Improved high-quality genome assembly and annotation of Tibetan hulless barley

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Abstract

Background

The Tibetan hulless barley (Hordeum vulgare L. var. nudum), also called “Qingke” in Chinese and “Ne” in Tibetan, is the staple food for Tibetans and an important livestock feed in the Tibetan Plateau. The Tibetan hulless barley in China has about 3500 years of cultivation history, mainly produced in Tibet, Qinghai, Sichuan, Yunnan and other areas. In addition, Tibetan hulless barley has rich nutritional value and outstanding health effects, including the beta glucan, dietary fiber, amylopectin, the contents of trace elements , which are higher than any other cereal crops.

Findings

Here, we reported an improved high-quality assembly of Tibetan hulless barley genome with 4.0 Gb in size. We employed the falcon assembly package ,scaffolding and error correction tools to finish improvement using PacBio long reads sequencing technology, with contig and scaffold N50 lengths of 1.563Mb and 4.006Mb, respectively, representing more continuous than the original Tibetan hulless barley genome nearly two orders of magnitude. We also re-annotated the new assembly, and reported 61,303 stringent confident putative protein-coding genes, of which 40,457 is HC genes. We have developed a new Tibetan hulless barley genome database (THBGD) to download and use friendly, as well as to better manage the information of the Tibetan hulless barley genetic resources.

Conclusions

The availability of new Tibetan hulless barley genome and annotations will take the genetics of Tibetan hulless barley to a new level and will greatly simplify the breeders effort. It will also enrich the granary of the Tibetan people.

Keyword: Tibetan hulless barley; Genome assembly; full-length cDNA; Tibetan hulless barley Genome Database

Introduction

Tibetan hulless barley (Hordeum vulgare L. var. nudum) is a cereal crop, a variant of barley, alias called hulless barley, yuan wheat, rice barley(**Fig.1**). Tibetan hulless barley has a long history of cultivation in the Qinghai-Tibet Plateau. The adaptation to extreme environmental conditions in high altitudes made it the staple food for Tibetans beginning at least 3,500–4,000 years ago [1]. It continues to be the predominant crop in Tibet, occupying ~70% of crop lands.

Genome sequences provide a substantial basis for understanding the biological essences of crops associated with biologically and economically essential traits[2]. Therefore, it is necessary and crucial to construct the genome of a species. In recent years, with the development of NGS sequencing technology, many crops have published the genome draft or near-finished genome sequences, especially the larger genome of the wheat family[3-13]. At the same time, two studies have published two versions of the Tibetan hulless barley genome in 2015[2] and 2017[14], respectively. And the former assembly strain is Lasa Goumang , the latter is Zangqing320, all excellent cultivars of Tibetan hulless barley. The estimated genome size of the Tibetan hulless barley is 4.48Gb[2], which is much smaller than the 5.1Gb[4, 10] of barley. Although the former is a subspecies of barley, considering that the barley genome is rich in repeats(about 80.8%-84%[2, 4, 10]), the difference in genome size may come from the numbers and length of transposons.

Although the publication of the Tibetan hulless barley genome has attracted a new upsurge in Tibetan hulless barley genetics research[15, 16], due to the current poor genome assembly, such as the particularly short contig, the very low contig N50, and the poor genome annotation, the functional genome studying of Tibetan hulless barley has caused some vital problems. In addition, with the development of single-molecule sequencing platforms, such as those by Pacific Biosciences and Oxford Nanopore Technologies, have become available to researchers and are currently being increasingly applied in *de novo* genome assembly and genome update , and other scaffolding techniques(such as BioNano optical mapping, Chicago, Hi-C and 10X genomics)[17-19], it provides a new opportunity for improving Tibetan hulless barley genome assembly.

Therefore, we *de novo* assembled a high-quality genome of Tibetan hulless barley using high-coverage Pacbio long reads, combining published Illumina paired-ends and mate-pairs reads. And then, we applied PacBio Iso-seq to get high confidence full-length transcripts and re-annotation Tibetan hulless barley protein-coding genes. This new assembly, annotations and newly online Tibetan hulless barley genomics database discussed below will serve as valuable resources for investigating the economic and genetic of Tibetan hulless barley and will also help the molecular breeders to continually improve Tibetan hulless barley or barley germplasm resources.

DNA and RNA sequencing

DNA isolation, libraries construction and sequencing

The seedling of Tibetan hulless barley (NCBI BioSample ID **SAMN09914874**) were collected from Tibet, China. After collection, tissues were immediately transferred into liquid nitrogen and stored until DNA extraction. DNA was extracted using the Cetyltrimethyl Ammonium Bromide (CTAB) method according to the protocal " Preparing Arabidopsis Genomic DNA for Size-Selected ~20 kb SMRTbell™ Libraries "(https://www.pacb.com/wp-content/uploads/2015/09/Shared-Protocol-Preparing-Arabidopsis-DNA-for-20-kb-SMRTbell-Libraries.pdf). The quality of the extracted genomic DNA was checked using 1% agarose gel electrophoresis, and the concentration was quantified using a Qubit fluorimeter (Invitrogen, Carlsbad, CA, USA).

Single-molecule Real-time long reads sequencing was performed at NextOmics Technology Corporation (Wuhan, China) with a PacBio Sequel Sequencer (Pacific Biosciences, Menlo Park, CA,USA). The SMRT Bell library was prepared using a DNA Template Prep Kit 1.0, and Six 20-kb SMRT Bell libraries were constructed(**Fig. S1**). Genomic DNA（10ug）was mechanically sheared using a Covaris g-Tube with a goal of DNA fragments of approximately 20kb. A Bioanalyzer 2100 12K DNA Chip assay was used to assess the fragment size distribution. Sheared genomics DNA(5ug) was DNA-damage repaired and end-repaired using polishing enzymes. A blunt-end ligation reaction followed by exonuclease treatment was conducted to generate the SMRT Bell template. A Blue Pippin device(Sage Science) was used to size select the SMRT Bell template and

enrich large fragments(>10kb).The size-selected library was quality inspected and quantified on an Agilent Bioanalyzer 12Kb DNA Chip (Agilent) and a Qubit fluorimeter (Life Technologies). A ready-to-sequence SMRT Bell Polymerase Complex was created using a Binding Kit 2.0 (PacBio p/n 100-862-200), according to the manufacturer's instructions. The PacBio Sequel instrument was programmed to load and sequenced the sample on PacBio SMRT cells v2.0 (Pacific Biosciences), acquiring one movie of 360 min per SMRT cell. The MagBead loading (Pacific Biosciences) method was used to improve the enrichment of the larger fragments. A total of 64 SMRT cells were processed yielding 300.24G subread sequences, average 4.69Gb per cell, and 9,358bp in length(**Table S1**).

For Illumina sequencing data, we applied previously published paired-ends and mate-pair library data[2](SRA no. SRA201388 , totally 819.37Gb clean data) for PacBio assembly error evaluation and scaffolding, respectively.

RNA isolation and sequencing

Fresh roots, and leaves of Tibetan hulless barley were sampled in Tibet, China. Totally, 12 samples ,different N condition (Prepared for unpublished paper) were used for Iso-seq sequencing. Total RNA was prepared by grinding tissue in TRIzol reagent (Invitrogen 15596026) on dry ice and processed following the protocol provided by the manufacturer. To remove DNA, an aliquot of total RNA was treated with RQ1 DNase (Promega M6101), followed by phenol/chloroform/isoamyl alcohol extraction, chloroform/isoamyl alcohol extraction using Phase Lock Gel Light tubes (5 PRIME 2302800) and ethanol precipitation. Precipitated RNA was stored at -20℃.

