Title

SEQdata-BEACON: a comprehensive database of sequencing performance and statistical tools for performance evaluation and yield simulation in BGISEQ-500

Yanqiu Zhou[*], Chen Liu[*], Rongfang Zhou[*], Anzhi Lu, Biao Huang, Liling Liu, Ling Chen, Bei Luo, Jin Huang, Zhijian Tian

BGI-Wuhan Clinical Laboratories, Wuhan, 430074, China

*These authors contributed equally to this paper.

Corresponding Author:

Jin Huang, Zhijian Tian

Building B2, No.666 Gaoxin Road, Wuhan East lake Hi-tech Development zone, Wuhan 430074, China

Email address: huangjin@genomics.cn, tianzhijian@genomics.cn

Short Title: SEQdata-BEACON: a database of sequencing performance in BGISEQ-500

Abstract

Background. BGISEQ-500 is based on DNBSEQ[TM] technology and superior in providing high outputs and requiring less cost. This sequencer has been widely used in various areas of scientific and clinical research. A better understanding of the sequencing process and sequencer performance is essential for stabilizing sequencing process, accurately interpreting sequencing results and efficiently solving sequencing troubles. To solve these problems, a comprehensive database SEQdata-BEACON was constructed to accumulate sequencing performance data in BGISEQ-500.

Methods. Totally 60 BGISEQ-500 sequencers in BGI-Wuhan lab were used to collect the sequencing performance data. Those lanes in paired-end 100 sequencing using 10bp

barcode were chosen, and each lane containing 66 metrics was assigned a unique entry

number as ID. The database was constructed in MySQL server 8.0 and the website was

built on Apache (2.4.33 win64 VC15 server). The statistical analysis and linear regression

models were generated by R program based on the data from November 2018 to April

2019.

Results. A total of 2236 entries were recorded in the database, including sample ID,

yield, quality, machine state and supplies information. According to correlation matrix,

the 52 numerical metrics were clustered into three groups signifying yield-quality,

machine state and sequencing calibration. The metrics distributions also delivered some

patterns and rendered clues for further explanation or analysis of the sequencing process.

Using the data of total 200 cycles, the linear regression model well simulated the final

outputs. Moreover, the predicted final yield could be provided in the 15th cycle of the

early stage of sequencing and the corresponding coefficient of determination R^2 of the

200th and 15th cycle models were 0.97 and 0.81 respectively. The data source, statistical

findings and application tools were all available in our website

http://seqBEACON.genomics.cn:443/home.html. These resources can be used as a

constantly updated reference for BGISEQ-500 users to comprehensively understand

DNBSEQ^{TM} technology, solve sequencing problems and optimize the sequencing

process.

Keywords: BGISEQ-500, Prediction tools, Data analysis, Database

Introduction

The next generation sequencing (NGS, also known as high-throughput sequencing)

has led us into a genomic era. In the past 15 years, the development of sequencing

technology was mainly committed to the reduction in cost and improvement in outputs,

accuracy and read length. Nowadays, the sequencer manufacturers Illumina and Beijing

Genomics Institute (BGI) provided high outputs and accuracy, while Pacific Bioscience

and Oxford Nanopore offered long read length (Ansorge 2018; Goodwin et al. 2016). The

first BGI sequencer BGISEQ-500 has launched in 2015 (http://en.mgitech.cn/), which

was based on two key technologies: DNA nanoball (DNB) and Combinatorial Probe-

Anchor Synthesis (cPAS). DNA library circles formed a single-stranded DNA molecule

named as DNB by rolling circle amplification (RCA). The DNBs were dispersed and

immobilized on a patterned array, then a probe was annealed to a DNA molecular anchor

on the DNB. In each cycle, DNA polymerase incorporated one base labeled with

fluorescence group, and the light signal was collected via a high-resolution imaging

system and converted into bases after basecalling (Drmanac et al. 2010).

Recently, BGISEQ-500 platform has been widely used in a variety of sequencing

types, such as whole genome sequencing (WGS), whole exome sequencing (WES),

RNA-seq, small RNA and metagenomics (Chen et al. 2017; Fehlmann et al. 2016; Han et

al. 2018; Huang et al. 2017; Xu et al. 2019). BGISEQ-500 sequencing platform has not

only participated in transcriptome analysis of plant nitrogen metabolism and resistance

response, but also in human clinical applications for the cancer genome sequencing and

TP53 mutations detection in high-grade serous ovarian cancer (Chen et al. 2018; Li et al.

2018; Liu et al. 2018; Patch et al. 2018). In addition, it is reported that BGISEQ-500 had

good performance in single cell resolution, such as scRNA-seq and scCAT-seq (Liu et al.

