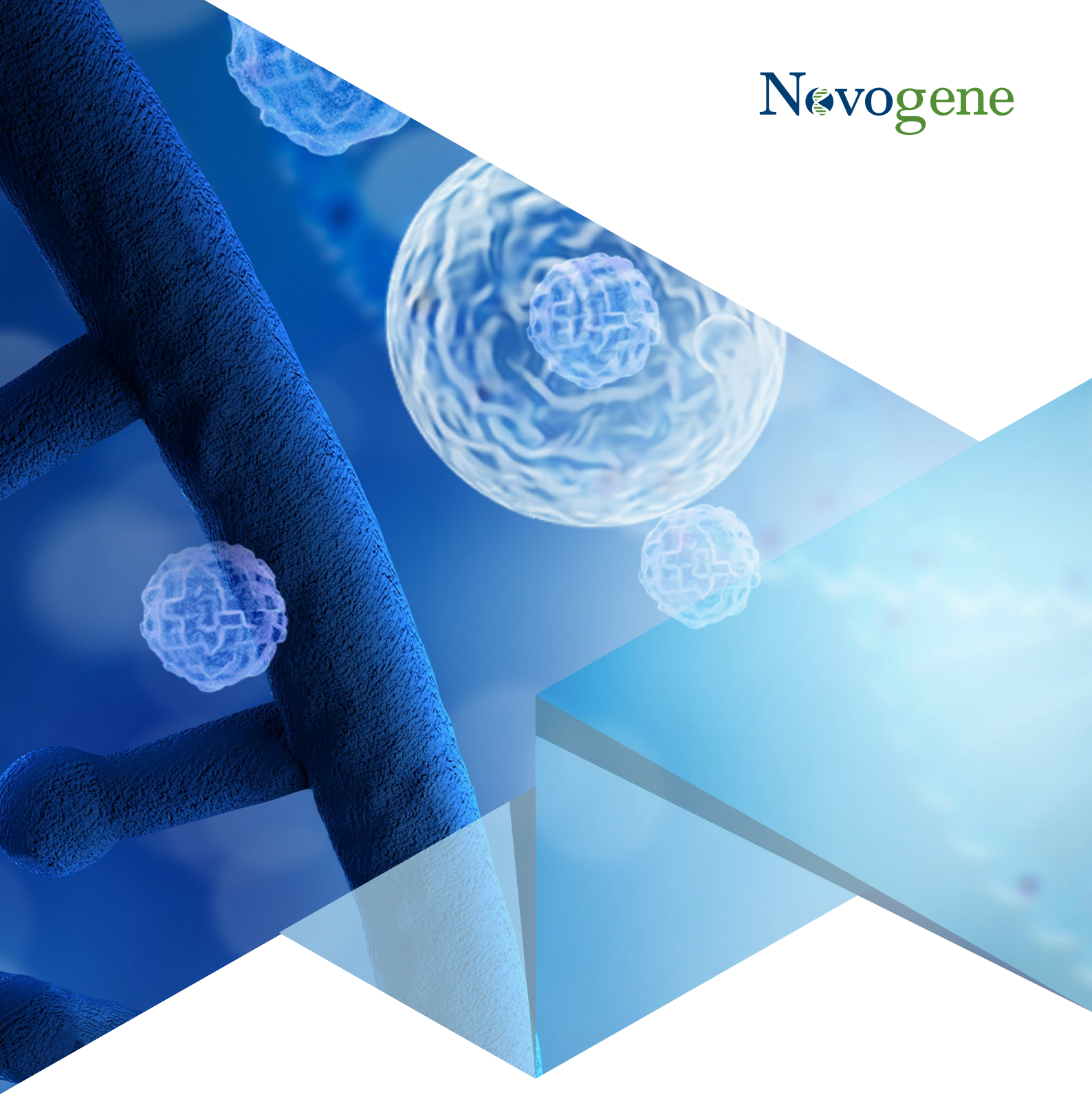

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APPLICATIONS OF SINGLE-CELL SEQUENCING IN STEM CELLS, CANCER AND SKIN RESEARCH