One microgram of total RNA per reaction per tissue was reverse transcribed using the Clontech SMARTer cDNA synthesis kit. Specific barcoded oligo dT in separate PCR tubes, to generate barcoded FL cDNA. Large-scale PCR products were purified with AMPure PB beads and quality control (QC) was performed on a 2100 BioAnalyzer (Agilent). Equimolar ratios of six cDNA libraries were pooled together. A total of 3.8 mg cDNA was subjected to size fractionation using the SageELF system. Size fractions eluted from the run were subjected QC and pooled in equimolar ratios for subsequent re-amplification to yield four libraries (1–2, 2–3, 3–6 and 5–10Kb). The pooled PCR products were purified using AMPure PB beads. One to five micrograms of purified

amplicons were subjected to Iso-Seq SMRT Bell library preparation (https://pacbio.secure.force.com/SamplePrep) .A total of 17 SMRT cells (unpublished data) were sequenced on the PacBio RS II platform using P6-C4 chemistry with 3–4 h movies.

Genome assembly

The Sequel raw bam files were converted into subreads in fasta format using the standard Pacbio software package BAM2fastx. Totally, 300.24Gb PacBio data, covered genome ~67X(estimated genome size 4.48Gb),was used to initially assemble.

Falcon assembly

we used falcon software package (https://github.com/PacificBiosciences/falcon) to construct primary assembly. Firstly, corrected the error reads by the overlap strategy (parameter length_cutoff control is used to correct the wrong seed reads length);Secondly, pre-assembled the corrected reads; Thirdly, Constructed contig with long reads obtained from pre-assembly (parameter length_cutoff_pr controls the pre-assembled reads length used to build contig); After many attempts, we determined that the following two reference settings can get better assembly results: length_cutoff = 5000;length_cutoff_pr = 10000. And, we get the initial assembly, 1.229Mb in length of contig N50.

Arrow polish

There are often errors in the preliminary assembly results of the genome, such as SNP or InDel, which can be fixed by certain algorithms. Arrow is a genomic polishing tool based on hidden Markov models. We use the following steps to polish the genome:

1) Align the PacBio sequencing reads to the genome assembly with blasr (pbalign --algorithm=blasr, the rest of the parameters default);

2) Use Arrow to polish based on the alignment result (variantCaller --algorithm=arrow, the rest of the parameters default).pbalign and variantCaller are from the SMRT Link 4 toolkit (https://www.pacb.com/support/software-downloads/)

Scaffolding

The Scaffolding tool can group, sort, and orient contigs to join contigs into longer sequence fragments, called scaffold. The sequence between some contigs may be unknown but the distance between them can be roughly known, which is also helpful for subsequent related work.

SSPACE[20] is a widely used tool for building scaffold. Firstly aligned the reads to contigs using alignment tools (such as BWA, bowtie), and then built scaffold based on the connection information between the contigs. This project uses Illumina data previously published and PacBio data to build scaffold.

Firstly , using Illumina mate-pair library (20kb and 40Kb fragment long) sequencing data to scaffolding contigs. Aligned the paired-end reads to contigs with bowtie[21]; then, constructed scaffold (parameter: -p 1 -g 2) with SSPACE-STANDARD-3.0.

Secondly, using PacBio data to continuously improving the scaffolds. Aligned PacBio reads to the scaffold version of Illumina data using blasr; and then, built scaffold with SSPACE-LongRead[22].

The final assembly produced 1,856 scaffolds, 1,092 of which greater than or equal to 1Mb, with a contig N50 length of 1.563Mb and scaffold N50 of 4.006Mb. Compared to the preliminary draft of the published two Tibetan hulless barley genomes[2, 14], the contiguity was improved more than ~80 and 243 times, respectively(**Table 1**;**Table S2-S3;Fig. S2**).

Assembly evaluation

First, the 682.57Gb Illumina paired-ends reads(library fragment 250,500 and 800,respectively) were mapped to new assembly, and 99.87-99.89% PE reads were mapped concordantly (**Table S4**).

Using these short reads, the estimated quality value (QV) of Tibetan hulless barley genome was calculated according to a previously described method[23]. The erroneous bases in the genome assembly were identified using the variant calling software Genome Analysis Toolkit (GATK) v4[24] with default parameters. The QV of

the Tibetan hulless barley genome was estimated to be 52(**Table S5**), which means that the accuracy of the assembly in base level is very accurate after base correction.

We randomly downloaded 4 *Hordeum vulgare subsp. vulgare* (domesticated barley) BAC clone sequencing data (NCBI ID: AC248476.1,AC250041.1,AC253057.1 and AC253422.1) to evaluate assembly quality. Using nucmer[25] mapping BAC data to new assembly, the results manifested very high consistency.(**Fig. S3**).

In addition, we also used BUSCO[26] to perform an assessment of genome completeness . BUSCO used a total of 1440 genes to evaluate the genome; the genome has 1312 complete BUSCO genes, accounting for 91.1%, of which 1288 have single copies, accounting for 89.4%, 24 have multiple copies, accounting for 1.7%; 34 gene have only a few fragments of the gene; 94 genes are missing from the genome.

Together, the results indicated that our dataset represented a genome assembly with a high level of coverage, quality and completeness.

Repetitive sequences annotation

Repetitive DNA accounts for much of the remarkable variation in plant genome sizes[27], especially some Gramineae crop plants, above 80% [10, 12, 28] of genome for repetitive elements . Repetitive sequences are divided into transposons and simple repeats. According to Tandem repeats finder program[29] , we marked simple repeat elements, 155,813,415 in length , 3.89% of genome. For transposons, we performed homology-based and de novo prediction, respectively. The software packages involved are RepeatMasker[30], RepeatProteinMask[30] , repeatmodeler (http://www.repeatmasker.org/RepeatModeler/) and ltr_finder[31]. The repetitive database used here is Repbase 21.01[32](**Fig. S4**). Long terminal repeat(LTR) retroelements, belong to class I elements, is accounted for 70.22% of genome. All repeats together accounted for 87.48% of the genome(**Table 2**;**Table S6**).

Gene prediction

For the annotation of protein-coding gene, we used a combination of multiple data sources, followed by a high confidence (HC) and low confidence (LC) classification of the annotated genes(**Fig. S4-5**).

Combination of multiple annotations

According to Iso-seq methods, we obtained 39,442 high-quality full-length transcripts. Theses high-quality transcripts were aligned to the genome using GMAP (version 2017-05-08)[33], with a total of 14,099 perfect alignments; TransDecoder v4.0.1(https://github.com/TransDecoder) was used to predict the coding region of 14,099 transcripts, of which 13,936 contained open reading frame (ORF), these ORF contained transcripts are ultimately assigned to 9,360 genes, called perfect genes. We employed Augustus v3.3[34],which used perfect genes as input train sets, to get 128,400 putative genes. At the same time ,we downloaded representative proteins of 7 related species (*Triticum aestivum A*[12], *Triticum aestivum D*[9], *Brachypodium distachyon*[35], *Hordeum vulgare*[10], *Oryza sativa*[36], *Sorghum bicolor*[37] and *Zea mays*[38]) from public database(**Table S7**). Aligning the protein sequences of each species to the Tibetan hulless barley genome using tblastn v2.6.0+[39]; and then, using Genewise v2.4.1[40] to predict gene structure and obtain homologous gene sequences from various species in the Tibetan hulless barley genome. In addition, using 16 transcriptome data (**Table S8**) from different tissues and different conditions, we mapped the reads to Tibetan hulless barley genome by hisat2 v2.1.0[41] and reference-based assembled the 47,490 transcripts by stringtie v1.2.4[42, 43]. The gene information extracted from PacBio Iso-seq, Augustus, Homolog and RNA-seq was integrated using EVidenceModeler[44], according to different weight parameters (**Table S9**), to obtain 129,269 putative genes. The same RNA-seq reads were de novo assembled using Trinity v2.4.0[45] to obtain 722,803 transcripts. Structural optimization of EVM integration results was performed using PASA v2.1.0[46] and Trinity assembly results.

129,269 transcripts were annotated with NR, Uniprot, and Pfam database. And filter the putative genes according to the function assignment result: (1) NR, Uniprot comparison identity>=50%, coverage>=50%; (2) Not satisfying (1), but protein length >= 50, and containing the Pfam domain. The tandem duplicated genes were identified using MCScanX. Only the longest protein of each subgroup was kept to represent the

corresponding gene. The combine Tibetan hulless barley genome annotation included 65,833 genes with names derived from BLAST analysis and genes with domain names derived from Pfam analysis.