2019; Natarajan et al. 2019; Zhao et al. 2018). Compared with Illumina platforms,

BGISEQ-500 demonstrated comparable SNP detection accuracy in WGS, similarly

consistency in variation detection in WES, and high concordance in transcriptome and

metagenomic studies (Fang et al. 2018; Patch et al. 2018; Xu et al. 2019; Zhu et al. 2018).

Therefore, DNBSEQ^{TM} technology had the advantages of cost-effective, high

reproducibility and low duplication rate which provided a new choice for resolve issues

in scientific research, agriculture, environment and clinical applications.

Among the sequencing performance metrics in BGISEQ-500, yield (eg., Reads, the

number of DNBs recognized by the Basecall software) and quality (eg., Q30, the

percentage of bases with an error rate below 0.001) were the most concerned. Besides,

other metrics regarding chemical reaction and instrument state were also recorded in the

sequencing summary. However, we still lack a profound understanding of the metrics

meaning especial the connection between them. As one of the world's largest sequencing

service providers, BGI performed thousands of sequencing runs each year. Accompanied

with enormous nucleotide data, massive sequencing performance data was highly

valuable for illustrating this complicated process and troubleshooting. Unfortunately, this

type of datasets or databases are rare currently and a comprehensive database is required

to integrate the abundant sequencer performance data.

In this study, we designed a database SEQdata-BEACON to comprehensively

collect sequencing performance data in BGISEQ-500 including the information of sample

ID, yield, quality, machine state and supplies. We calculated Pearson's correlation of 52

numerical metrics for hierarchical clustering and analyzed their distribution patterns. We

also used linear regression to establish the yield simulation models to investigate the

connection between yield correlated metrics, and attempted to predict the final yield in

the early stage of sequencing. All the data and statistical analysis results were exhibited in

our open access website. These resources can be an updating reference dataset for

BGISEQ-500 users from enterprises or colleges to gain a deeper understanding of

DNBSEQ^{TM} technology.

Materials and Methods

Data Collection and Database Construction

We chose 60 BGISEQ-500 sequencers in BGI-Wuhan lab and collected all available

files generated after chemical reaction and basecalling. We accumulated the lanes in

PE100 sequencing since it is the major sequencing type in BGISEQ-500. Each lane was

assigned a unique entry number as ID and other 65 metrics were evaluated and extracted

to form a database 'SEQdata-BEACON'. No detailed sample information was entered

into the database to protect the privacy of customers. The database was constructed in

MySQL server 8.0 and the architecture was shown in Fig. 1.

Web Visual Interface and Statistical analysis

A user-friendly interface of SEQdata-BEACON was built on Apache (2.4.33 win64

VC15 server). An explorer Google Chrome 68.0.3440.106 was suggested to access the

website. The statistical analysis and the figures in this study were generated based on the

data from November 2018 to April 2019 by program R version 3.5.0 x64 reserved the

function to install modules/package.

Yield simulation model

We tried to investigate the metrics correlated to yield and used them to simulate the

final outputs. First, considering the sequencing principle, DNB was the decisive source of

yield. The number of successfully fixed DNBs on the patterned array determined the

ability to produce reads (Drmanac et al. 2010; Wang et al. 2019). TotalEsr (Total

Effective Spot Rate) multiplied by Dnbnumber excluded those spots without DNB or

with dark DNB not showing light signal, and represented the maximum signal that could

be successfully collected. Second, some metrics that clustered with yield (Reads) may

reflect the low-quality reads resulting from chemical reaction, light intensity, camera

acquiring and basecalling in the sequencing process. At the end of sequencing, the

sequencers filtered out these reads with low quality score which resulted in the loss of

final yield. Thus, we constructed a linear regression model with TotalEsr*Dnbnumber,

BIC, accGRR, SNR and FIT as variables of inputs, and output was yield (Reads). We

used Turkey's boxplot to pinpoint the possible outliers which defined as observations that

fall below Q_1-1.5 IQR or above Q_3+1.5 IQR, and these outliers were excluded from the

model construction. The LR model formula was as follows (Eqs. (1)):

$Y = \beta_0 + \beta_1 TD + \beta_2 B + \beta_3 G + \beta_4 S + \beta_5 F + \varepsilon, \varepsilon \sim \text{Normal}(0, \sigma)$ (1)

Y is the value of the dependent variable yield. T is the value corresponding to the

current cycle in TotalEsr. D is the value of Dnbnumber at the beginning of the

sequencing. B, G, S and F are the average values of BIC, accGRR, SNR and FIT for

the first 200 cycles respectively. ε is observed error and obeys normal distribution. The

model parameters β_n are the coefficient value and estimated using a regression model.