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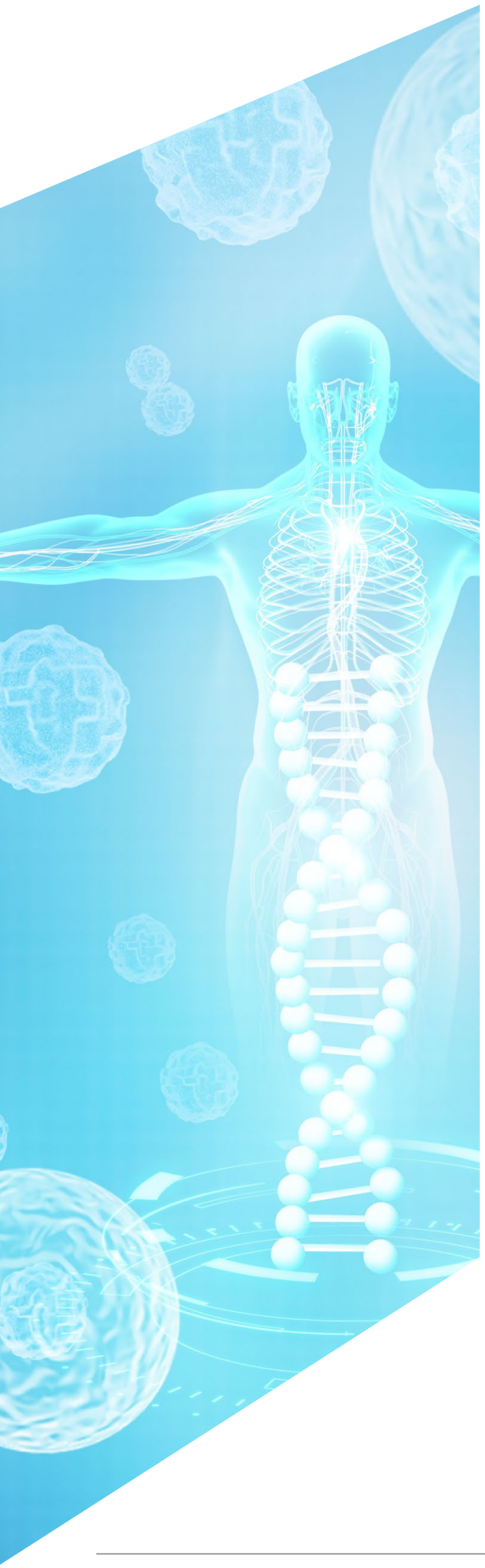
Introduction

Single-cell RNA-sequencing (scRNA-seq) uses next-generation sequencing (NGS) technologies to examine the sequence information contained within single cells. This advancement in technology enables us to examine the genome and transcriptome and provide other multi-omics information, giving us a better understanding of the function of an individual cell and uncovering mechanisms that could otherwise go unnoticed when examining multiple cells at once. Single-cell sequencing can also reveal differences between populations of cells and study cellular evolutionary relationships. This makes it a useful tool for studying cellular function as well as for examining the development of tissues and tumors.

Using Single-cell Sequencing To Discover More About Stem Cells

Single-cell sequencing technologies have advanced our understanding of the occurrence and development of stem cells by examining the differences in gene expression due to their differentiation into different terminal cells.

In a recent study, researchers wanted to learn more about human embryonic yolk sac and fetal liver megakaryocytes by asking questions about the transcriptome, cellular heterogeneity, and megakaryopoiesis or how megakaryocytes develop[1]. To answer these questions, they used scRNA-seq to examine individual cells. Droplet-based scRNA-seq datasets were produced using a Chromium



system (10X Genomics, PN120263). Briefly, Gel beads in Emulsion (GEMs) were generated by loading suspended cells onto a Chromium Controller (10X Genomics). Cells were then lysed to release RNA before reverse transcription, including all barcoding steps, in individual GEMs to create a cDNA library. For Smart-seq2 datasets, megakaryocytes were sorted using fluorescence-activated cell sorting (FACS) according to their phenotype (CD41a+, CD42b+) and lysed before reverse transcription and PCR could be carried out to enrich fragments for cDNA library construction. The cDNA libraries were amplified using KAPA Hyper Prep Kit and then sequenced on an Illumina Hiseq X Ten platform. The raw data were then aligned using Cell Ranger software from 10X Genomics.