High-confidence Genes

Stringent confidence classification was applied to all predicted genes to discriminate between loci representing high-confidence (HC) protein-coding genes and less reliable low-confidence (LC) genes, which potentially consisted of gene fragments, putative pseudogenes and non-(protein)-coding transcripts. We assigned confidence values to a gene model in a two-step procedure by using the similar criteria and methods described previously[10](**Fig. S5**). Finally, we obtained 61,303 stringent confident putative genes, of which 40,457 is HC genes(**Table 3**; **Fig. S5**).

Functional annotation of all 61,303 protein sequences was performed using NR, KEGG, SwissProt, Trembl, GO, PFAM, and InterPro databases. 96.57% of all protein sequences were assigned to at least one database(**Table S10**).

Non-coding Gene prediction

In the process of annotation of non-coding RNAs, tRNAscan-SE[47] software is used to search for tRNA sequences in the genome according to the structural characteristics of tRNA; since rRNA is highly conserved, the rRNA sequence of related species can be selected as a reference sequence, through BLASTN Alignment to find rRNA in the genome; in addition, using the Rfam family's covariance model, Rfam's own INFERNAL software[48] can predict miRNA and snRNA sequence information on the genome(**Table 4**).

Annotation evaluation

The BUSCO[26] assessment showed that 91% of the 1,440 genes were completed annotated. And RNA-seq reads rates mapping to gene set also have a significant improvement, from 60% previously annotation to exceed 70%(**Table S8**).

In summary, we also acquired a high-quality genome annotation, and it will greatly benefit future function genomics research.

Genome database

A new Tibetan hulless barley Genome Database(THBGD) has been developed to facilitate studies on Qinghai-Tibet Plateau crop plant genomics. The database currently provides access to Tibetan hulless barley genome assembly V1,de novo assembly using NGS sequencing and V2,de novo assembly improvement using combined TGS and NGS. Meanwhile, it also provides access to genome annotation, including repetitive element annotation, ncRNA prediction and protein-coding genes prediction(**Fig. 2**). The THBGD embedded JBrowse[49] visual genomic display module to display genome and gene annotation information. One can click on the gene name on the screen to display specific information about the gene. One also can use online tools Blast v2.4.0+ to enter the query sequence in fasta format, align and return the visual aligned results. In the future, we will focus on optimizing the interactive operation and visualization of the THBGD.

You can access to the THBGD by visiting http://show.genebang.com/project/index

Discussion

Tibetan hulless barley is a local species of barley and is of great significance to the life and culture of the Tibetan. Its genome and annotation updates help future research on functional genomics, and also help breeders to simplify their work and enrich the genomics resources of barley. The generation of Tibetan hulless barley high-quality reference genomes also demonstrates the superiority of PacBio long reads sequencing technology for larger genome assembly[50]. Although the genome has a long contig, the scaffold is still a bit short at the state-of-art of the current genome assembly, mainly because of the complexity and data volume of the scaffolding mate-pair library is relatively few. In the future, we will use BioNano optical mapping, Hi-C, and akin weapon to complete the genomic construction at the chromosome level[17]. Having an excellent Tibetan hulless barley genomics resources, it will enable geneticists to focus more on functional genomics itself and breeding research.

Availability of supporting data

Raw genomic sequence reads are available in the NCBI Sequence Read Archive under project number **SRS3725794**. Genome assembly and annotation file are available in Tibetan hulless barley Genome Database (THBGD) or contact the correspondence author. Supplementary files can be downloaded from the GigaScience Website.

Abbreviations

BLAST: Basic Local Alignment Search Tool;

BUSCO: Benchmarking Universal Single-Copy Orthologs;

QV:quality value;

PacBio: Pacifc Biosciences;

RNA-seq: RNA sequencing;

NGS: Next generation sequencing

TGS: Third generation sequencing

THBGD: Tibetan hulless barley Genome Database

Conflicts of Interest

The authors declare no conflict of interest.

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Sampling and experimentation: X.Z.,Y.W.,H.Y.,Q.X.,S.Z.,Z.W.,W.Q.,Y.B.,J.D.,M.Y..

Genome assembly and Genome annotation guidance and performance : X.L.(Co-leader) and his team.

Data analysis: T.X., X.Z.,Y.W.,H.Y.,Q.X.,S.Z.,Z.W.,W.Q.,Y.B.,J.D.,M.Y..

Genome database construction: T.X. and his team

Data submission: T.X.

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Reference

1. Fu DX, Ruan RW, Dai XM and Liu YM. Astudy of ancient barley, wheat and millet discovered at Changguo in Tibet. 2000.
2. Zeng X, Long H, Wang Z, Zhao S, Tang Y, Huang Z, et al. The draft genome of Tibetan hulless barley reveals adaptive patterns to the high stressful Tibetan Plateau. Proceedings of the National Academy of Sciences of the United States of America. 2015;112 4:1095-100. doi:10.1073/pnas.1423628112.
3. Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, et al. Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature. 2012;491 7426:705-10. doi:10.1038/nature11650.
4. International Barley Genome Sequencing C, Mayer KF, Waugh R, Brown JW, Schulman A, Langridge P, et al. A physical, genetic and functional sequence assembly of the barley genome. Nature. 2012;491 7426:711-6. doi:10.1038/nature11543.
5. Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, et al. Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature. 2013;496 7443:91-5. doi:10.1038/nature12028.
6. Ling HQ, Zhao S, Liu D, Wang J, Sun H, Zhang C, et al. Draft genome of the wheat A-genome progenitor Triticum urartu. Nature. 2013;496 7443:87-90. doi:10.1038/nature11997.
7. International Wheat Genome Sequencing C. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science. 2014;345 6194:1251788. doi:10.1126/science.1251788.

8. Avni R, Nave M, Barad O, Baruch K, Twardziok SO, Gundlach H, et al. Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science. 2017;357 6346:93-7. doi:10.1126/science.aan0032.
9. Luo MC, Gu YQ, Puiu D, Wang H, Twardziok SO, Deal KR, et al. Genome sequence of the progenitor of the wheat D genome Aegilops tauschii. Nature. 2017;551 7681:498-502. doi:10.1038/nature24486.
10. Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, et al. A chromosome conformation capture ordered sequence of the barley genome. Nature. 2017;544 7651:427-33. doi:10.1038/nature22043.
11. Zhao G, Zou C, Li K, Wang K, Li T, Gao L, et al. The Aegilops tauschii genome reveals multiple impacts of transposons. Nat Plants. 2017;3 12:946-55. doi:10.1038/s41477-017-0067-8.
12. Ling HQ, Ma B, Shi X, Liu H, Dong L, Sun H, et al. Genome sequence of the progenitor of wheat A subgenome Triticum urartu. Nature. 2018;557 7705:424-8. doi:10.1038/s41586-018-0108-0.
13. Appels R, Eversole K, Feuillet C, Keller B, Rogers J, Stein N, et al. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science. 2018;361 6403 doi:10.1126/science.aar7191.
14. Dai F, Wang X, Zhang XQ, Chen Z, Nevo E, Jin G, et al. Assembly and analysis of a Tibetan hulless barley reference genome demonstrate its close genetic relation to modern cultivated barley. Plant Biotechnol J. 2018;16 3:760-70. doi:10.1111/pbi.12826.
15. Wei Z, Zeng X, Qin C, Wang Y, Bai L, Xu Q, et al. Comparative transcriptome analysis revealed genes commonly responsive to varied nitrate stress in leaves of Tibetan hulless barley. Frontiers in plant science. 2016;7:1067.
16. Zeng X, Bai L, Wei Z, Yuan H, Wang Y, Xu Q, et al. Transcriptome analysis revealed the drought-responsive genes in Tibetan hulless barley. BMC genomics. 2016;17 1:386.
17. Sedlazeck FJ, Lee H, Darby CA and Schatz MC. Piercing the dark matter: bioinformatics of long-range sequencing and mapping. Nat Rev Genet. 2018;19 6:329-46. doi:10.1038/s41576-018-0003-4.
18. Yuan Y, Bayer PE, Batley J and Edwards D. Improvements in Genomic Technologies: Application to Crop Genomics. Trends Biotechnol. 2017;35 6:547-58. doi:10.1016/j.tibtech.2017.02.009.
19. Paajanen P, Kettleborough G, Lopez-Girona E, Giolai M, Heavens D, Baker D, et al. A critical comparison of technologies for a plant genome sequencing project. bioRxiv. 2017; doi:10.1101/201830.
20. Boetzer M, Henkel CV, Jansen HJ, Butler D and Pirovano W. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics. 2011;27 4:578-9. doi:10.1093/bioinformatics/btq683.
21. Langmead B. Aligning short sequencing reads with Bowtie. Curr Protoc Bioinformatics. 2010;Chapter 11:Unit 11.7. doi:10.1002/0471250953.bi1107s32.
22. Boetzer M and Pirovano W. SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information. BMC Bioinformatics. 2014;15 1:211. doi:10.1186/1471-2105-15-211.

