BMKGENE

Transcriptome Sequencing

Product Manual

Nanopore Based Full-length mRNA Sequencing

BMKGENE

RNA sequencing has been an invaluable tool for comprehensive transcriptome analysis. Doubtlessly, traditional short-read sequencing achieved numerous important development in here. Nevertheless, it often encounters limitations in full-length isoform identifications, quantification, PCR bias

Nanopore sequencing distinguishes itself from other sequencing platforms, in that the nucleotides are read directly without DNA synthesis and generates long read at tens of kilobases. This empowers direct read-out crossing full-length transcripts and tackling the challenges in isoform-level studies

Service Specifications

Library	Platform	Data recommended	Data QC	Sample requirements
cDNA-PCR (ployA)	PromethION P48	6 Gb	Average quality score: Q10 Full-length ratio≥70%	Conc. ≥ 40 ng/μl Total amount ＞ 1 μg RIN ≥ 8-7.5 Limited or no DNA and protein contamination

Bioinformatics Analysis Content

- Raw data QC
- Full-length transcripts identification
- Reference genome alignment
- Isoform-level expression

• Functional Interpretation on DEGs and DETs

√ Expression in isoform level √ Differentially expressed genes (DEGs) √ Differentially expressed transcripts(DETs)

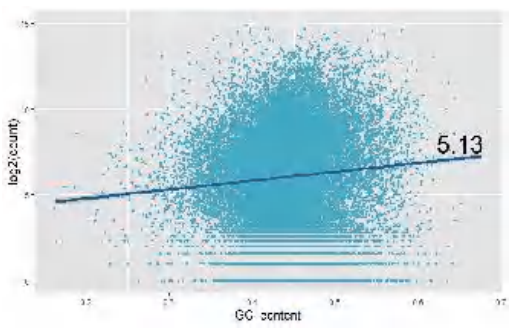
√ Function annotation and enrichment √ Fusion genes √ Alternative splicing analysis and differential alternative splicing events

√ Alternative poly-adenylation (APA) √ Gene structure optimization √ Novel transcripts prediction

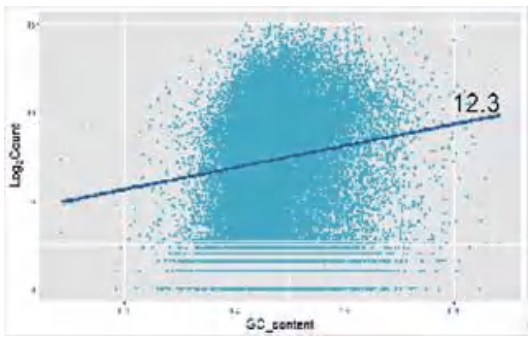
Service Highlights

• Low sequence-specific bias

GC-bias, a constant issue in traditional methods, leads to under-representation of sequences with low or high-level GC contents. Nanopore overcome the challenges by introducing much less-biased read with limited PCR reactions.



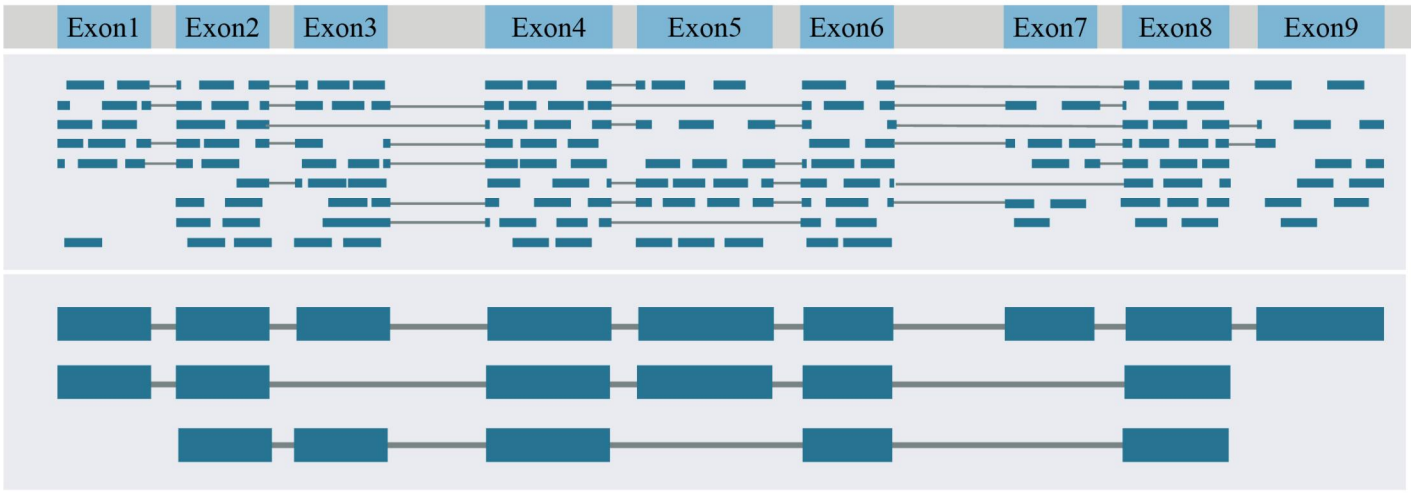
Nanopore



Next Generation Sequencing

• Identification of multiple isoforms per gene

Assembly-based alternative splicing events identification is always problematic due to in identifying all constituent exons. enables generating of complete transcript isoform sequences, which achieves isoform-level resolution. This makes complex transcriptome structural studies possible.

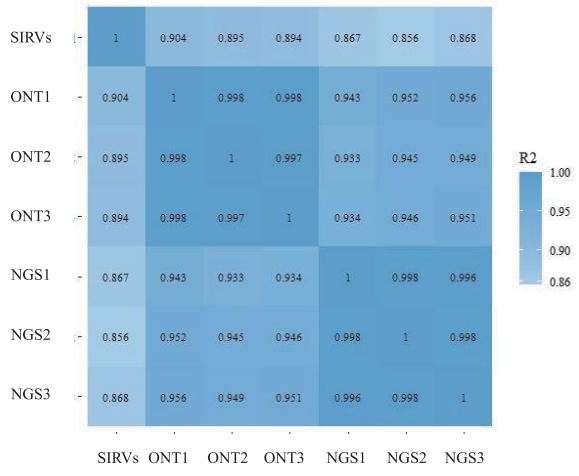


• Less data required to cover same number of transcripts

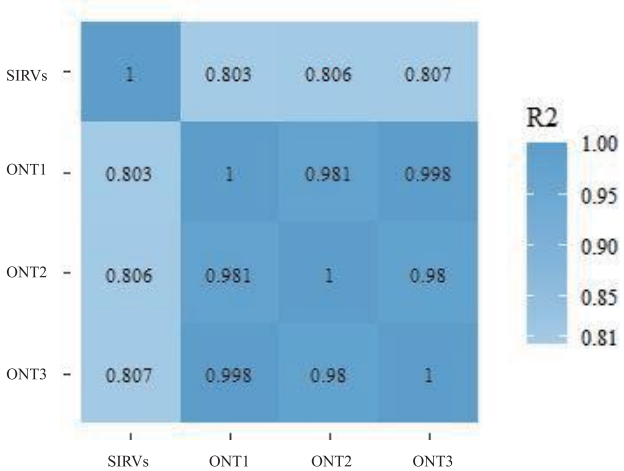
Full-length read out of cDNA molecule could largely reduced multiple-locus alignments, which increased data usage. Compared to short-read technologies, nanopore requires 7-folder fewer data to cover the same number of transcripts.

• Expression quantification in isoform level

Differential expression analysis in gene level is very likely to mask the changes in isoform level. With reliable isoform identification capacity, nanopore empowers both more accurate gene expression quantification and that in transcripts. In-house data of BMK demonstrated that by introducing SIRVs as known standards, the accuracy of Nanopore-based accuracy achieved over 90%, which is higher than that of NGS-based expression quantification. The performance in isoform level expression was also shown to be impressive , which achieved 80%.



