 bp | 4,120,796,736 bp | 1,769,374,127 bp | 15,208,567,933 bp |
| Coverage depth | 160 | 40 | 65 | 106 |
| Number of reads | 87,217 | 383,544 | 215,026 | 1,514,730 |
| Read length | | | | |
| Average | 8,582 | 10,744 | 8,229 | 10,040 |
| Maximum | 44,113 | 48,211 | 45,400 | 41,579 |
| Top 5% of reads | >21 kb | >24 kb | >18 kb | >23 kb |
| Half of data in reads | >6 kb | >9 kb | >7 kb | >6 kb |

**Remark**: It can be seen from Table S3 that the current PacBio sequencer can boast average read lengths over 10 kb (for both *C. elegans* and *D. melanogaster* datasets), with an N50 of more than 6 kb (that is, over half of all data are in reads longer than 6 kb), which is drastically longer than the maximum read length (only paired-end 250 bp) generated by Illumina HiSeq 2500 [3].

**Table S4**. Statistics of two PacBio CCS datasets

| **Datasets** | ***E. coli* K12 MG1655** | ***E. coli* C227-11** |
|---|---|---|
| Total number bases | 217,871,193 bp | 223,962,022 bp |
| Depth | 45 | 41 |
| Number of reads | 231,629 | 502,157 |
| Read length | | |
| Average | 940 | 446 |
| Standard deviation | 332 | 168 |
| Maximum | 2,627 | 1,864 |
| Minimum | 200 | 116 |

**Table S5**. The proportions of different type errors in these four CLR raw datasets (%)

| **Type of data** | **Type of errors** | **Maximum** | **Minimum** | **Average** | **Standard deviation** |
|---|---|---|---|---|---|
| ***E. coli K12*** | Match | 92.971 | 73.770 | 88.345 | 3.089 |
| | Substitution | 17.656 | 0.278 | 2.300 | 1.857 |
| | Insert | 18.674 | 0.304 | 2.953 | 2.120 |
| | Deletion | 21.368 | 1.483 | 6.403 | 2.761 |
| ***C. elegans*** | Match | 91.871 | 83.364 | 86.543 | 1.325 |
| | Substitution | 9.150 | 1.607 | 6.326 | 0.940 |
| | Insert | 5.777 | 0.273 | 2.839 | 1.321 |
| | Deletion | 7.227 | 1.669 | 4.292 | 1.127 |
| ***A. thaliana*** | Match | 93.097 | 71.719 | 80.051 | 2.960 |
| | Substitution | 21.811 | 0.913 | 11.559 | 2.912 |
| | Insert | 11.748 | 0.348 | 2.525 | 1.034 |
| | Deletion | 17.822 | 2.080 | 5.865 | 1.521 |
| ***D. melanogaster*** | Match | 93.449 | 71.296 | 83.147 | 4.896 |
| | Substitution | 19.644 | 0.268 | 6.907 | 4.080 |
| | Insert | 12.599 | 0.172 | 3.133 | 1.633 |
| | Deletion | 17.936 | 1.195 | 6.813 | 1.930 |

From Table S5 it can be seen that although the accuracy of different datasets varies, the match accuracy is in the range of 83% to 88%, which is near to the error rate PacBio reported [4].

**Table S6**. The proportions of different type errors in these two CCS raw datasets (%)

| **Type of data** | **Type of errors** | **Maximum** | **Minimum** | **Average** | **Standard deviation** |
|---|---|---|---|---|---|
| ***E. coli* K12 MG1655** | Match | 100.00 | 73.312 | 97.730 | 2.538 |
| | Substitution | 11.978 | 0.00 | 3.740 | 1.585 |
| | Insert | 9.317 | 0.00 | 0.939 | 1.165 |
| | Deletion | 10.777 | 0.00 | 0.957 | 1.084 |
| ***E. coli* C227-11** | Match | 100.00 | 76.145 | 98.231 | 2.417 |
| | Substitution | 10.624 | 0.00 | 0.808 | 1.406 |
| | Insert | 8.148 | 0.00 | 1.034 | 1.154 |
| | Deletion | 9.567 | 0.00 | 0.456 | 1.071 |