23. Xia M, Han X, He H, Yu R, Zhen G, Jia X, et al. Improved de novo genome assembly and analysis of the Chinese cucurbit Siraitia grosvenorii, also known as monk fruit or luo-han-guo. Gigascience. 2018;7 6 doi:10.1093/gigascience/giy067.
24. Heldenbrand JR, Baheti S, Bockol MA, Drucker TM, Hart SN, Hudson ME, et al. Performance benchmarking of GATK3.8 and GATK4. bioRxiv. 2018; doi:10.1101/348565.
25. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, et al. Versatile and open software for comparing large genomes. Genome Biology. 2004;5 2:R12. doi:10.1186/gb-2004-5-2-r12.
26. Simao FA, Waterhouse RM, Ioannidis P, Kriventseva EV and Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31 19:3210-2. doi:10.1093/bioinformatics/btv351.
27. Paterson AH. Leafing through the genomes of our major crop plants: strategies for capturing unique information. Nat Rev Genet. 2006;7 3:174-84. doi:10.1038/nrg1806.
28. Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, et al. The B73 maize genome: complexity, diversity, and dynamics. Science. 2009;326 5956:1112-5. doi:10.1126/science.1178534.
29. Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Research. 1999;27 2:573-80.
30. Tarailo-Graovac M and Chen N. Using RepeatMasker to Identify Repetitive Elements in Genomic Sequences. Current Protocols in Bioinformatics. 2009;25 1:4.10.1-4..4. doi:doi:10.1002/0471250953.bi0410s25.
31. Xu Z and Wang H. LTR_FINDER: an efficient tool for the prediction of full-length LTR retrotransposons. Nucleic Acids Research. 2007;35 suppl_2:W265-W8. doi:10.1093/nar/gkm286.
32. Bao W, Kojima KK and Kohany O. Repbase Update, a database of repetitive elements in eukaryotic genomes. Mobile DNA. 2015;6 1:11. doi:10.1186/s13100-015-0041-9.
33. Wu TD and Watanabe CK. GMAP: a genomic mapping and alignment program for mRNA and EST sequences. Bioinformatics. 2005;21 9:1859-75. doi:10.1093/bioinformatics/bti310.
34. Stanke M and Waack S. Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics. 2003;19 suppl 2:ii215-ii25.
35. Initiative IB. Genome sequencing and analysis of the model grass Brachypodium distachyon. Nature. 2010;463 7282:763.
36. Du H, Yu Y, Ma Y, Gao Q, Cao Y, Chen Z, et al. Sequencing and de novo assembly of a near complete indica rice genome. Nature communications. 2017;8:15324.
37. McCormick RF, Truong SK, Sreedasyam A, Jenkins J, Shu S, Sims D, et al. The Sorghum bicolor reference genome: improved assembly, gene annotations, a transcriptome atlas, and signatures of genome organization. The Plant Journal. 2018;93 2:338-54.

38. Jiao Y, Peluso P, Shi J, Liang T, Stitzer MC, Wang B, et al. Improved maize reference genome with single-molecule technologies. Nature. 2017;546 7659:524.
39. Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ. Basic local alignment search tool. Journal of molecular biology. 1990;215 3:403-10. doi:10.1016/s0022-2836(05)80360-2.
40. Birney E, Clamp M and Durbin R. GeneWise and Genomewise. Genome Research. 2004;14 5:988-95. doi:10.1101/gr.1865504.
41. Kim D, Langmead B and Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nature Methods. 2015;12:357. doi:10.1038/nmeth.3317
https://www.nature.com/articles/nmeth.3317#supplementary-information.
42. Pertea M, Pertea GM, Antonescu CM, Chang T-C, Mendell JT and Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nature Biotechnology. 2015;33:290. doi:10.1038/nbt.3122
https://www.nature.com/articles/nbt.3122#supplementary-information.
43. Pertea M, Kim D, Pertea GM, Leek JT and Salzberg SL. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nature Protocols. 2016;11:1650. doi:10.1038/nprot.2016.095
https://www.nature.com/articles/nprot.2016.095#supplementary-information.
44. Haas BJ, Salzberg SL, Zhu W, Pertea M, Allen JE, Orvis J, et al. Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. Genome Biology. 2008;9 1:R7. doi:10.1186/gb-2008-9-1-r7.
45. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology. 2011;29:644. doi:10.1038/nbt.1883
https://www.nature.com/articles/nbt.1883#supplementary-information.
46. Haas BJ, Delcher AL, Mount SM, Wortman JR, Smith Jr RK, Hannick LI, et al. Improving the Arabidopsis genome annotation using maximal transcript alignment assemblies. Nucleic acids research. 2003;31 19:5654-66.
47. Lowe TM and Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic acids research. 1997;25 5:955-64.
48. Nawrocki EP, Burge SW, Bateman A, Daub J, Eberhardt RY, Eddy SR, et al. Rfam 12.0: updates to the RNA families database. Nucleic acids research. 2014:gku1063.
49. Skinner ME, Uzilov AV, Stein LD, Mungall CJ and Holmes IH. JBrowse: a next-generation genome browser. Genome Res. 2009;19 9:1630-8. doi:10.1101/gr.094607.109.
50. Li C, Lin F, An D, Wang W and Huang R. Genome Sequencing and Assembly by Long Reads in Plants. Genes. 2017;9 1 doi:10.3390/genes9010006.

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Tables

Table 1 Comparison of the new genome with previously published assemblies of the Tibetan hulless barley genome.

	This study	**Zeng *et al.*[2]**	**Dai *et al.*[14]**
Sequenced genome size (Gb)	4.00	3.89	3.73
Contig N50 (Kb)	1,563.00	18.07	5.94
Scaffold N50 (Kb)	4,006.00	242.00	171.11
Repeat proportion(%)	87.48	81.39	NA
Annotated protein-coding genes	61,303*	36,151	46,787

* Only refer to stringent confident putative protein-coding genes.

Table 2 Repetitive element annotation statistics.

Type	**Repeat Size**	**% of genome**
Trf	155,813,415	3.89
Repeatmasker	2,935,487,965	73.27
Proteinmask	19,934,506	0.50
De novo	3,359,143,070	83.85
Total	3,504,452,012	**87.48**

Table 3 Protein-coding genes prediction summary.

Method	Software and gene set	Gene number
PacBio	Smrtlink	39,442
	Gmap	14,099
	TransDecoder	13,936
Ab initio	Augustus	128,400
Homology-based	Triticum aestivum A	42,601
	Brachypodium distachyon	33,911
	Hordeum vulgare	46,312
	Oryza sativa	38,426
	Sorghum bicolor	29,909
	Triticum aestivum D	44,897
	Zea mays	29,872
RNA-seq	StringTie	47,490
Integration	EVidenceModeler	129,269
	PASA	129,269
Filter	NR、SwissProt、PFAM	68,018
	After MCScanX moved	65,833
	After TE moved	**61,303**

Table 4 ncRNA genes annotation statistics.