Correlatio among standards (SIRVs), Nanopore (ONT) and NGS in gene expression



Correlation between standards and Nanopore in Transcript quantification

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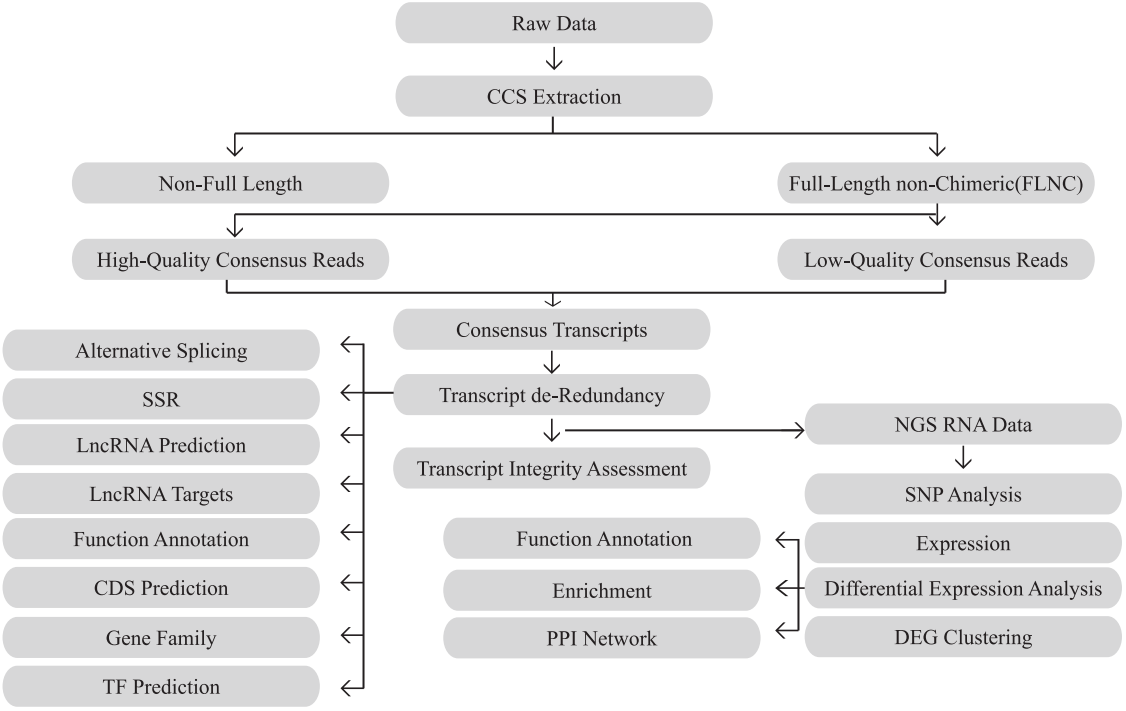
De novo Full-length transcriptome Sequencing -PacBio

De novo full-length transcriptome sequencing, also known as De novo Iso-Seq takes the advantages of PacBio sequencer in read length, which enables sequencing of full-length cDNA molecules without any breaks. This completely avoids any errors generated in transcript assembly steps and constructs unigene sets with isoform-level resolution. This unigene sets provides powerful genetic information as "reference genome" at transcriptome-level. In addition, combining with next generation sequencing data, this service empowers an accurate quantification of isoform-level expression.

- ☑ Raw data processing
- ☑ Transcript identification
- ☑ Sequence structure
- ☑ Expression quantification
- ☑ Function annotation
- ☑ Differential analysis



Service Advantages

- **Direct read-out of full-length cDNA molecule from 3'- end to 5'- end**
- **Iso-form level resolution in sequence structure**
- **Transcripts with high accuracy and integrity**
- **Highly compatible to vaiours species**
- **Large sequencing capacity with 4 PacBio Sequel II sequencing platforms equipped**
- **Highly experienced with over 700 Pacbio-based RNA sequencing projects**

Sample Requirements

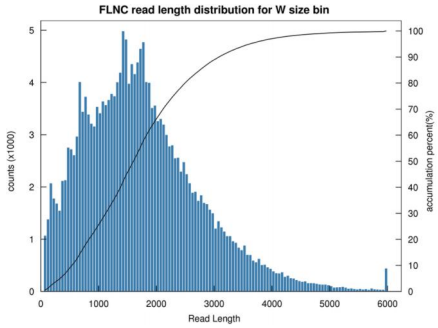
Purity	Integrity	Amount
OD260/280≥1.8; OD260/230≥1.0; clear peak at 260 nm	For plants: RIN≥7.5; For animals: RIN≥8; 28S/18S≥1.0; limited or no baseline elevation	Total ≥ 1 μg;Volume ≥ 10 μl;Conc. ≥100 ng/μl;

Specifications

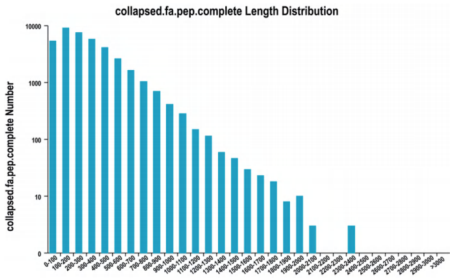
Library	Platform	Recommended data yield	Data QC
Poly-A enriched mRNA CCS library	PacBio Sequel II	20 Gb/sample (Depending on species)	FLNC（%）： ≥75%

*FLNC: Full-length non-chimeric transcripts

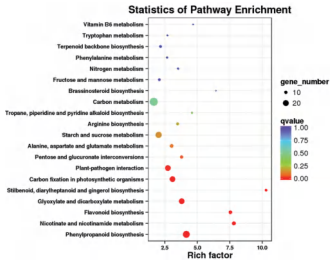
Demo Results



FLNC length distribution



Complete ORF region length distribution



Differential expressed transcripts -KEGG

Case Study

Title: The developmental dynamics of the Populus stem transcriptome

Published: Plant Biotechnology Journal, 2019

Sequencing strategy:

Sample collection: stem regions: apex, first internode(IN1), second internode(IN2), third internode(IN3), internode(IN4) and internode(IN5) from Nanlin895

NGS-sequence: RNA of 15 individuals were pooled as one biological sample. Three biological replicates of each points were processed for NGS sequence

TGS-sequence: Stem regions were divided into three regions, i.e. apex, IN1-IN3 and IN4-IN5. Each region was processed for PacBio sequencing with four types of libraries: 0-1 kb, 1-2 kb, 2-3 kb and 3-10 kb.

Key results

1.A total of 87150 full-length transcripts were identified, in which, 2081 novel isoforms and 62058 novel alternative spliced isoforms were identified.

2.1187 lncRNA and 356 fusion genes were identified.

3.From primary growth to secondary growth, 15838 differentially expressed transcripts from 995 differentially expressed genes were identified. In all DEGs, 1216 were transcription factors, most of which has not yet been reported.

4.GO enrichment analysis revealed importance of cell division and oxidation-reduction process in primary and secondary growth.

*Chao Q, Gao ZF, Zhang D, et al. The developmental dynamics of the Populus stem transcriptome. Plant Biotechnol J. 2019;17(1):206-219. doi:10.1111/pbi.12958

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mRNA Sequencing (NGS)

mRNA sequencing adopts next-generation sequencing technique (NGS) to capture the messenger RNA(mRNA) from Eukaryote at specific period that some special functions are activating.

Standard bioinformatics analyses include:

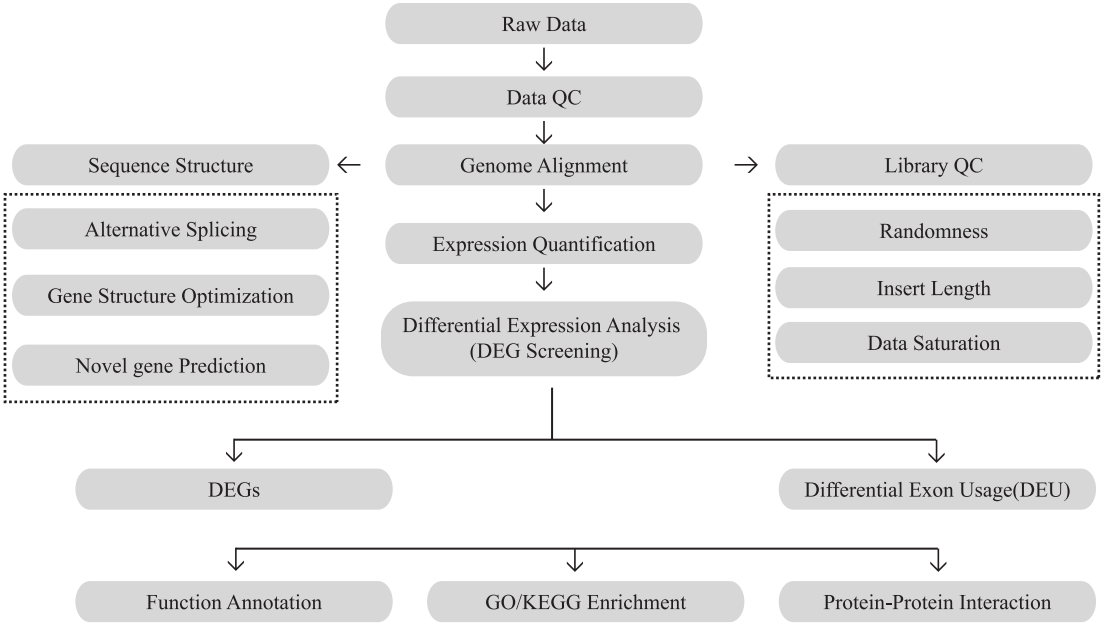
(1)Reference genome alignment

(2)gene structure and novel gene prediction

(3)Gene expression quantification

(4)Differential expression analysis

(5)Function annotation, enrichment and interaction networks



Service Advantages

1. Comprehensive bioinformatics including sequence structure, differential expression analysis and function interpretation using diverse database.