The researchers sequenced 11,021 cells from the yolk sac samples and 17,677 cells from the fetal liver, enabling them to identify nine-cell clusters and 15 cell populations. The use of scRNA-seq enabled the researchers to identify essential genes for megakaryopoiesis. Furthermore, the researchers discovered significant differences between megakaryocytes separated from the fetal liver and those found in the embryonic yolk. Single-cell RNA-seq has also enabled researchers to make important advances in other areas. For example, Luo et al. (2021) used scRNA-seq to understand more about human FOXP3+ regulatory (Treeg) cells, which are central to immune tolerance[2]. The researchers discovered novel Treg differentiation and heterogeneity information by studying individual cells. Before confining the cells in one lane of 10X Chromium equipment, the researchers collected samples and utilized FACS analysis by sorting the cells into several subtypes. The libraries were then prepared using a variety of techniques that depended on the information needed. These include a Chromium Single Cell 3' GEM, Library and Gel bead kit V3, and Chromium Single Cell 5′ Library & Gel Bead Kit. All samples were prepared following the 10X Genomics scRNA-seq protocol and sequenced by Illumina Platform. The raw reads were then demultiplexed and mapped to the human genome GRCh38 using CellRanger software. This research added to our understanding of Treg differentiation and heterogeneity.

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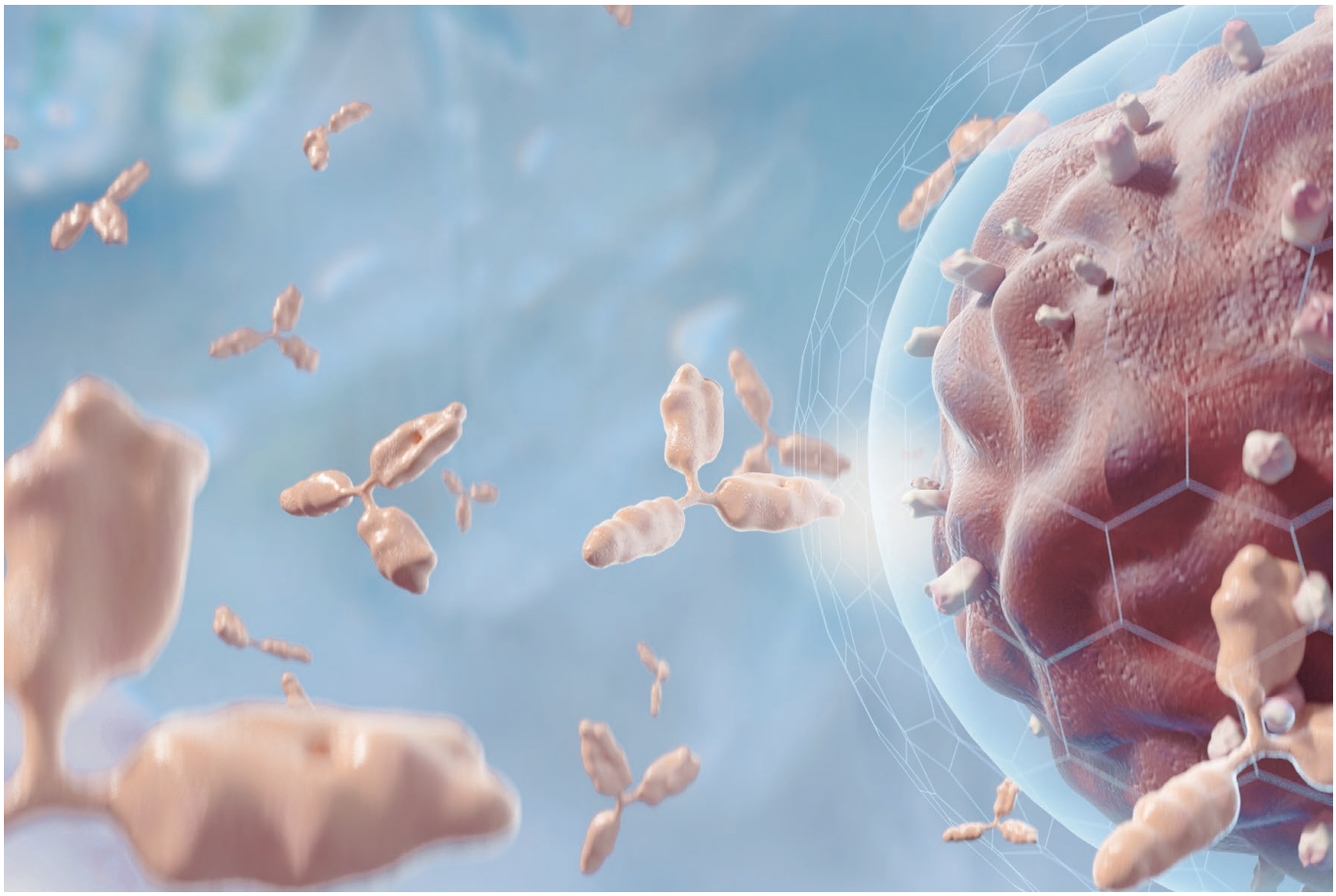
Advancing Our Understanding of Cancer Using Single-cell Sequencing