Type		Copy	Average length(bp)	Total length(bp)	% of genome
miRNA		5,530	129.27	714,857	0.0178
tRNA		2,477	74.71	185,064	0.0046
rRNA	**Total**	1,583	151.69	240,131	0.0060
	18S	63	1,076.60	67,826	0.0017
	28S	174	133.20	23,177	0.0006
	5.8S	39	147.33	5,746	0.0001
	5S	1,307	109.70	143,382	0.0036
snRNA	**Total**	1,156	115.14	133,097	0.0033
	CD-box	831	103.44	85,960	0.0021
	HACA-box	132	133.48	17,620	0.0004
	splicing	193	152.94	29,517	0.0007

Figures

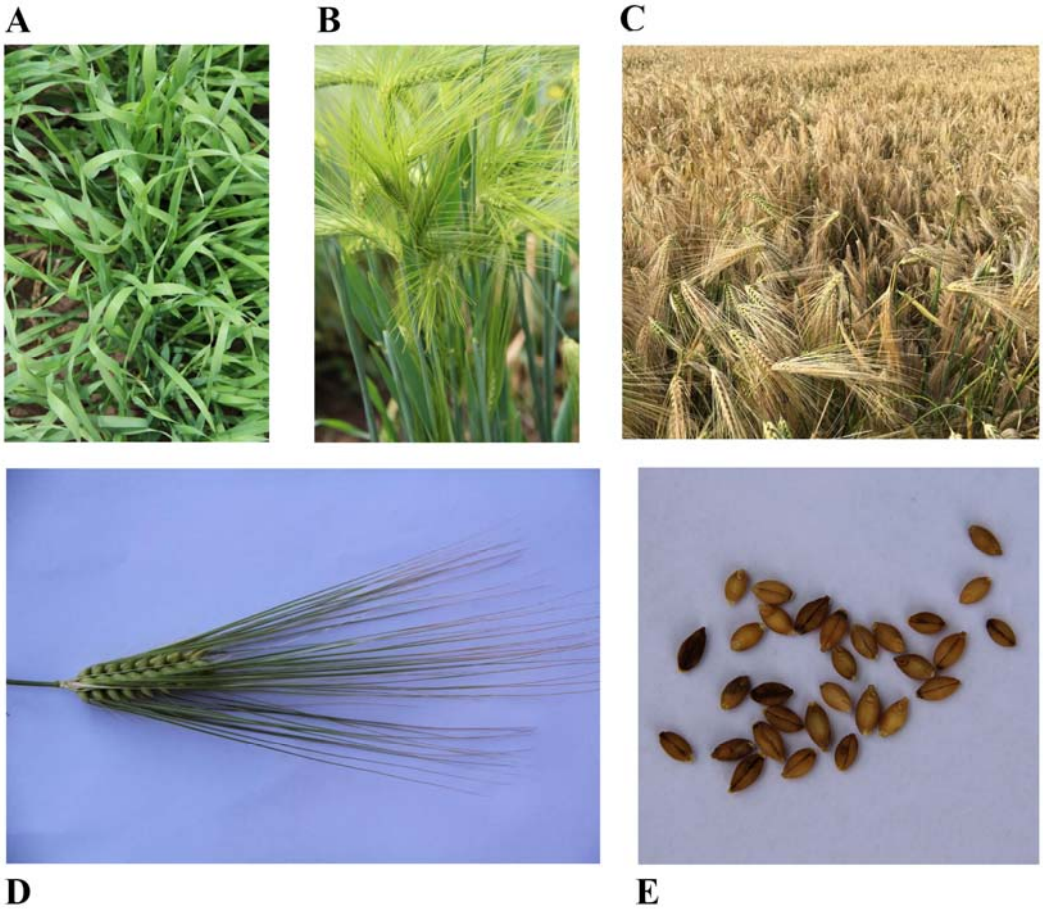


Figure 1 Morphological characteristics of the Tibetan hulless barley, Tibetan hulless barley. These pictures show (A),seedling;(B), heading stage; (C), mature stage;(D), filling stage spike and (E), grain of Tibetan hulless barley.

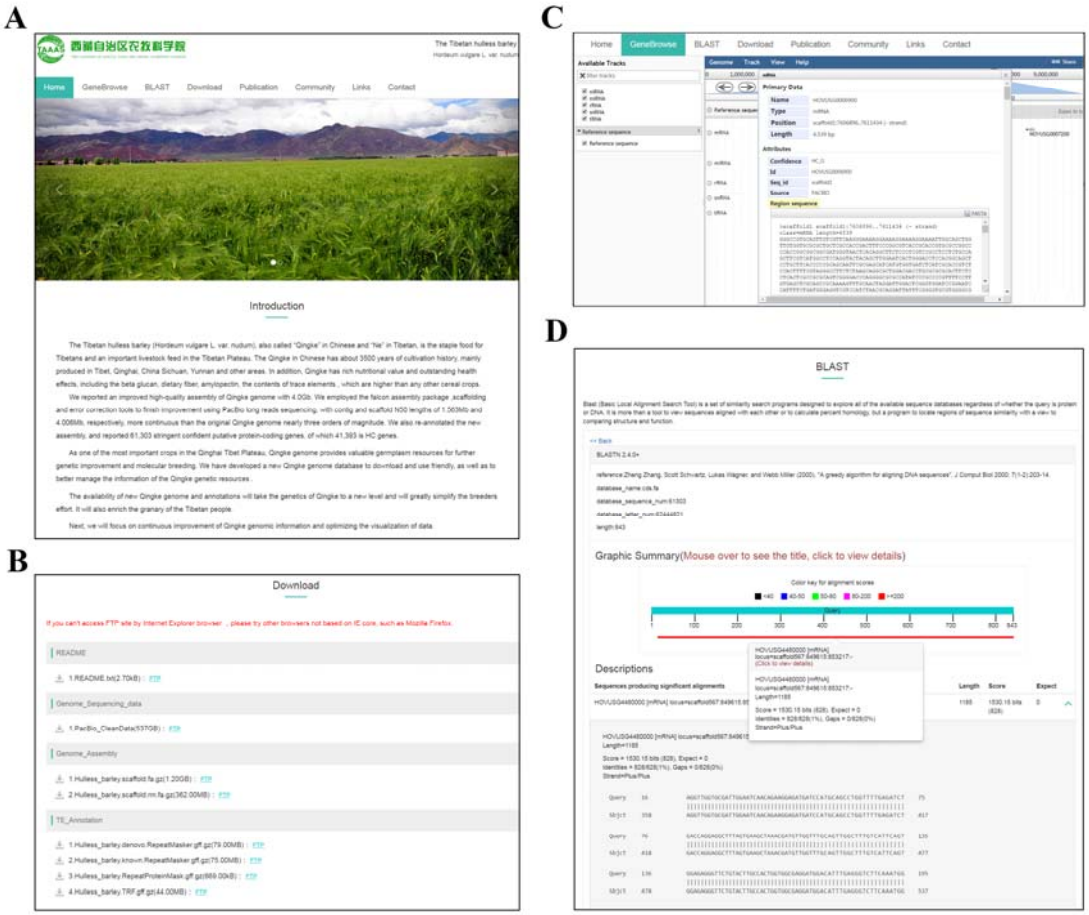


Figure 2 Tibetan hulless barley Genome Database(THBGD). (A),the home page of THBGD;(B), Data download page;(C),gene browse page and (D),sequences BLAST page.