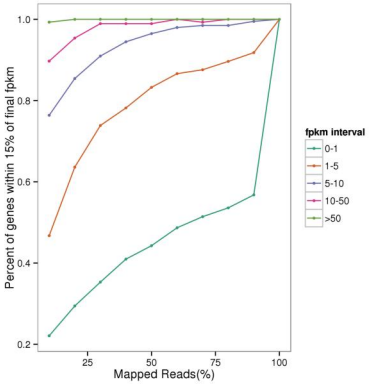
2. Experienced: processed over 200K samples, sample type include cell, tissue, body fluid and hair follicle et al.

3. Strict quality control system: strict QC standard of all steps in a project(Sample QC, library QC, data QC and NT Blast)

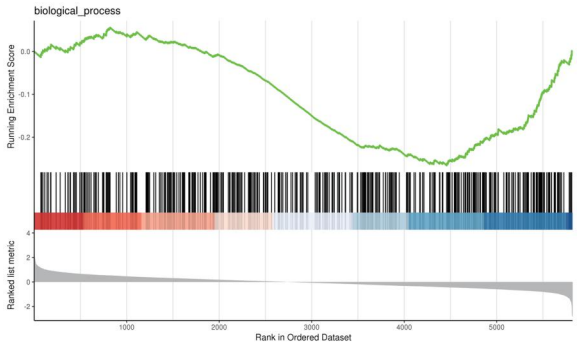
Sample Requirements

Purity	Integrity	Amount
OD260/280:1.7-2.5； OD260/230≥0.5-2.5；	For plants: RIN≥6.5; For animals: RIN≥7;	Conc. ≥30 ng/μl; Volume ≥ 10 μl; Total ≥0.6 μg

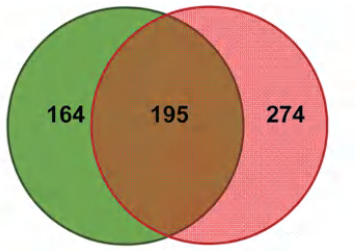
Domo Resuits



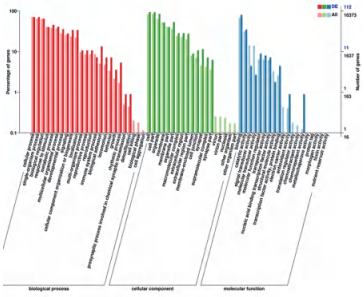
Data Saturation analysis



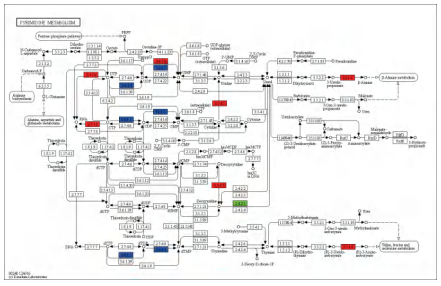
GSEA Analysis



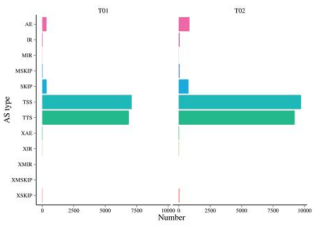
Venn diagram



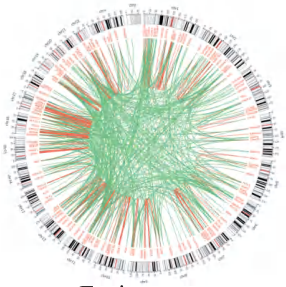
GO classifications



KEGG pathway



Alternative splicing



Fusion gene

Case Study

Title: Transcriptome analysis of bagging-treated red Chinese sand pear peels reveals light-responsive pathway functions in anthocyanin accumulation

1.In total, 8,870 differentially expressed genes were further analysed by a weighted gene coexpression network analysis and early-middle and late light responsive genes were identified.

2. The “blue” module was highly correlated with the total anthocyanin content (r=0.93, p=2×10−9), and 11 structural genes involved in anthocyanin biosynthesis and transportation.

3.A Gene Ontology (GO) enrichment analysis of the early light-responsive genes identified 19 significantly enriched GO terms. Most of them were related to photosynthesis and light response.To obtain more detailed information, a pathway analysis was carried out using MapMan.

* Bai S , Sun Y , Qian M , et al. Transcriptome analysis of bagging-treated red Chinese sand pear peels reveals light-responsive pathway functions in anthocyanin accumulation[J]. Scientific Reports.10.1038/s41598-017-00069-z

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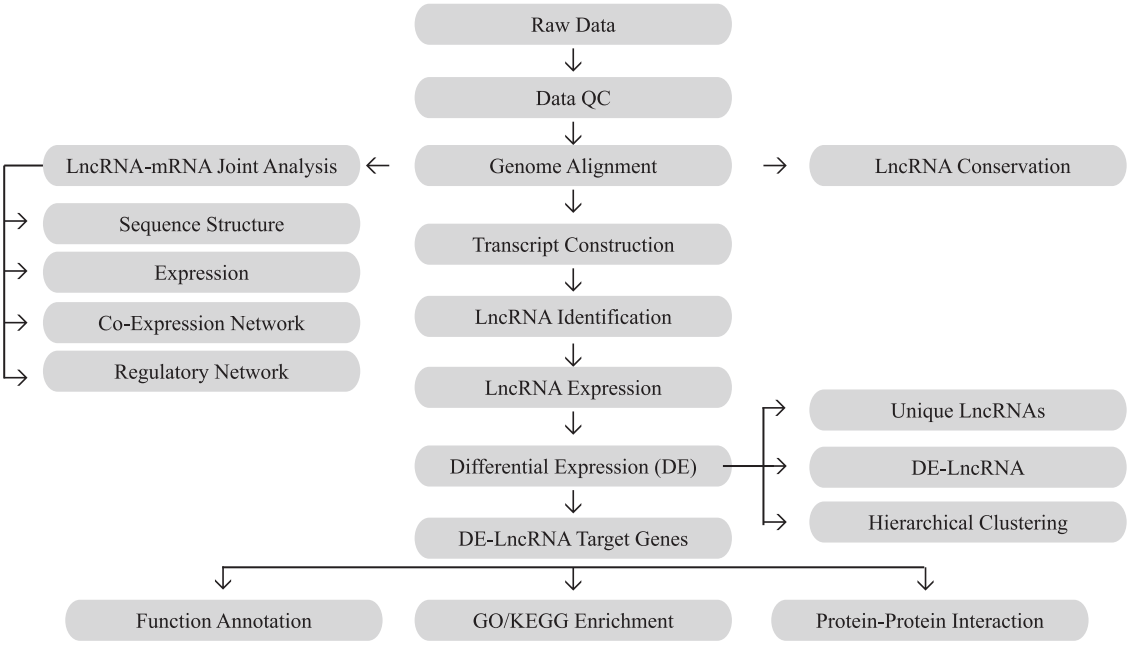
Lnc-RNA Sequencing

LncRNA is a kind of low encoding potential RNA molecule that comprised of longer than 200nt. It is an important part of non-coding RNA. It is widely distributed in the nucleus or cytoplasm, and proved as a significant role in life activities. LncRNA sequencing is a powerful tool in Cell differentiation, Ontogenesis and Human diseases.



Service Advantages

1. High-quality service guaranted by optimized experimental SOP with 5 strict quality control points across entire work flow.