Further, we simulated the final yield based on the metric values in every 5 cycles.

The formula was as follows (Eqs. (2)):

$Y_i = \beta_{0,i} + \beta_{1,i} T_i D_i + \beta_{2,i} B_i + \beta_{3,i} G_i + \beta_{4,i} S_i + \beta_{5,i} F_i + \varepsilon_i, \varepsilon_i \sim \text{Normal}(0, \sigma)$ (2)

Y_i is the value of the dependent variable yield. T_i is the value corresponding to the

current cycle i in TotalEsr. D_i is the value of Dnbnumber at the beginning of the

sequencing. B_i, G_i, S_i and F_i are the average values of BIC, accGRR, SNR and FIT

for the first i cycles respectively. ε_i is observed error in the i cycle and obeys normal

distribution. The model parameters $\beta_{n,i}$ are the coefficient value and estimated using a

regression model. Both linear regression and backward elimination method of stepwise

regression were conducted by program R version 3.5.0 x64 reserved the function to

install modules/package.

Results

Database Construction

DNA libraries were circularized, made into DNBs and loaded onto the patterned

array. After successive chemical reaction, signal acquisition and basecalling, the

sequencers normally generated a series of folders and files in each cycle or at the end of

sequencing process. In BGISEQ-500, over 10 files recorded the information about the

entire sequencing process, such as InputInfo_*.txt, RunInfo.txt, summaryReport.html,

fovReport.QC.txt, BarcodeStat.txt and fq.fqStat.txt etc. The metrics values in these files

were the preliminary dataset for 'SEQdata-BEACON', and totally 66 metrics were

extracted and entered into our database (Fig. 1). From November 2018 to April 2019,

'SEQdata-BEACON' accumulated a total of 2236 entries in PE100 sequencing using

10bp barcode from 60 sequencers in BGI-Wuhan lab. The database primarily collected

the information of sample ID, yield, quality, machine status, and supplies. The unique

identifier for each entry, sequencing type and other sample information were stored as

Sample ID. Regular quality control metrics such as Reads, Bases related to yield and Q30

related to quality were stored. Machine state such as Signal, Intensity and Theta were

stored into the database. Sequencing reagents and sequencing time were also stored in the

database.

Web Visual Interface

To provide open access to our data, we designed a comprehensive website

'SEQdata-BEACON' with Home, Browse, Tools, Download and Guide pages to display

the database and data-mining applications. The 'Home' page allowed the users to

overview the introduction of our website and schematic architecture of our database (Fig.

2A). The 'Browse' page allowed the users to look through the numerical metric features

including heatmap of Pearson's correlation matrix and the distribution. For example, the

distribution of FIT and its change per cycle were both manifested in charts for observing

the distribution patterns and fluctuations (see the section 'Statistical Finding: Metric

Features' for details). The 'Tools' page allowed the users to test our simulation model

(Fig. 2B and C). Users could enter specified metric values in our website in the example

format and click 'Start', then the expected yield confidential intervals will be shown (see

the section 'Statistical Finding: Yield simulation model' for details). The 'Download'

page allowed the users to obtain the data in EXCEL format according to our update time,

while all the data and analysis results will be updated every two months. The 'Guide'

page supplied a guideline for regular operation.

Statistical Finding: Metric Features

Based on the data from November 2018 to April 2019, Pearson's correlation of numerical metrics was calculated and the 52*52 correlation matrix was exhibited in the heatmap (Fig. 3). These 52 metrics were mainly clustered into three groups with 20, 15 and 17 metrics respectively, and the first group could be further divided into two branches which represented yield and quality. The other two groups represented machine state (e.g., Theta, tells the angle between the moving direction of the array stage and the track line on the array), and sequencing calibration (e.g., Lag, Runon, record the percentage of read strands being out of phase with the current cycle). Red blocks indicated for the positive correlation and blue blocks for the negative ones. It is shown that yield was positively correlated with quality, and the sequencing calibration were negatively correlated with them both. It is also denoted that some metrics were redundant, for example, Reads was highly correlated with other five yield related metrics, and metrics about CG contents were highly correlated with each other. Only one of the redundant metrics was reserved in further data analysis.