**Table S7** Statistics of the simulated reads with PBSIM, FASTQSim and NPBSS for genome of *E. coli K12*

| Methods | Match rate (%) | Insertion rate (%) | Deletion rate (%) | Substitution rate (%) | Total error rate (%) | Average length (bp) |
|---|---|---|---|---|---|---|
| Raw Data | 88.345 | 2.953 | 6.403 | 2.300 | 11.656 | 8,517 |
| PBSIM | 87.031 | 2.751 | 6.878 | 2.652 | 12.281 | 8,826 |
| FASTQSim | 89.117 | 2.614 | 6.028 | 2.246 | 10.915 | 8,421 |
| NPBSS | 88.976 | 2.809 | 6.563 | 2.437 | 11.809 | 8,596 |

**Table S8** Statistics of the simulated reads with PBSIM, FASTQSim and NPBSS for genome of *C. elegans*

| Methods | Match rate (%) | Insertion rate (%) | Deletion rate (%) | Substitution rate (%) | Total error rate (%) | Average length (bp) |
|---|---|---|---|---|---|---|
| Raw Data | 86.543 | 2.839 | 4.292 | 6.326 | 13.457 | 10,132 |
| PBSIM | 86.162 | 2.893 | 4.712 | 7.047 | 14.652 | 10,547 |
| FASTQSim | 87.065 | 2.743 | 4.039 | 6.156 | 12.938 | 9,986 |
| NPBSS | 86.324 | 2.851 | 4.076 | 6.482 | 13.409 | 10,476 |

**Table S9** Statistics of the simulated reads with PBSIM, FASTQSim and NPBSS for genome of *A. thaliana*

| Methods | Match rate (%) | Insertion rate (%) | Deletion rate (%) | Substitution rate (%) | Total error rate (%) | Average length (bp) |
|---|---|---|---|---|---|---|
| Raw Data | 80.051 | 2.525 | 5.865 | 11.559 | 19.949 | 8,241 |
| PBSIM | 79.347 | 2.612 | 6.149 | 11.661 | 20.422 | 8,309 |
| FASTQSim | 80.187 | 2.634 | 5.126 | 12.056 | 19.816 | 8,117 |
| NPBSS | 80.195 | 2.498 | 5.931 | 11.587 | 20.016 | 8,275 |

**Table S10** Statistics of the simulated reads with PBSIM, FASTQSim and NPBSS for genome of *D. melanogaster*

| Methods | Match rate (%) | Insertion rate (%) | Deletion rate (%) | Substitution rate (%) | Total error rate (%) | Average length (bp) |
|---|---|---|---|---|---|---|
| Raw Data | 83.147 | 3.133 | 6.813 | 6.907 | 16.853 | 10,049 |
| PBSIM | 84.696 | 3.678 | 6.361 | 6.972 | 17.011 | 11,032 |
| FASTQSim | 84.925 | 3.016 | 6.124 | 5.941 | 15.081 | 9,978 |
| NPBSS | 83.172 | 3.652 | 6.308 | 6.836 | 16.796 | 10,592 |

**Table S11**. Optional arguments of NPBSS

| Options | Description [default] |
|---|---|
| -dep | sequencing depth [20] |
| -n | number of reads to simulate |
| -min | minimum sequence length [100] |
| -max | maximum sequence length (default:45000). |
| -lg mean std | the mean and standard deviation for log-normal [8500 6930] |
| -len | average sequence length [8500] |
| -sub | substitution error rate between 0 to 1 [0.06] |
| -ins | insertion error rate between 0 to 1 [0.03] |
| -del | deletion error rate between 0 to 1 [0.06] |
| -samp | sample the read length from a FASTA file provided by users |
| -sam | create SAM format output [0] |
| -model | error model provided by users, not recommended to change |
| -qv | QVs selection table, not recommended to change |