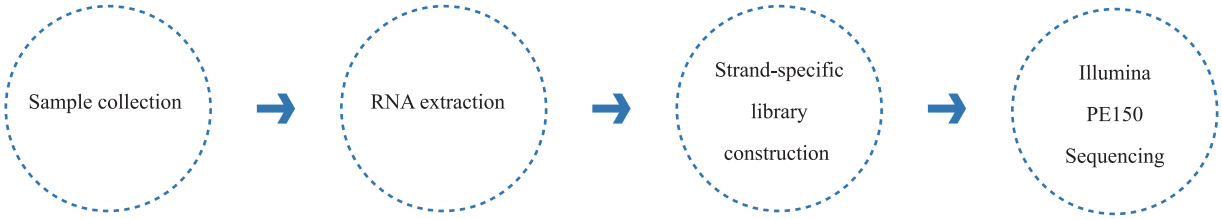
2. Comprehensive lncRNA analysis, consisting lncRNA profiling, lncRNA target prediction and joint analysis with mRNA.

3. Extensive experience on lncRNA sequencing with over 20,000 samples processed covering diverse species and sample types.

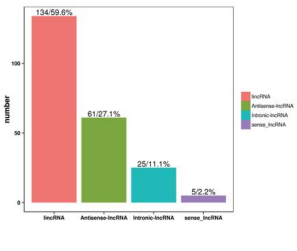
Sample Requirements

Integrity	Purity	Amount
For plants: RIN≥6.5;For animals: RIN≥7	OD260/280: 1.7-2.5； OD260/230: 0.5-2.5；	Conc. ≥100 ng/μl; Volume≥ 10 μl; Total ≥ 1 μg

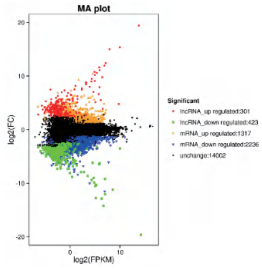
Service Workflow



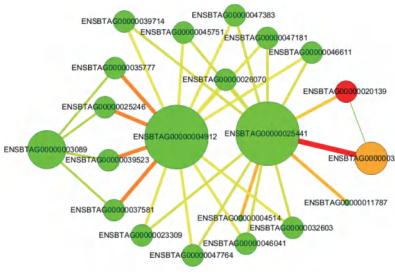
Demo Results



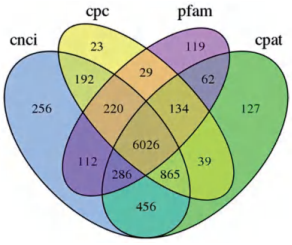
LncRNA identification



differential between LncRNA and mRNA



Protein-protein interaction network of DE-LncRNAs



Venn diagram on predicted lncRNA

Case Study

Title:Deregulated lncRNA expression profile in the mouse lung adenocarcinomas with KRAS‐G12D mutation and P53 knockout

Published: Journal of Cellular and Molecular Medicine, 2019

This study investigate the aberrantly expressed lncRNAs in the mouse lung adenocarcinoma with P53 knockout and the KrasG12D mutation.

1.6424 lncRNAs were differentially expressed (≥ 2-fold change, $P < 0.05$).

2.Among all 210 lncRNAs(FC≥8), 11 lncRNAs' expression was regulated by P53, 33 lncRNAs by KRAS and 13 lncRNAs by hypoxia in the primary KP cells, respectively.

3.NONMMUT015812, which was remarkably up-regulated in the mouse lung adenocarcinoma and negatively regulated by the P53 re-expression, was detected to analyse its cellular function.

4.Knockdown of NONMMUT015812 by shRNAs decreased proliferation and migration abilities of KP cells. NON-MMUT015812 was a potential oncogene.

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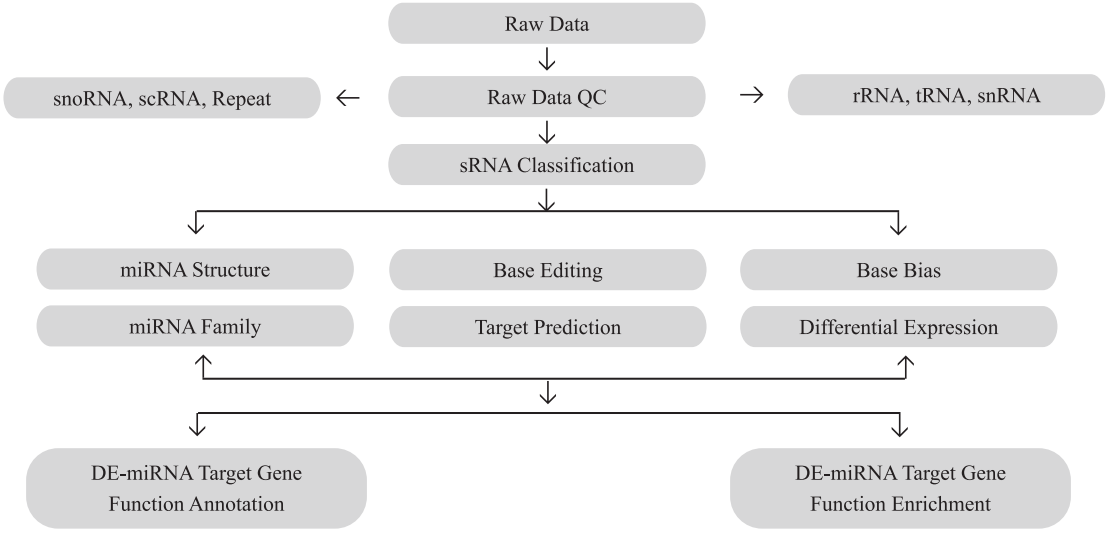
Small RNA Sequencing

Small RNA refers to a class of non-coding RNA molecules that are usually less than 200nt in length, including micro RNA (miRNA), small interference RNA (siRNA), and piwi-interacting RNA (piRNA).
MicroRNA (miRNA) is a class of endogenous small RNA with a length of about 20-24nt. miRNA invole in many life processes which revealing tissue - specific and stage - specific expression and highly conserved in different species.



Service Advantages

1.Extensive experience in sRNA sequencing with hundreds of closed projects covering 50+ species.
2.Strict quality control system monitoring entire project process.
3.Comprehensive bioinformatics analysis on small RNA expression as well as target molecule prediction.
4.Joint analysis available for miRNA+mRNA; miRNA+lncRNA; miRNA+circRNA+lncRNA, etc.

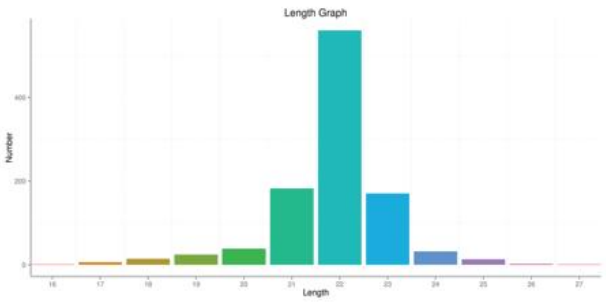
Service Specifications

Library	Platform	Recommended Data Output	Quality Control
Size-selection based small RNA library	Illumina NovaSeq 6000	10M/ 20M Reads	Q30≥85%

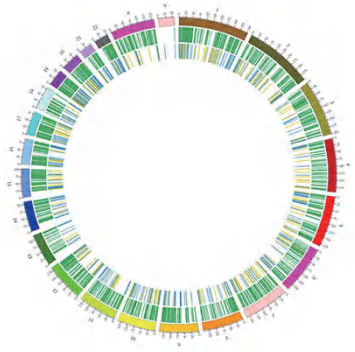
Sample Requirements

Purity	Integrity	Amount
OD260/280:1.7-2.5； OD260/230≥0.5-2.5；	For plants: RIN≥6.5; For animals: RIN≥7;	Conc. ≥300 ng/μl; Volume ≥ 10 μl; Total ≥ 1 μg

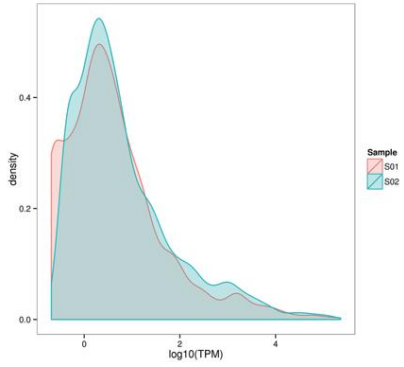
Demo Results



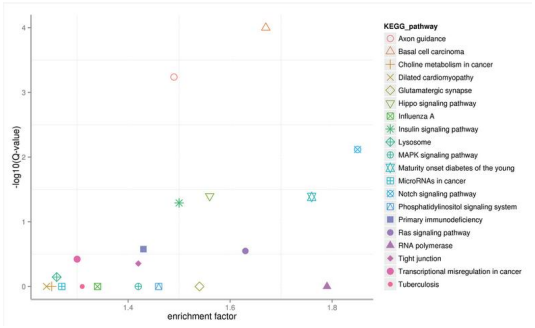
Small RNA Identification



Small RNA base editing



miRNA expression distribution



DE-miRNA target gene annotation

Case Study

Title:Integrated Analysis of MiRNA and Genes Associated with Meat Quality Reveals that Gga-MiR-140-5p Affects Intramuscular Fat Deposition in Chickens

Published: Cellular Physiology and Biochemistry, 2018

Integrated Analysis of MiRNA and Genes Associated with Meat Quality Reveals that Gga-MiR-140-5p Affects Intramuscular Fat Deposition in Chickens

Late laying-period hens exhibited lower global expression levels of miRNAs than juvenile hens.