Next, we investigated the distribution pattern of the above metrics. It is displayed in the scatterplot of Q30 versus Reads that yield was ranging from 350-850M, and Q30 was above 70%. The histogram on the upper and right part of chart also exhibited the distribution of Reads and Q30 respectively (Fig. 4A). According to the normal performance of BGISEQ-500, Reads was greater than 650M and Q30 was over 85% in each lane (http://en.mgitech.cn/). In the scatter plot, totally 2026 lanes located in the normal range, and the proportion of outliers was less than 10%, which suggested that the instrument performance was relatively stable. Furthermore, the distribution pattern of FIT was manifested in a histogram, and it was mostly around 0.80 (Fig. 4B). FIT value was presented cycle by cycle, and it was found to slowly decrease from 0.811 to 0.757 in read1 and from 0.835 to 0.763 in read2 with more deviation in the beginning to less deviation in the end of each read (Fig. 4C). The metric FIT suggested the distribution of

206 differences between signal and noise for each base and the performance of optical path

207 during the sequencing process (Wang et al. 2019). The deviation in each cycle may reflect

208 the changing pattern of FIT in the sequencing process and hint the signal or optical path

209 status indirectly.

Statistical Finding: Yield simulation model

In this study, considering the BGISEQ-500 sequencing principle and Pearson's

correlation matrix clustering results, the linear regression model included the variables

TotalEsr, Dnbnumber, BIC, accGRR, SNR and FIT as predictors. Statistical program R

was applied to construct the model using the data from November 2018 to April 2019.

The LR model was Eq. (3) and Table 1 displayed the standard error, t-value and p-value

of this function in regression results. It is indicated that the contribution of accGRR was

not significant. Subsequently, backward elimination method of stepwise regression was

used to build the model to find an optimum solution and the final LR model was Eq. (4).

The regression results of this function pointed out that the contributions of

TotalEsr*Dnbnumber, BIC, SNR, FIT were significant at the 1% probability level (Table

1). Here we compared the yield with predicted results, taking the coefficient of

determination (R^2) range from 0 to 1 to measure the accuracy of our model, which

showed the value for R^2 was 0.97 (Fig. 5). It is suggested that five metrics

TotalEsr*Dnbnumber, BIC, SNR, FIT successfully simulated yield in a LR model.

In addition, we hoped that this simulation model can be used to predict the final

yield at the early stage of sequencing. In the sequencing process, the yield was assumed

to be a function of TotalEsr, Dnbnumber, BIC, accGRR, SNR and FIT at the current

running cycle. The model was conducted every 5 cycles using backward elimination

regression by statistical program R, and totally 40 models were established from the cycle

1 to 200. Comparing the residual deviation of these models, the residuals of prediction

models were fluctuated in the beginning of read1 and read2, which was mainly due to the

establishment of the algorithm matrix by the sequencer (Fig. S1). However, the value for

R^2 of model in the 15th cycle was 0.81, indicating that the final yield could be effectively

simulated at this stage. The corresponding LR model was Eq. (5).

$$Y = -129.692 + 0.983TD + 1.400B + 0.143G - 3.334S + 26.997F \qquad (3)$$

$$Y = -129.787 + 0.983TD + 1.402B - 3.334S + 27.027F \qquad (4)$$

$$Y_{15} = -141.021 + 1.270TD + 4.743B - 604.450G - 3.465S + 28.882F \qquad (5)$$

Discussion

Continuous improvement in sequencing technologies and sequencers has provided

us the choice to producing higher throughput data with less sequencing cost. With the

upgrade of DNBSEQ^{TM} technology, BGI has introduced more efficient sequencing

platforms, such as MGISEQ-2000 and MGISEQ-T7. Meanwhile, because of the excellent

performance of DNBSEQ^{TM} technology, this series of sequencers had been widely used

both in science research and clinical aspects. The sequencer performance metrics brought

us a wealth of information of the sequencer, but our understanding of this information

was seriously lacking. To gain insight into the sequencing performance in BGISEQ-500,

we constructed a database 'SEQdata-BEACON' which has already contained 2236

entries with 66 metrics and comprehensively recorded the information of sample ID,

yield, quality, machine state and supplies. The method of automatically collecting metric

values from sequencing summary could effectively lighten human labor work, shorten

time cost and improve data accuracy.

At present, in our 60 BGISEQ-500 sequencers in BGI-Wuhan lab, sequencing

libraries were derived from plant, animal, microbial and human DNA or RNA samples,

while PE100 sequencing using 10 bp barcode was for WGS, WES and RNA-seq.

Therefore, the data we collected including libraries from most species and major types of

sequencing applications. By analyzing the distribution characteristics of the numerical

metrics, in Q30 versus Reads scatterplot, 90.6% lanes had reads more than 650M and

Q30 above 85% which proved that BGISEQ-500 was stable and reliable in the massively

parallel sequencing. Without the risk of index hopping, DNBSEQ^{TM} could provide

excellent sequencing data with less duplication and error, which could have extensive

application in population scale sequencing projects, such as the 10KP (10,000 Plants)

Genome Sequencing Project (Cheng et al. 2018; Li et al. 2019).