A

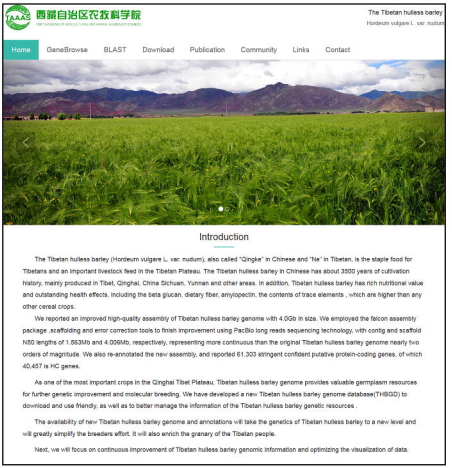
B

C

D

E

A


西藏自治区农牧科学院
The Tibetan hulless barley
Hordeum vulgare L. var. nudum
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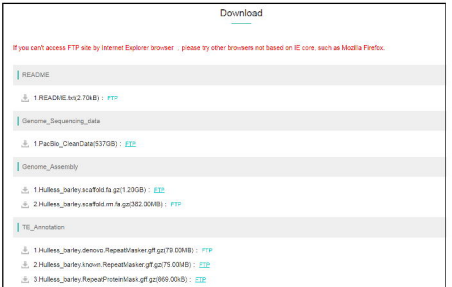
Introduction
The Tibetan hulless barley (Hordeum vulgare L. var. nudum), also called "Qingke" in Chinese and "Ne" in Tibetan, is the staple food for Tibetans and an important livestock feed in the Tibetan Plateau. The Tibetan hulless barley in Chinese has about 3500 years of cultivation history, mainly produced in Tibet, Qinghai, China Sichuan, Yunnan and other areas. In addition, Tibetan hulless barley has rich nutritional value and outstanding health effects, including the beta glucan, dietary fiber, amylopectin, the contentions of trace elements , which are higher than any other cereal crops.
We reported an improved high-quality assembly of Tibetan hulless barley genome with 4.0Gb in size. We employed the falcon assembly package ,scaffolding and error correction tools to finish improvement using PacBio long reads sequencing technology, with contig and scaffold N50 lengths of 1.563Mb and 4.006Mb, respectively, representing more continuous than the original Tibetan hulless barley genome nearly two orders of magnitude. We also re-annotated the new assembly, and reported 61,303 stringent confident putative protein-coding genes, of which 40,457 is HC genes.
As one of the most important crops in the Qinghai Tibet Plateau, Tibetan hulless barley genome provides valuable germplasm resources for further genetic improvement and molecular breeding. We have developed a new Tibetan hulless barley genome database(THBGD) to download and use friendly, as well as to better manage the information of the Tibetan hulless barley genetic resources .
The availability of new Tibetan hulless barley genome and annotations will take the genetics of Tibetan hulless barley to a new level and will greatly simplify the breeders effort. It will also enrich the granary of the Tibetan people.
Next, we will focus on continuous improvement of Tibetan hulless barley genomic information and optimizing the visualization of data.

B


Download
If you can't access FTP site by Internet Explorer browser , please try other browsers not based on IE core, such as Mozilla Firefox.
README
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Genome_Sequencing_data
1.PacBio_CleanData(537GB) : FTP
Genome_Assembly
1.Hulless_barley.scaffold.fa.gz(1.23GB) : FTP
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TE_Annotation
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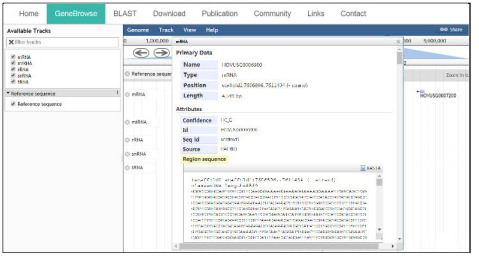
Home
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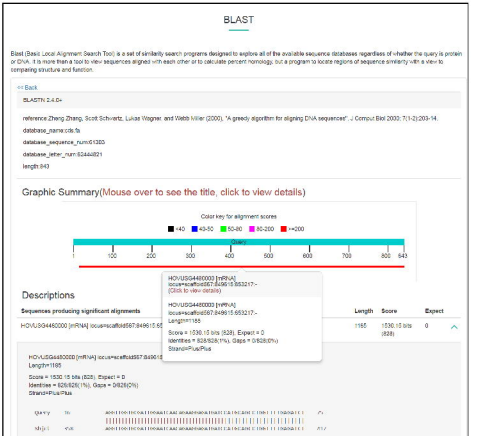
BLAST
Blast (Basic Local Alignment Search Tool) is a set of similarity search programs designed to explore all of the available sequence databases regardless of whether the query is protein or DNA. It is more than a tool to view sequences aligned with each other or to calculate percent homology, but a program to locate regions of sequence similarity with a view to comparing structure and function.
<< Back
BLASTN 2.4.0+
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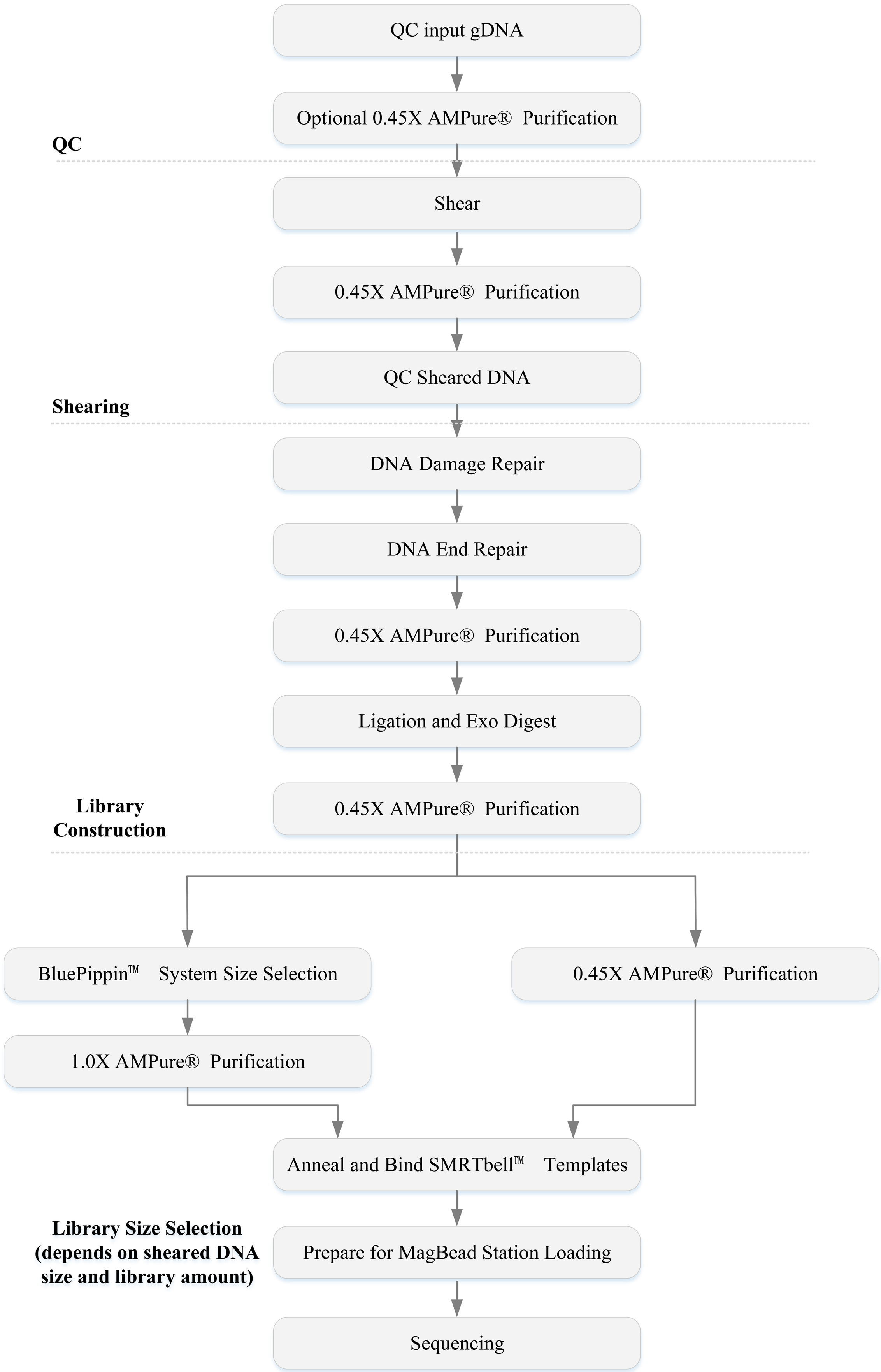
Descriptions
Sequences producing significant alignments
Length
Score
Expect