A regulatory network of mirNA-mrna that may affect meat quality was constructed.

gga-miR-140-5p promotes adipocyte differentiation by down regulating RXRG.

*Zhang M , Li D H , Li F , et al. Integrated Analysis of MiRNA and Genes Associated with Meat Quality Reveals that Gga-MiR-140-5p Affects Intramuscular Fat Deposition in Chickens[J]. Cellular Physiology and Biochemistry, 2018:2421-2433. DOI： 10.1159/000489649

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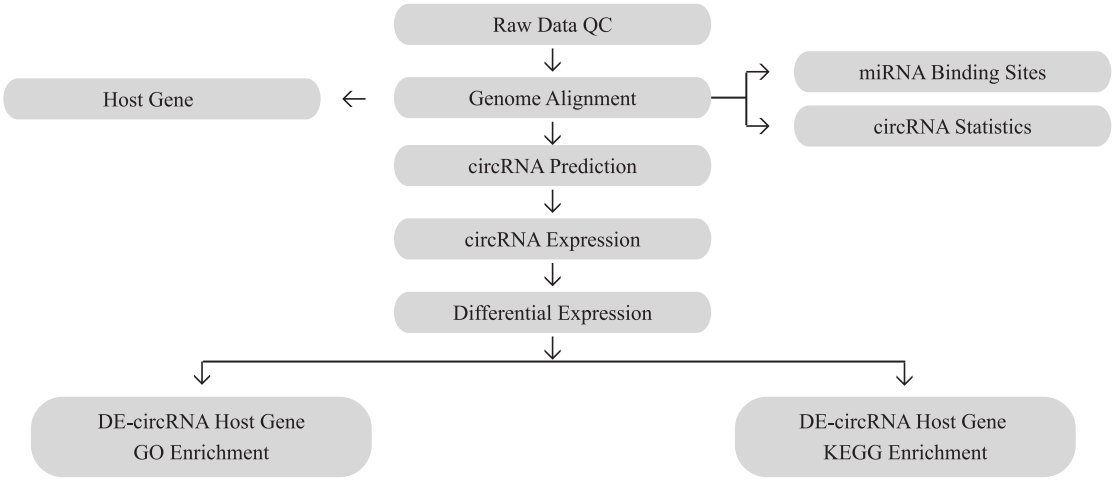
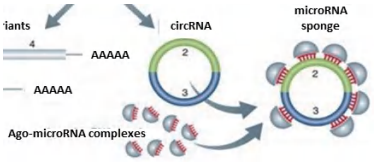
circRNA Sequencing

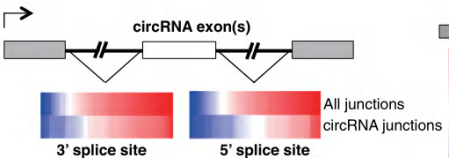
CircRNAs (Circular RNAs) are one class of non-coding RNA molecules that short of 5' end cap and 3' end poly(A) tail. CircRNAs preform circular structure by covalent bond which is status to against RNA exonuclease digestion.



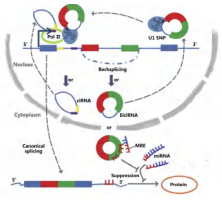
Applications



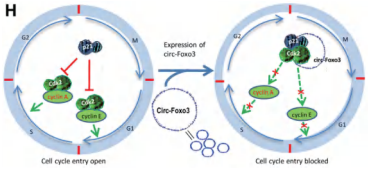
Mechanism of miRNA Sponge



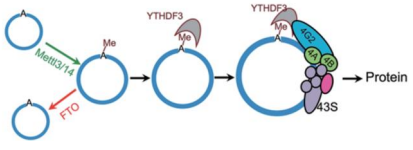
Regulating Alternative Splicing



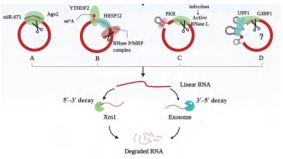
Regulating gene expression



Binding functional protein



Translation



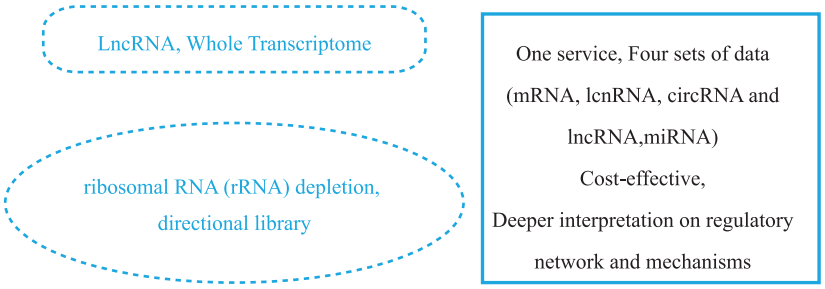
Mediating RNA degradation

Sample Requirements

Purity	Integrity	Amount
OD260/280: 1.7-2.5；OD260/230: 0.5-2.5；	For plants: RIN≥6.5; For animals: RIN≥7;	Conc. ≥100 ng/μl; Volume ≥ 10 μl; Total ≥ 2 μg

Research Strategy

In circRNA sequening projects, ribosomal RNA(rRNA) depletion directional library becames more popular compared to linear RNA depletion, in that this strategy saves information on other RNA molecules, including mRNA, lncRNA, etc. Joint analysis on these dataset provides rich information on revealing

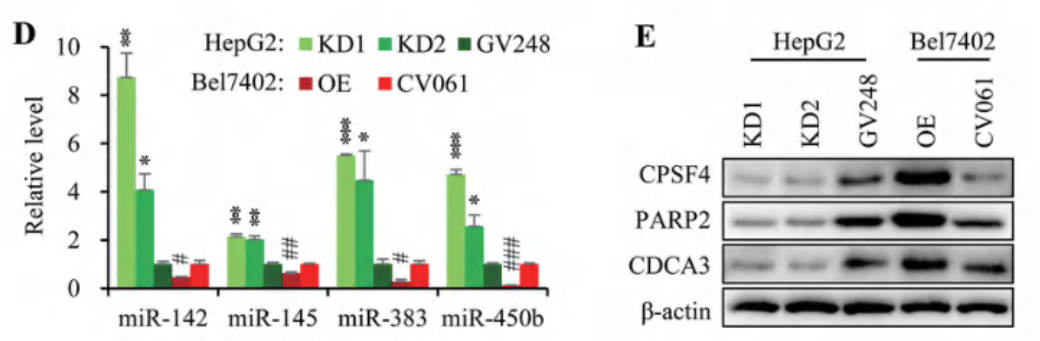


Whole Transcriptome Sequencing

Case Study

Title:CPSF4 regulates circRNA formation and microRNA mediated gene silencing in hepatocellular carcinoma
Published: Oncogene, 2021

1. CPSF4 expression is upregulated in HCC and that high expression of CPSF4 is associated with poor prognosis in HCC patients
2. CPSF4 reduces the levels of circRNAs, which possess a polyadenylation signal sequence and this decrease in circRNAs reduces the accumulation of miRNA and disrupts the miRNA-mediated gene silencing in HCC
3. Experiments in cell culture and xenograft mouse models showed that CPSF4 promotes the proliferation of HCC cells and enhances tumorigenicity. Moreover, CPSF4 antagonizes the tumor suppressor effect of its downstream circRNA in HCC



miRNA levels and protein levels

*Wang X , Dong J , Li X , et al. CPSF4 regulates circRNA formation and microRNA mediated gene silencing in hepatocellular carcinoma[J]. Oncogene.