In order to study the correlation of yield-correlated metrics, we used the backward

elimination method of stepwise regression to establish a yield simulation model with the

value for R^2 was 0.97. The model presented good simulation, which suggested that

TotalEsr, Dnbnumber, BIC, SNR and FIT were contributed to the yield. While predicting

the final production, we still used all six parameters to construct 40 prediction models

using stepwise regression. From the residual deviation of the models, it is found that the

final yield can be predicted in the 15th cycle at the early stage of sequencing, and the

small change in the residual within read1 and read2 implied the little fluctuation of the

metrics during the sequencing process. The study of yield related metrics and their

relationship began with constructing linear regression model which is a common

statistical technique for simulating the associations between variables. However, it is not

excluded that neural network models, machine learning model or other methods which

may be utilized to get better simulation results. Furthermore, we wanted to investigate the

quality associated metrics and establish a quality simulation model. Combined with yield

simulation model, these two models may effectively simulate the sequencing results and

bring us more ideas to increase the sequencing performance. With the updating of the

database and website every two months, it is expected that the accumulation of data,

enrichment of metrics together with the upgrade of algorithms will develop more data-

mining applications.

We have established the first reported BGISEQ-500 sequencing performance

database and website to comprehensively collect and summarize performance data. We

paid more attention to the data accumulation, and hope to explore the data patterns by

statistical analysis and interpret sequencing results. The database presented data and

statistical results through the website, providing an ongoing reference dataset which gave

users opportunities to promptly understand the performance and advantages of the

sequencer. Recently, some clues were found in monitoring the instrument degradation by

calculating error rates in Nextseq 500 (Manley et al. 2016). We also expected that our

charts of metrics distributions could imply the troubles in the sequencing process. Recent

investigations reported that MGISEQ-2000 had comparable SNP detection accuracy in

WGS and high gene detection in scRNA-seq compared with Illumina platforms

(Gorbachev et al. 2019; Senabouth et al. 2019). In the future, we plan to gather more

sequencing platforms of DNBSEQ^{TM} technology, which will provide an integrated

statistical reference for BGI sequencers, and is beneficial to fully understand the

sequencer performance of this series. Moreover, we also hope to add sequencer PacBio

Sequel II and Oxford Nanopore PromethION to get deeper understanding of single-

molecule sequencing technology. We expected SEQdata-BEACON to be a

comprehensive platform: with data accumulation and data-mining, it could present the

performance of the sequencing platform from various aspects; by adding functional

modules such as QC metrics models and metrics standards, it also could provide users

useful suggestions for optimization and troubleshooting to solve their problems.

Conclusion

Widespread application of NGS resulted in large amount of data, including

nucleotide sequences and sequencing process performance. We designed a database

SEQdata-BEACON to accumulate sequencing performance data in BGISEQ-500

containing 66 metrics of sample ID, yield, quality, machine state and supplies. The

correlation matrix of 52 numerical metrics was clustered into three groups covering the

information of yield-quality, machine state and sequencing calibration. The distribution

of numerical metrics presented some features and provided clues for further interpreting

the metric meanings and analysis. We also constructed linear regression models to well

simulate the final yield using metrics values in 200th and 15th cycle. The data source,

statistical findings and application tools were all available in our website

(http://seqBEACON.genomics.cn:443/home.html), which can facilitate BGISEQ-500

users from enterprises or colleges to understand and interpret their sequencing results.

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References

Ansorge WJ. 2018. Next Generation DNA Sequencing (II): Techniques, Applications. *Top 10 Contributions on Bioinformatics & Systems Biology*. DOI 10.4172/2469-9853.S1-005.

Chen K, Liu J, Liu S, Xia M, Zhang X, Han D, Jiang Y, Wang C, and Cao X. 2017. Methyltransferase SETD2-Mediated Methylation of STAT1 Is Critical for Interferon Antiviral Activity. *Cell* 170:492-506 e414. DOI 10.1016/j.cell.2017.06.042.

Chen Q, Xu X, Jiang J, and Li J. 2018. Transcriptome resequencing analysis of the responses of Ty-5-Mediated resistance to TYLCV via in resistant vs. susceptible tomato cultivars. *PeerJ Preprint*. DOI 10.7287/peerj.preprints.26578v1.

Cheng S, Melkonian M, Smith SA, Brockington S, Archibald JM, Delaux PM, Li FW, Melkonian B, Mavrodiev EV, Sun W, Fu Y, Yang H, Soltis DE, Graham SW, Soltis PS, Liu X, Xu X, and Wong GK. 2018. 10KP: A phylodiverse genome sequencing plan. *Gigascience* 7:1-9. DOI 10.1093/gigascience/giy013.