QC input gDNA
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QC Sheared DNA
Shearing
DNA Damage Repair
DNA End Repair
0.45X AMPure® Purification
Ligation and Exo Digest
0.45X AMPure® Purification
Library Construction
BluePippin™ System Size Selection
0.45X AMPure® Purification
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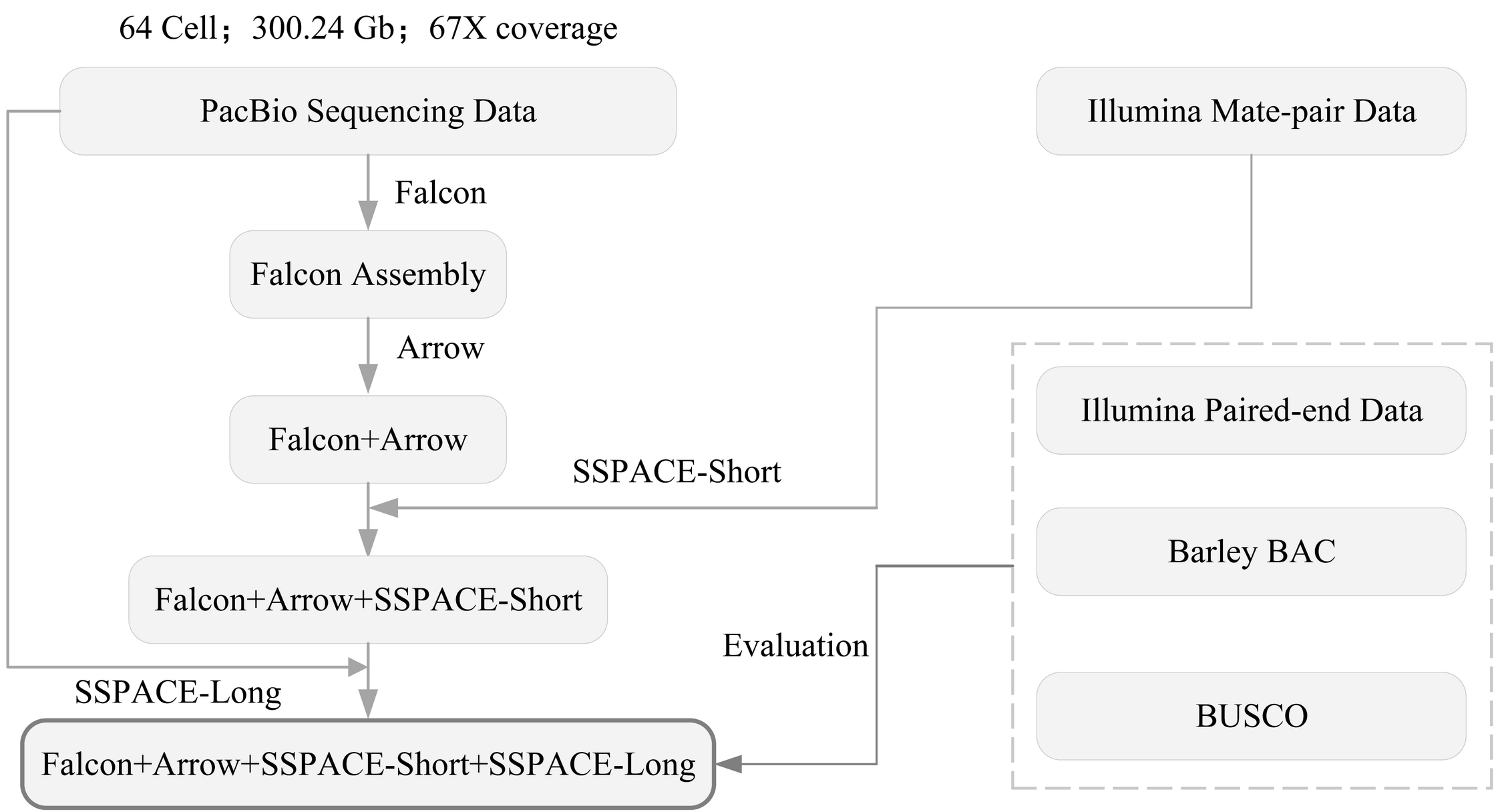
Sequencing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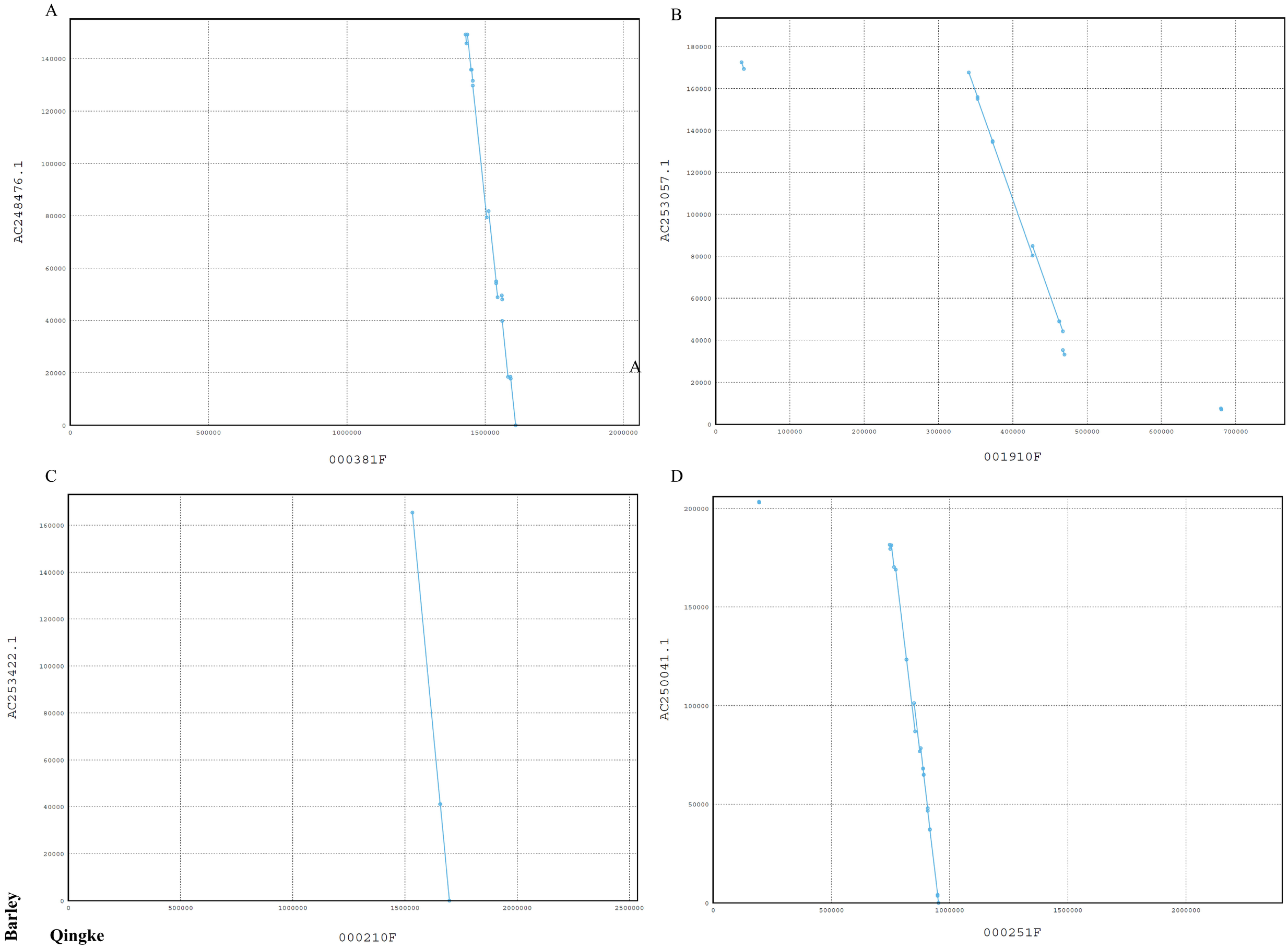
Barley BAC
BUSCO
Evaluation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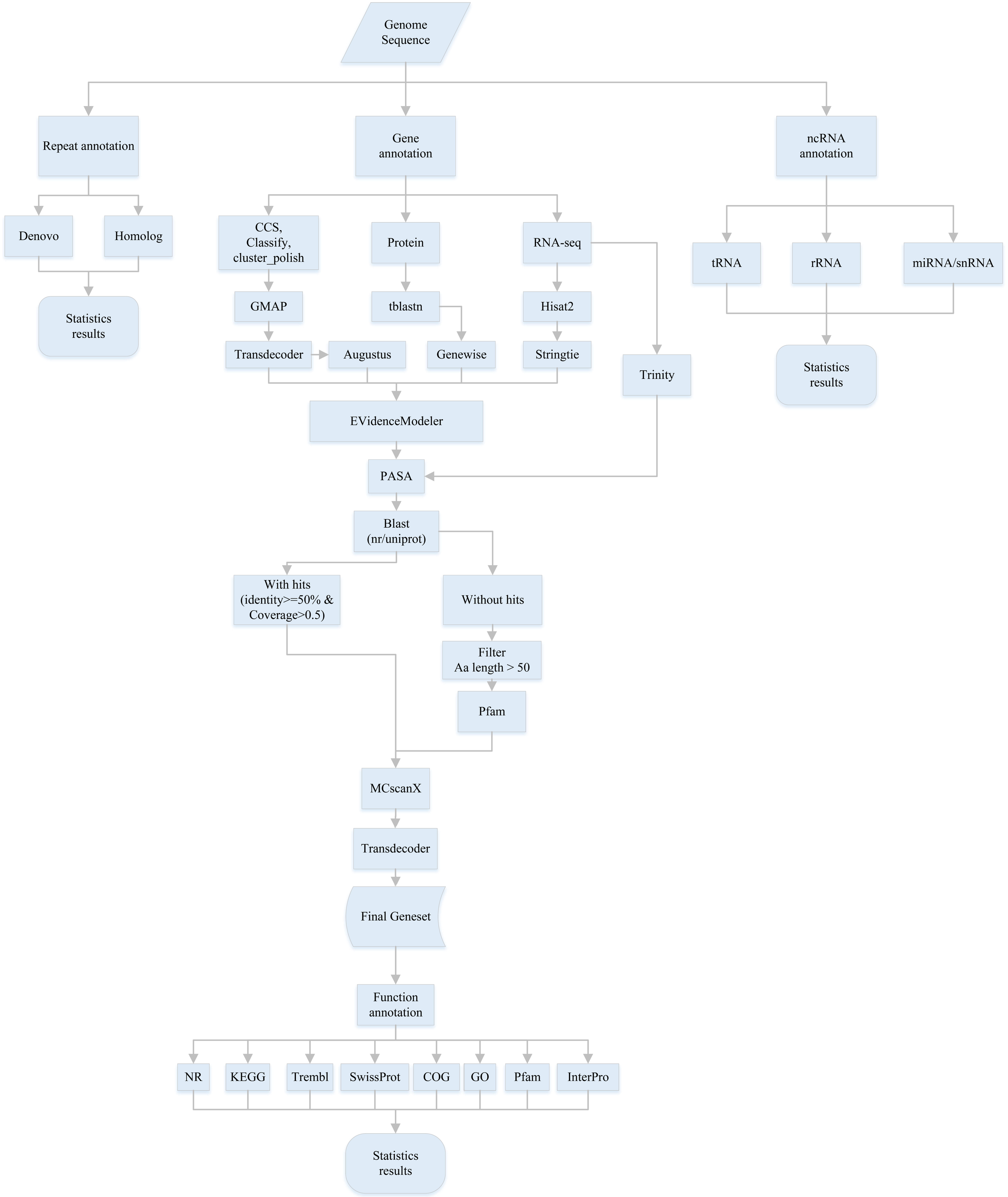
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Gene annotation
ncRNA annotation
Denovo
Homolog
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CCS, Classify, cluster_polish
Protein
RNA-seq
GMAP
tblastn
Hisat2
Transdecoder
Augustus
Genewise