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Whole Transcriptome Sequencing

Whole transcriptome refers to collection of all RNA molecules under specific condition, including mRNA and non-coding RNA. Biomarker Technologies provides whole transcriptome sequencing service that includes detection of expression and structure of mRNA, lncRNA, circRNA and miRNA in samples, which aims at profiling of all transcription products. Combining with ceRNA mechanisms, whole transcriptome sequencing can provide a more comprehensive view on regulatory networks across different types of RNA molecules.

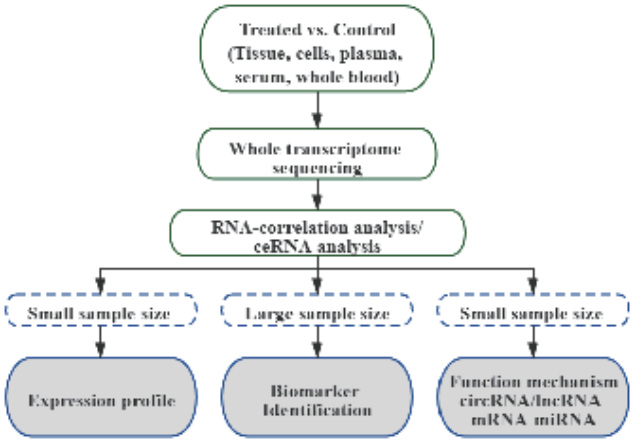
Service advantages

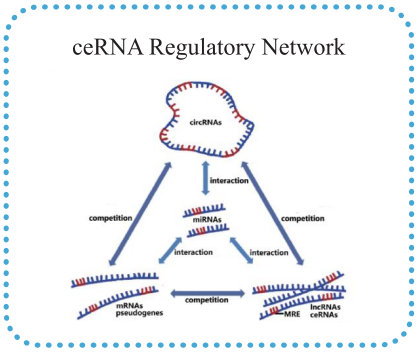
- **All RNA molecules in One-go:** 4 datasets, including mRNA, lncRNA, smallRNA and circRNA will be generated in one experiement.
- **Applicable to diverse sample type:** Tissue, blood, body fluids, hair follicle, etc.
- **Highly-experienced in library construction:** Low sample amount required for library construction; Library with ultra-low RNA contents is available for rare samples.
- **Comprehensive bioinformatics analysis:** BMKCloud-based bioinformatics analysis allows easy personalized parameter design and analysis; advanced analysis on regulatory network available.

Applications	Disease mechanism, regulation of tissue development, pharmacology, biomolecular targets
Research strategy	Transcriptomics: Deeper interpretation on RNA-level regulatory mechanisms Multi-omics: Joint analysis with proteomics, metabolomics, etc. to obtain a boarder image on RNA interactions and regulations.
Material	Tissue: RNA expression or DNA methylation is tissue-specific and time-specific. Materials should be representative to a specific condition. Blood: Blood disorders or for molecular diagnosis

Whole transcriptome study

Whole transcriptomic sequencing enables quantification of both mRNA and non-coding RNA expression. In addition, sequence-based and expression-correlation based network analysis helps reveal regulatory network among mRNA, lncRNA, miRNA and circRNA, as indicated in ceRNA regulatory mechanism. This analysis has been widely applied in research on mechnisms of diseases, cancer, development, etc., which could quickly identify target genes and relevant up- or down- streams genes that relevant to functions of interests.





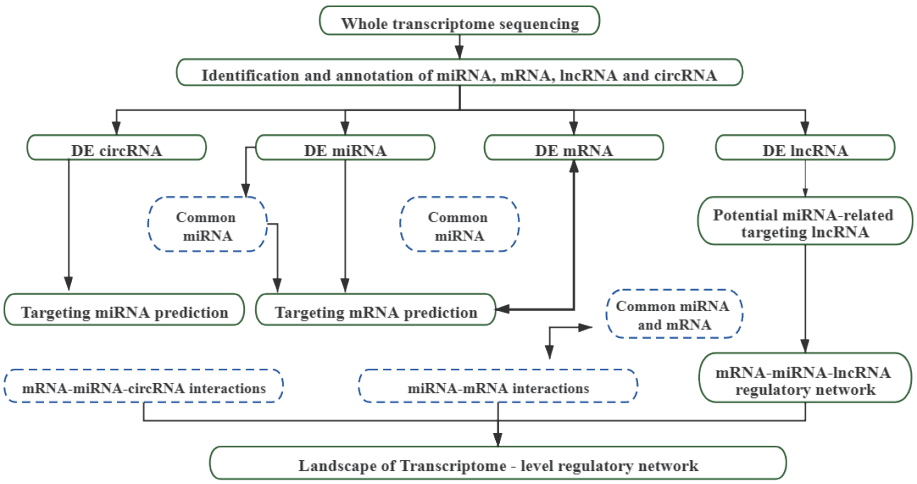
Affects of ceRNA on targeted gene expression:
Validation on competing domain at protein or RNA level
ceRNA affects on targeted genes relying on miRNA:
Validation on relationship with miRNA regulation/Validation on miRNA binding sites
Functional validation experiments: Tissue culture/ animal experiments/Clinical tests

Service specifications

Library	Platform	Data volume	Sample
Whole transcriptome library	Illumina PE150 & SE50	circRNA/lncRNA/mRNA: 16 Gb miRNA: 10 M reads	RNA: ≥ 1.5 μg Cell culture: ≥ 1×10^7 Blood: 2-3 ml

Bioinformatics work flow

Combining with BMKCloud online bioinformatics platform, BMK provides whole transcriptome sequencing with comprehensive data interpretation service including standard sequencing data analysis as well as customized data mining based on specific research goals.



Case study

Title: The circRNA circAGFG1 acts as a sponge of miR-195-5p to promote triple-negatiev breast cancer progression through regulating CCNE1 expression
Molecular Cancer, IF=10.679

In this study, Triple-negative breast cancer(TNBC) tissue and adjacent tissue were subjected to whole transcriptome sequencing, in which 354 differentially expressed circRNA and 3255 DEGs were identified. Expression-based correlation analysis indicated an increased expression of circAGFG1 and CCNE1 gene in TNBC tissue. Sequenced-based prediction identified common binding sites among circAGFG1, CCNE1 and miR-195-5p. The results indicated that circAGFG1 may act as a ceRNA of miR-195-5p that promote expression of CCNE1, which may further lead to TMBC cell proliferation.

Title: Lnc-TALC promotes O6-methylguanine-DNA methyltransferase expression via regulating the c-Met pathway by competitively binding with miR-20b-3p.
Nature communication, IF=11.878

In this study, temozolomide(TMZ) resistant glioblastoma(GBM) and normal glioblastoma were processed for high-throughput sequencing on RNA molecules. A total of 33 up- or down- regulated lncRNA and correlated mRNA were identified from sequencing data. The results indicated that lnc-TALC was significantly associated with TMZ resistance. In addition, sequence-based correlation analysis identified common binding sites among miR-20b-3p, lncTALC and 3'UTR of MET. This study revealed lnc-TALC as a novel therapeutic target to overcome TMZ resistance.

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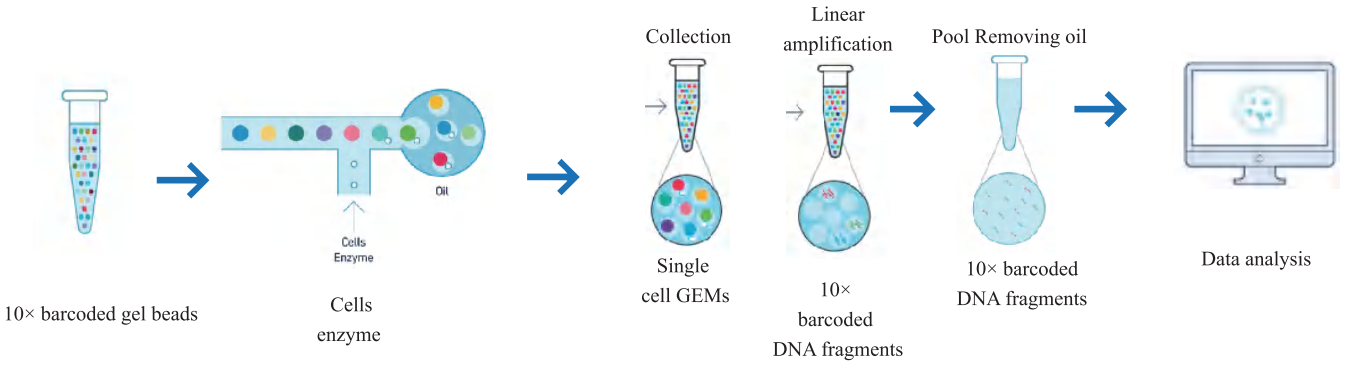
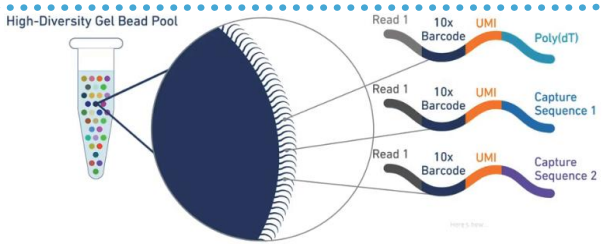
Single-cell RNA Sequencing

The advance in single cell capturing and individual library construction technique combining with high-throughput sequencing allows gene expression studies on cell-by-cell basis. It enables a deeper and complete system analysis on complex cell populations, in which it largely avoid masking of their heterogeneity by taking average of all cells, as done in bulk RNA sequencing. BMK provides 10× Genomics ChromiumTM based single-cell RNA sequencing service. This service has been widely used in studies on disease related studies, such as immune cell differentiation, tumor heterogeneity, tissue development, etc.