Drmanac R, Sparks AB, Callow MJ, Halpern AL, Burns NL, Kermani BG, Carnevali P, Nazarenko I, Nilsen GB, Yeung G, Dahl F, Fernandez A, Staker B, Pant KP, Baccash J, Borcherding AP, Brownley A, Cedeno R, Chen L, Chernikoff D, Cheung A, Chirita R, Curson B, Ebert JC, Hacker CR, Hartlage R, Hauser B, Huang S, Jiang Y, Karpinchyk V, Koenig M, Kong C, Landers T, Le C, Liu J, McBride CE, Morenzoni M, Morey RE, Mutch K, Perazich H, Perry K, Peters BA, Peterson J, Pethiyagoda CL, Pothuraju K, Richter C, Rosenbaum AM, Roy S, Shafto J, Sharanhovich U, Shannon KW, Sheppy CG, Sun M, Thakuria JV, Tran A, Vu D, Zaranek AW, Wu X, Drmanac S,

Oliphant AR, Banyai WC, Martin B, Ballinger DG, Church GM, and Reid CA. 2010. Human
genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science*
327:78-81. DOI 10.1126/science.1181498.

Fang C, Zhong H, Lin Y, Chen B, Han M, Ren H, Lu H, Luber JM, Xia M, Li W, Stein S, Xu X, Zhang
W, Drmanac R, Wang J, Yang H, Hammarstrom L, Kostic AD, Kristiansen K, and Li J. 2018.
Assessment of the cPAS-based BGISEQ-500 platform for metagenomic sequencing. *Gigascience*
7:1-8. DOI 10.1093/gigascience/gix133.

Fehlmann T, Reinheimer S, Geng C, Su X, Drmanac S, Alexeev A, Zhang C, Backes C, Ludwig N, Hart
M, An D, Zhu Z, Xu C, Chen A, Ni M, Liu J, Li Y, Poulter M, Li Y, Stahler C, Drmanac R, Xu X,
Meese E, and Keller A. 2016. cPAS-based sequencing on the BGISEQ-500 to explore small non-
coding RNAs. *Clinical Epigenetics* 8:123. DOI 10.1186/s13148-016-0287-1.

Goodwin S, McPherson JD, and McCombie WR. 2016. Coming of age: ten years of next-generation
sequencing technologies. *Nature Review Genetics* 17:333-351. DOI 10.1038/nrg.2016.49.

Gorbachev A, Kulemin N, Naumov V, Belova V, Kwon D, Rebrikov D, and Korostin D. 2019.
Comparative analysis of novel MGISEQ-2000 sequencing platform vs Illumina HiSeq 2500 for
whole-genome sequencing. DOI 10.1101/577080.

Han M, Hao L, Lin Y, Li F, Wang J, Yang H, Xiao L, Kristiansen K, Jia H, and Li J. 2018. A novel
affordable reagent for room temperature storage and transport of fecal samples for metagenomic
analyses. *Microbiome* 6:43. DOI 10.1186/s40168-018-0429-0.

Huang J, Liang X, Xuan Y, Geng C, Li Y, Lu H, Qu S, Mei X, Chen H, Yu T, Sun N, Rao J, Wang J,
Zhang W, Chen Y, Liao S, Jiang H, Liu X, Yang Z, Mu F, and Gao S. 2017. A reference human
genome dataset of the BGISEQ-500 sequencer. *Gigascience* 6:1-9. DOI
10.1093/gigascience/gix024.

Li Q, Zhao X, Zhang W, Wang L, Wang J, Xu D, Mei Z, Liu Q, Du S, Li Z, Liang X, Wang X, Wei H,
Liu P, Zou J, Shen H, Chen A, Drmanac S, Liu JS, Li L, Jiang H, Zhang Y, Wang J, Yang H, Xu X,
Drmanac R, and Jiang Y. 2019. Reliable multiplex sequencing with rare index mis-assignment on
DNB-based NGS platform. *BMC Genomics* 20:215. DOI 10.1186/s12864-019-5569-5.

Li S, Tian Y, Wu K, Ye Y, Yu J, Zhang J, Liu Q, Hu M, Li H, Tong Y, Harberd NP, and Fu X. 2018.
Modulating plant growth-metabolism coordination for sustainable agriculture. *Nature* 560:595-600.
DOI 10.1038/s41586-018-0415-5.

Liu D, Zhang XX, Li MC, Cao CH, Wan DY, Xi BX, Tan JH, Wang J, Yang ZY, Feng XX, Ye F, Chen
G, Wu P, Xi L, Wang H, Zhou JF, Feng ZH, Ma D, and Gao QL. 2018. C/EBPbeta enhances
platinum resistance of ovarian cancer cells by reprogramming H3K79 methylation. *Nat Commun*
9:1739. DOI 10.1038/s41467-018-03590-5.