Stringtie
Trinity
EVidenceModeler
PASA
Blast (nr/uniprot)
With hits (identity>=50% & Coverage>0.5)
Without hits
Filter Aa length > 50
Pfam
MCscanX
Transdecoder
Final Geneset
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Pfam
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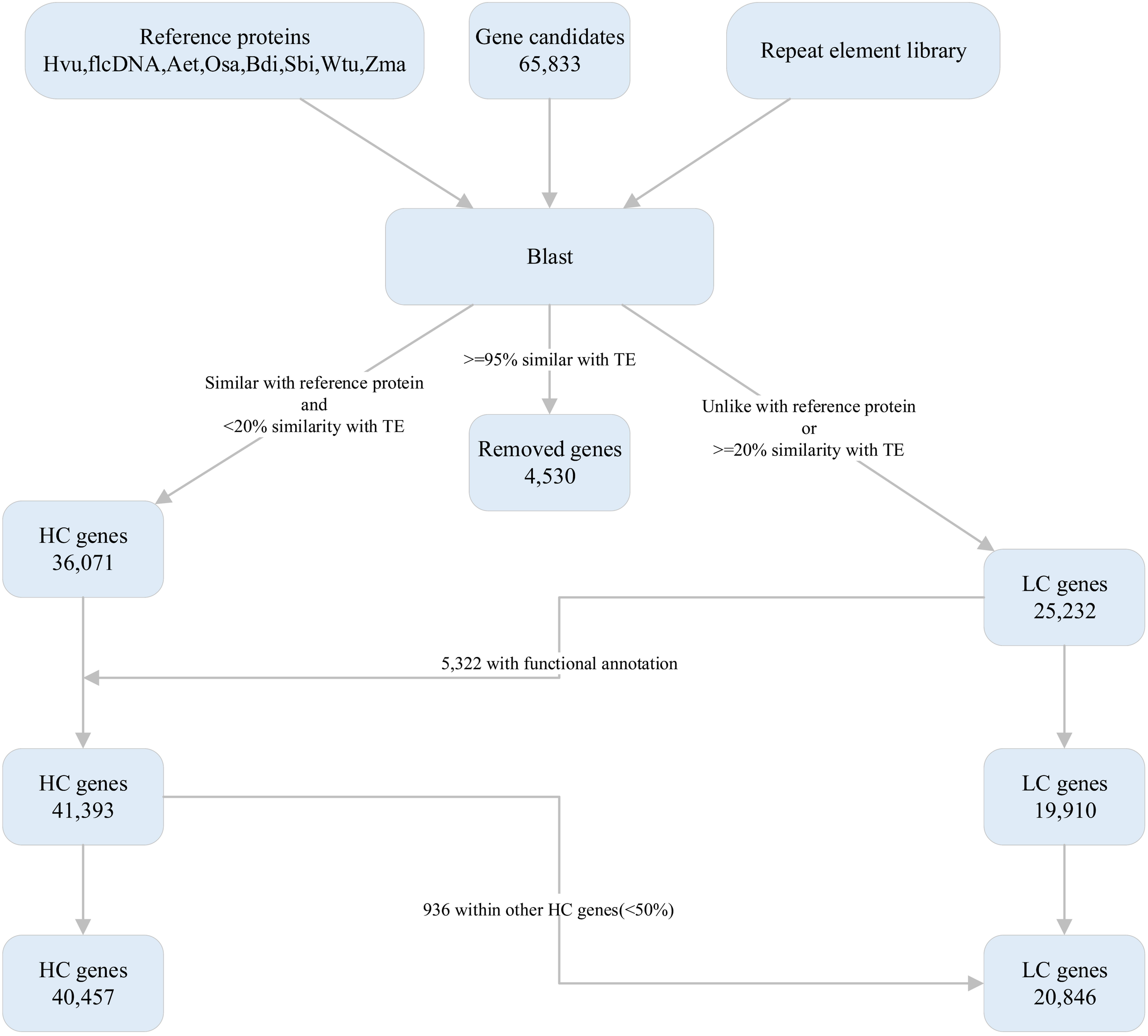
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Blast
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and
<20% similarity with TE
>=95% similar with TE
Unlike with reference protein
or
>=20% similarity with TE
Removed genes
4,530
HC genes
36,071
LC genes
25,232
5,322 with functional annotation
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LC genes
19,910
936 within other HC genes(<50%)
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LC genes
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