Technical work flow

The isolation of cells is achieved by 10× Genomics ChromiumTM , which consists eight-channel microfluidics system with double crossings. In this system, a gel beads with barcodes and primer, enzymes and a single cell are encapsulated in nanoliter-sized oil drop, generating Gel Bead-in-Emulsion(GEM). Once GEM are formed, cell lysis and release of barcodes are performed in each GEM. mRNA are reverse transcribed into cDNA molecules wih 10× barcodes and UMI, which are further subject to standard sequencing library construction.

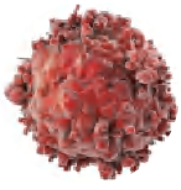


Service advantages

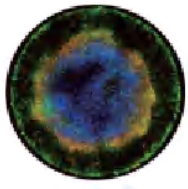
- **Highly-efficient single-cell capturing:** Chromium suite enables high-throughput capturing and labeling of 500 to 10,000 cells per library.
- **Diverse bioinformatics analysis:** In addition to basic bioinformatics, the robust bioinformtics group can also provide advanced and personalized data interpretation, such as cell differentiation trajectory.
- **Highly-experienced in library construction:** BMK is one of the earliest 10× Genomics related service providers. We have accumulated massive experience in single-cell library construction and reverse transcription and library construction on ultra-low RNA samples.
- **Integrated service:** BMK provides integrated service single-cell RNA sequencing service pack containing single cell capturing, library construction, sequencing, standard bioinformatics analysis, advanced bioinformatics analysis and customized data analysis.

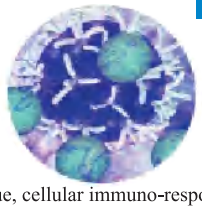
Applications

Cancer, Tumor micro-environment



Drug targets,Drug resistance





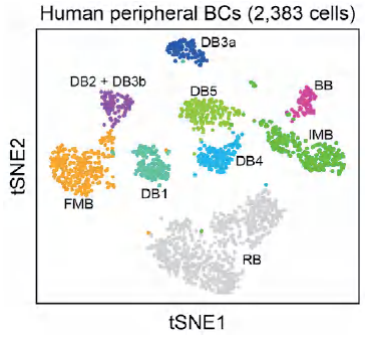
Tissue, cellular immuno-responses

Neuro science。Neuro-oncology
Head damage

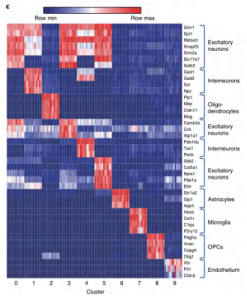
Service specifications

Library	Platform	Data volume	Sample
10× Genomics single-cell library	10× Genomics Illumina PE150	100,000 reads/cell approx. 100-200 Gb	Cell number: >2× 10^5 Cell conc. at 700-1,200/μl Cell diameter: 10-40 μm Cell viability: approx. 90%

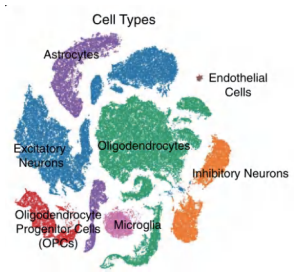
Demo results



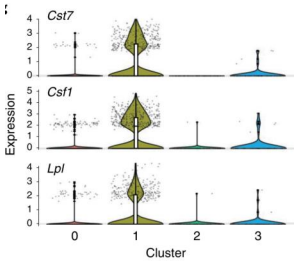
t-SNE cell clustering



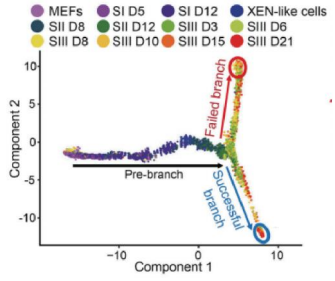
Heatmap: gene expression clustering across cell sub-populations



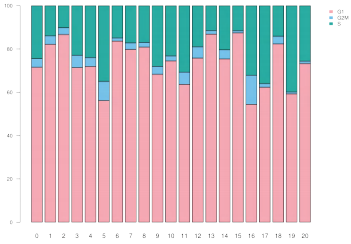
Cell sub-population identification



Gene expression distribution of specific genes



Cell trajectory analysis/pseudotime

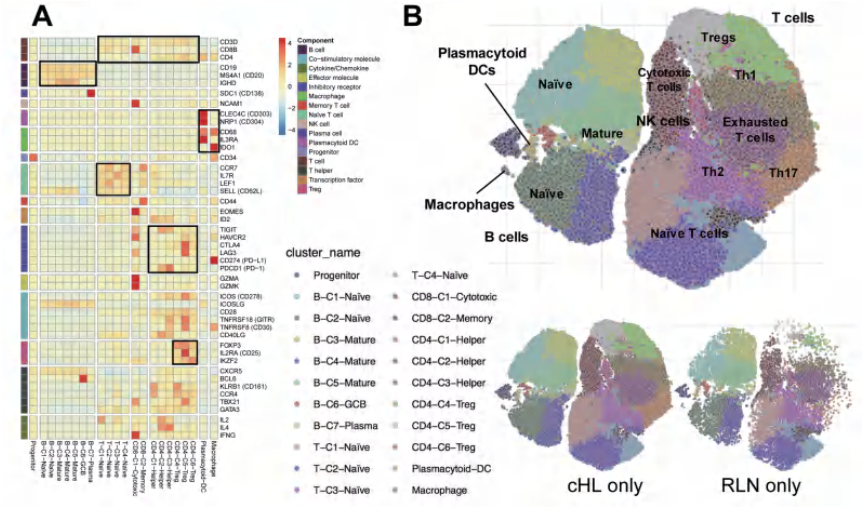


Cell cycle identification of sub-population

Case study

Title: Single-cell transcriptome analysis reveals disease defining T-cell subsets in the tumor microenvironment of classic Hodgkin Lymphoma. Cancer Discovery, IF=29.497

In this study, 22 tissue specimen of Hodgkin Lymphoma(HL) and 5 reactive lymph nodes were processed for single-cell RNA sequencing. Over 127,000 cells in total were isolated and subjected to single-cell RNA sequencing, which, for the first time, revealed HL-specific phenotypes in immuno microenvironment at single-cell resolution. Gene expression profiling at single-cell level identified a novel HL-related T-cell subset, which shown significant expression of the inhibitory receptor LAF3. Functional analyses furter confirmed LAG3+ T-cell subsets as immunosuppression mediator. Spacial assessment of LAG3+ T-cells and HRS cells shown that LAG3+ T-cells were significantly increased around MHC II-deficient tumor cells. Application of single-cell RNA sequencing in HL microenviornment study provided new insights in immune cell position in tumor microenvironment at single-cell resolution, which further suggested new biomarkers and novel ideas in treatments targeting immune checkpoints.



Immune cell atlas of the Hodgkin lymphoma(HL) microenvironment revealed by single-cell RNA sequencing

Spatial Transcriptome Sequencing

BMKGENE

Spatial transcriptome sequencing is a novel technology that has promising applications in diverse research arena, including cancer, immunology, tissue microenvironment, neuroscience, etc. It empowers resolving of mRNA profile while retaining information of spatial position. Matching gene expression profile with individual tissue section leads to more comprehensive molecular understanding of tissues, such as spatial-specific expression of functional genes.