Liu L, Liu C, Quintero A, Wu L, Yuan Y, Wang M, Cheng M, Leng L, Xu L, Dong G, Li R, Liu Y, Wei
X, Xu J, Chen X, Lu H, Chen D, Wang Q, Zhou Q, Lin X, Li G, Liu S, Wang Q, Wang H, Fink JL,
Gao Z, Liu X, Hou Y, Zhu S, Yang H, Ye Y, Lin G, Chen F, Herrmann C, Eils R, Shang Z, and Xu
X. 2019. Deconvolution of single-cell multi-omics layers reveals regulatory heterogeneity. *Nat*
Commun 10:470. DOI 10.1038/s41467-018-08205-7.

Manley LJ, Ma D, and Levine SS. 2016. Monitoring Error Rates In Illumina Sequencing. *J Biomol Tech*
27:125-128. DOI 10.7171/jbt.16-2704-002.

Natarajan KN, Miao Z, Jiang M, Huang X, Zhou H, Xie J, Wang C, Qin S, Zhao Z, Wu L, Yang N, Li
B, Hou Y, Liu S, and Teichmann SA. 2019. Comparative analysis of sequencing technologies for
single-cell transcriptomics. *Genome Biology* 20. DOI 10.1186/s13059-019-1676-5.
Patch AM, Nones K, Kazakoff SH, Newell F, Wood S, Leonard C, Holmes O, Xu Q, Addala V, Creaney
J, Robinson BW, Fu S, Geng C, Li T, Zhang W, Liang X, Rao J, Wang J, Tian M, Zhao Y, Teng F,
Gou H, Yang B, Jiang H, Mu F, Pearson JV, and Waddell N. 2018. Germline and somatic variant
identification using BGISEQ-500 and HiSeq X Ten whole genome sequencing. *PLoS One*
13:e0190264. DOI 10.1371/journal.pone.0190264.
Senabouth A, Anderson S, Shi Q, Shi L, Jiang F, Zhang W, Wing K, Daniszewski M, Lukowski S, Hung
S, Nguyen Q, Fink L, Beckhouse A, Pebay A, Hewitt A, and Powell J. 2019. Comparative
performance of the BGI and Illumina sequencing technology for single-cell RNAsequencing. DOI
10.1101/552588.
Wang O, Chin R, Cheng X, Wu KYM, Mao Q, Tang J, Sun Y, Anderson E, Lam HK, Chen D, Zhou Y,
Wang L, Fan F, Zou Y, Xie Y, Y. ZR, Drmanac S, Nguyen D, Xu C, Villarosa C, Gablenz S, Barua
N, Nguyen S, Tian W, Liu JS, Wang J, Liu X, Qi X, Chen A, Wang H, Dong Y, Zhang W, Alexeev
A, Yang H, Wang J, Kristiansen K, Xu X, Drmanac R, and Peters BA. 2019. Efficient and unique
co-barcoding of second-generation sequencing reads from long DNA molecules enabling cost
effective and accurate sequencing, haplotyping, and de novo assembly. *Genome Research*. DOI
10.1101/gr.245126.118.
Xu Y, Lin Z, Tang C, Tang Y, Cai Y, Zhong H, Wang X, Zhang W, Xu C, Wang J, Wang J, Yang H, Yang
L, and Gao Q. 2019. A new massively parallel nanoball sequencing platform for whole exome
research. *BMC Bioinformatics* 20:153. DOI 10.1186/s12859-019-2751-3.
Zhao Y, Li X, Wang J, Wan Z, Gao K, Yi G, Wang X, Wu J, Fan B, Zhang W, Chen F, Yang H, Wang J,
Xu X, Li B, Liu S, Zhao W, Hou Y, and Liu X. 2018. Single-cell Transcriptomic Landscape of
Nucleated Cells in Umbilical Cord Blood. *bioRxiv*. DOI 10.1101/346106.
Zhu FY, Chen MX, Ye NH, Qiao WM, Gao B, Law WK, Tian Y, Zhang D, Zhang D, Liu TY, Hu QJ,
Cao YY, Su ZZ, Zhang J, and Liu YG. 2018. Comparative performance of the BGISEQ-500 and
Illumina HiSeq4000 sequencing platforms for transcriptome analysis in plants. *Plant Methods*
14:69. DOI 10.1186/s13007-018-0337-0.
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Additional Information and Declarations

Competing Interests

The authors declare there are no competing interests.

Author Contributions

Yanqiu Zhou, Chen Liu and Rongfang Zhou constructed the database, analyzed the data,

prepared figures and/or tables, drafted the work or revised it critically for important content.