**Table S12**. The proportions of each quality value for CLR datasets

| QVs | Proporation | | | | Average |
|---|---|---|---|---|---|
| | *E. coli K12* | *A. thaliana* | *C. elegans* | *D. melanogaster* | |
| 0 | 0.0002767 | 0.000201605 | 0.0001635 | 0.0002863 | 0.0002863 |
| 1 | 0.0083244 | 0.011920808 | 0.0087469 | 0.0125234 | 0.0125234 |
| 2 | 0.0253281 | 0.022706822 | 0.0180355 | 0.0265938 | 0.0265938 |
| 3 | 0.0323038 | 0.018465691 | 0.018373 | 0.0221681 | 0.0221681 |
| 4 | 0.0306581 | 0.021156354 | 0.0205046 | 0.025743 | 0.025743 |
| 5 | 0.0359439 | 0.035515384 | 0.0307281 | 0.0406372 | 0.0406372 |
| 6 | 0.0405824 | 0.037991929 | 0.038162 | 0.0427691 | 0.0427691 |
| 7 | 0.0479063 | 0.035942184 | 0.0454116 | 0.0412257 | 0.0412257 |
| 8 | 0.0606712 | 0.036334864 | 0.0604958 | 0.0426037 | 0.0426037 |
| 9 | 0.0689327 | 0.04788296 | 0.0690272 | 0.0537985 | 0.0537985 |
| 10 | 0.0724405 | 0.074258115 | 0.0757777 | 0.0807213 | 0.0807213 |
| 11 | 0.0895916 | 0.104679742 | 0.1078311 | 0.1141539 | 0.1141539 |
| 12 | 0.1214606 | 0.156821558 | 0.1033919 | 0.1626757 | 0.1626757 |
| 13 | 0.2060074 | 0.318315629 | 0.2074021 | 0.2754374 | 0.2754374 |
| 14 | 0.1595721 | 0.077806355 | 0.1959488 | 0.0586628 | 0.0586628 |

**Table S13**. The corresponding error rate values for CLR datasets

| QVs | Theoretical Error Rate* | Actual Error Rate | | | |
|---|---|---|---|---|---|
| | | ***E. coli K12*** | ***A. thaliana*** | ***C. elegans*** | ***D. melanogaster*** |
| 0 | 1.0 | 0.363441 | 0.335061 | 0.356124 | 0.398278 |
| 1 | 0.794328 | 0.342079 | 0.392718 | 0.38506 | 0.406250 |
| 2 | 0.630957 | 0.286841 | 0.279143 | 0.230829 | 0.308902 |
| 3 | 0.501187 | 0.176615 | 0.178972 | 0.155073 | 0.210356 |
| 4 | 0.398107 | 0.148211 | 0.139980 | 0.135561 | 0.147830 |
| 5 | 0.316228 | 0.112745 | 0.084524 | 0.093556 | 0.094655 |
| 6 | 0.251189 | 0.097835 | 0.083806 | 0.064551 | 0.089055 |
| 7 | 0.199526 | 0.086878 | 0.084875 | 0.058410 | 0.087177 |
| 8 | 0.158489 | 0.068557 | 0.073402 | 0.046792 | 0.076213 |
| 9 | 0.125893 | 0.058395 | 0.059289 | 0.043206 | 0.065260 |
| 10 | 0.1 | 0.055463 | 0.047066 | 0.038546 | 0.053380 |
| 11 | 0.079433 | 0.048612 | 0.045522 | 0.033444 | 0.047343 |
| 12 | 0.063096 | 0.042561 | 0.044553 | 0.031186 | 0.048163 |
| 13 | 0.050119 | 0.030721 | 0.034546 | 0.026841 | 0.037958 |
| 14 | 0.039811 | 0.020678 | 0.025923 | 0.021077 | 0.030345 |

*The Theoretical Error Rate is computed by: $p=10^{-QV/10}$, where QV∈ [0,14]

**Table S14**. The actual overall error probability ($P_{error}$) of each QV.

| QVs | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $P_{error}$ | 0.398 | 0.317 | 0.253 | 0.202 | 0.161 | 0.129 | 0.103 | 0.083 | 0.067 | 0.054 | 0.044 | 0.035 | 0.029 | 0.024 | 0.020 |

**Table S15.** The characteristics of each simulator.

| Simulators | Advantages | Limitations |
|---|---|---|
| PBSIM | Easy parameter settings.<br>Low computational complexity.<br>Generating both CLR and CCS sequences. | No SAM-formatted output.<br>Don't consider the relationship between. error rate and QVs. |
| FASTQSim | Platforms-independent (Illumina, 454, PacBio and IonTorrent).<br>For both read analysis and simulation.<br>Generating both CLR and CCS sequences. | High running time.<br>No SAM-formatted output.<br>Difficult to change parameters directly.<br>Don't consider the relationship between error rate and QVs. |
| SimLoRD | Easy parameter settings.<br>Specified for CCS sequence simulation.<br>Low computational complexity. | Normal performance for CLR reads.<br>Don't consider the relationship between error rate and QVs. |
| NPBSS | Consider the relationship between error rate and QVs.<br>Easy parameter settings.<br>Generating both CLR and CCS sequences. | Medium computational complexity. |

## References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