Bulk RNA Seq

Acquisition of average gene expression level of cell bulk

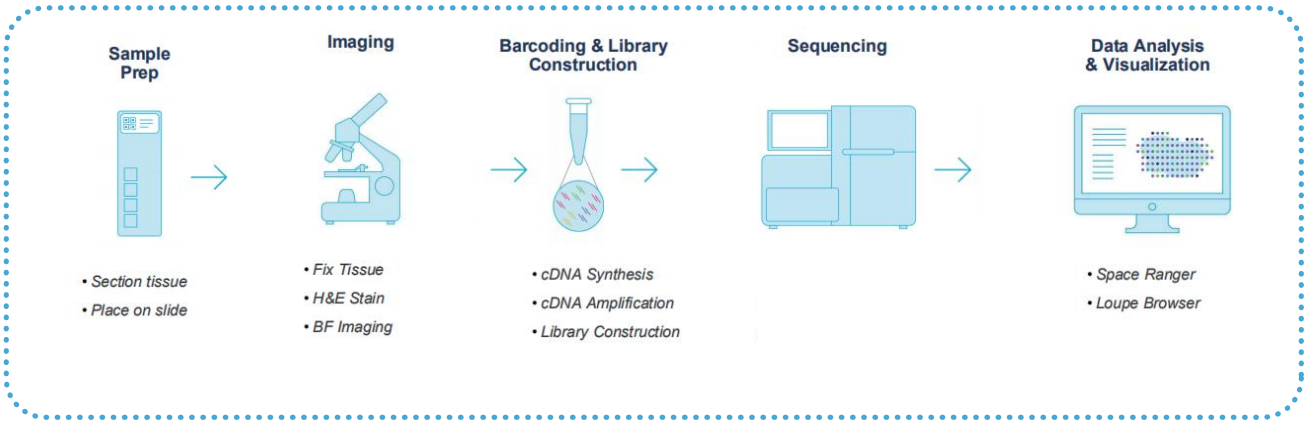
Single-cell RNA Seq

Single-cell transcriptome technologies reveals gene expression profile in each single cell.

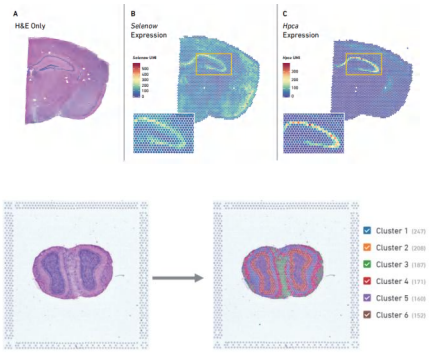
Spatial RNA Seq

Combination of 10X Visium and transcriptome sequencing enables matching of RNA-Seq data with particular position information, spatial-ly resolving gene expression profile.

Technical Scheme



Applications



Pathology

Gene expression with morphological context

Immunology

Immune cell infiltrating, gene expression pattern in immune organs

Neuroscience

Gene expression profile on each section of brain

Oncology

Tumour microenvironment, tumour infiltrating lymphocytes

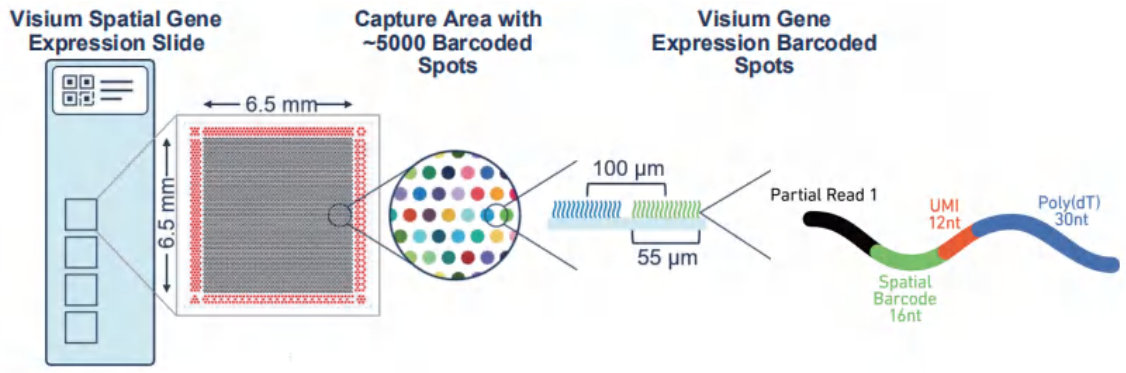
Developmental biology

Tissue-based studies on genes associated with morphology

Service Specification

Library	Platform	Recommended data yield (Gb)	Sample delivery
Spatial mRNA library	10X Visium Illumina PE150	≥ 50 k reads per spot	OCT embedded FFPE tissue (Diameter: approx. 0.8 cm)

10X Visium



Each Visium slide for library construction contains 4 RNA capture regions with area of 6.5 x 6.5 mm2, containing 5000 barcoded spots, i.e. each spot owns a unique barcode. The diameter of each barcoded spot is 55 µm and the center-to-center distance between each two spots is 100 µm.

Cellular mRNA in each tissue section is released and moved into each spot, where mRNA will be ligated to corresponding barcode. These barcoded mRNA is further processed from library construction and sequencing.

Finally, data analysis is based on the barcode information on each reads to trace back where each mRNA is originated, which achieves spatial-specific study on gene expression.

Latest Spatial Transcriptome Studies

Title	Journal (IF)	Published in	Tissue
An in vitro model of early anteroposterior organization during human development.	Nature 42.778	2020.06	Human embryonic stem cell
Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids.	Nature 42.778	2020.06	Embryo
Onco-fetal Reprogramming of Endothelial Cells Drives Immunosuppressive Macrophages in Hepatocellular Carcinoma.	Cell 38.637	2020.10	Human liver
Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease.	Cell 38.637	2020.10	Human brain
Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma.	Cell 38.637	2020.06	Squamous cell carcinoma
Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas.	Nat Biotechnol 36.558	2020.03	Pancreatic catheter

Demo Results

Spatial mRNA expression and clustering of mouse kidney

A. HE staining of tissue section. B. Merging of total UMI and tissue spatial information. C. Merging of total genes and tissue spatial information. D. Spatial clustering of total differentially expressed genes. Top 10 DEGs in cluster 2 are listed on the right. E, F, G, H show spatially expression of four genes.

Spatial resolved gene expression of mouse brain

A. HE staining of tissue section. B and C show spatially expression of two hippocampus marker genes: Tmsb4x and Selenow. As shown in the figure, a significant higher expression of two genes can be observed in hippocampus region, which meets the expected

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Innovation Leads to Wonders

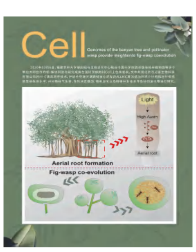
BMKGENE

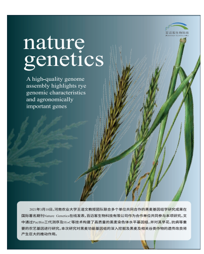
Biomarker Technologies(BMKGENE)

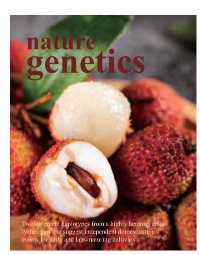
Biomarker Technologies (BMKGENE) has been actively engaged in innovation and developing high-throughput sequencing technologies and bioinformatics for over 12 years. We have brought in the most cutting-edge high-throughput sequencing platforms, including Illumina, Nanopore, PacBio, 10X Genomics, and high-performance computing platforms.

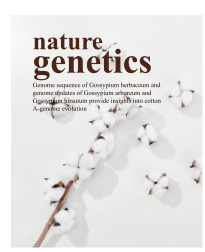
500+	50000+	3000+	13 years
Institutes in Collaboration	Projects Successfully Completed	Cooperative Papers Published	of Professional Experience

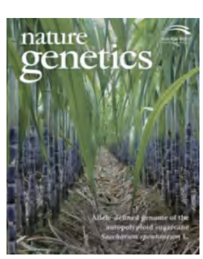
BMKGENE has a passionate and highly-skilled R&D team with over 500 members and owns over 60 nation patents of inventions and over 200 software copyrights on bioinformatics analyses. BMKGENE has a robust ability in tackling scientific and technical issues and has contributed to thousands of high-impact publications. BMKGENE is committed to providing the most comprehensive services covering genomics, transcriptomics, single-cell omics, microbial genomics and epigenetics.

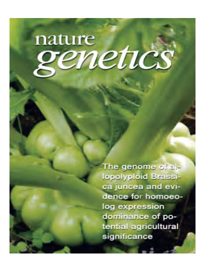












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