Anzhi Lu Biao Huang, Liling Liu, Ling Chen, Bei Luo contributed reagents/materials/analysis

tools.

Jin Huang, Zhijian Tian conceived and designed the experiments, approved the final draft of the

manuscript submitted for review and publication.

Supplemental Information

S1 Fig. Residual deviation of LR model every 5 cycles. Box plot displayed the residuals of all

the 40 LR models, each box manifested the median and first and third quartiles, star means the

standard deviation.

Figure 1. Schematic architecture of SEQdata-BEACON.

Figure 2. Web visual interface of SEQdata-BEACON. (A) "Home" page showed the database instruction and its five pages including Home, Browse, Tools, Download and Guide. (B) "Tools" page showed the input windows of metrics for users to test our yield simulation model. (C) "Tools" page showed and the resulting confidential intervals of predicted yield.

Figure 3. Numerical metrics correlation. Hierarchical clustering of the Pearson's correlation matrix between 52 metrics.

Figure 4. Distribution of Metrics in SEQdata-BEACON. (A) Q30 versus Reads, histogram of Q30 and Reads shown in grey, density profiles shown in blue. (B) Histogram of FIT in all entries. (C) Scatter plot of FIT through 200 cycles.

Figure 5. Cross-correlation of yield between predicted and actual values. A linear regression line is shown in blue line.

Figure S1. Residual deviation of LR model every 5 cycles. Box plot displayed the residuals of all the 40 LR models, each box manifested the median and first and third quartiles, star means the standard deviation.

Table 1. Model summaries of regressions linear for prediction of yield outputs.

Independent variables	Outputs in Eq. (3)			Outputs in Eq. (4)			Outputs in Eq. (5)		
	Std. error	t-Value	P-value	Std. error	t-Value	P-value	Std. error	t-Value	P-value
Constant	11.424	-11.353	< 2e-16**	10.662	-12.173	< 2e-16**	39.411	-3.578	0.00035**
TotalEsr*Dnbnumber	0.010	95.192	< 2e-16**	0.010	96.130	< 2e-16**	0.033	38.837	< 2e-16**
BIC	0.192	7.278	4.73e-13**	0.178	7.873	5.46e-15**	0.724	6.556	6.91e-11**
accGRR	6.175	0.023	0.981	---	---	---	59.009	-10.243	< 2e-16**
SNR	0.388	-8.586	< 2e-16**	0.388	-8.596	< 2e-16**	0.653	-5.307	1.23e-07**
FIT	4.757	5.675	1.57e-08**	4.577	5.905	4.08e-09**	11.249	2.567	0.010*

**Significant at 1% probability level.

* Significant at 5% probability level.

TotalEsr: ESR (Effective Spot Rate), the percentage of filtered Reads among the DNBs recognized by Basecalling. ESR = Total Reads/theoretical maximum reads number of one sequencing lane. TotalEsr calculated ESR value in the first 15 cycles in read1 and read2, and kept constant in the rest of each read.

Dnbnumber: The theoretical maximum number of DNBs on the patterned array.

BIC: Basecall information content, the percentage of DNBs that can be used for Basecalling among the DNBs recognized by the optical system. BIC = (numbers of DNB that can be used for Basecalling/numbers of DNB that can be recognized by the optical systems) $\times 100\%$.

accGRR: Accumulated Good Reads Rate, taking chastity greater than 0.6 as the filtering criteria, the percentage of filtered Reads among the DNBs recognized by Basecalling. accGRR = Total Reads/theoretical maximum reads number of one sequencing lane. This value is only a statistical indicator which reflects the overall quality of the read (multi-cycle state).

SNR: Signal to Noise Ratio, taking the SNR calculation of a single DNB as an example, base A (maximum light intensity) is used as the signal, the CGT is the background, and the variance of the CGT light intensity is noise. A_SNR=A_mean/CGT_dev.

FIT: FIT value represents the distribution of differences between signal and noise for each base. The FIT value is higher when the

distribution of differences between signal to noise for each channel/color are more concentrated.

SEQdata-BEACON

A

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Life is beautiful. Exploring the mystery of life will certainly make our life more beautiful and the world of life more beautiful!

B

*Dnbnumber: <u>Example format</u>
934364420

*TotalEsr:
0.965139972359

*BIC:
94.7004530713#94.4740412654#94.7259253686#94.3860635074#94.368247

*FIT:
0.866919358722#0.876793940418#0.872680660319#0.872026165848#0.873

*accGRR:
1#0.984478575553#0.973636308354#0.962293452703#0.953431246929

C

Predicted yield confidential intervals

649   701 714   756   798 811   862

95%
99%