Understanding intra-tumor heterogeneity is one of the key challenges in cancer research, and an accurate assessment is essential for developing effective treatment strategies. Single-cell RNA-sequencing has enabled researchers to investigate the interactions between tumor and non-tumor cells with more precision, advancing our understanding of cancers and how they interact with the cells around them.

In one recent study, researchers used this technique in conjunction with functional assays to examine the transcriptomic heterogeneity and intercellular crosstalk present in gallbladder cancer[3]. Using scRNA-seq in this scenario enabled the researchers to demonstrate the extent of heterogeneity present in cancer cells and identify why some cancers are harder to treat, resulting in reduced survival. Single-cell RNA-seq libraries were generated by extracting cells from tissue samples before loading and converting them using Chromium Single Cell 3' Reagent Kits. Libraries were then sequenced using Illumina NovaSeq instruments. Sequencing generated single-cell transcriptomes of 114,927 cells from 13 gall bladder cancer samples. By using scRNA-seq, the researchers found 16 distinct cellular types, which they classified based on the expression of specific markers. The researchers were able to identify a previously unknown mutation in the ErbB pathway, which causes immunosuppressive macrophage differentiation and regulatory T cell activation and is linked to reduced survival in patients where the mutation is present.

In another study, researchers used single-cell RNA sequencing to examine the developmental trajectory of thyroid cancer[4]. The use of this technology was necessary to separate the cells and examine which genes were being transcribed at what point to understand how the cancer cells progressed. Single-cell RNA-seq libraries were generated from cells from extracted thyroid tissues. Prepared cells were loaded onto a 10X Genomics Chromium Controller machine to generate GEMs. A Chromium Single Cell 3' reagent kit (10X Genomics, V2 chemistry) was then used to prepare barcoded complementary DNAs. The cDNA was then recovered, purified, and amplified to ensure sufficient quantities for library preparation. Libraries were run on Illumina's NovaSeq platform for PE150 sequencing.

The researchers examined 46,205 cells and their transcriptomes, identifying several cluster markers. Overall, this enabled them to identify 16 main clusters, which included 3 clusters for thyroid cancer cells. The use of scRNA-seq also enabled the researchers to understand the progression of cells from papillary thyroid cancer cells to anaplastic thyroid cancer cells.



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Getting Under Your Skin Using Single-cell RNA Sequecing

scRNA-seq has also been used to examine the development and heterogeneity of our skin cells, advancing our understanding of how the immune system in our skin develops and providing novel information that could be used to treat skin diseases.

The first study looked at the transcriptome of CD45+ hematopoietic cells in human fetal skin to examine the developmental dynamics of these cells[5]. The researchers used scRNA-seq to understand more about the development of the immune system during gestation. To do this, samples were collected and processed to obtain single cells as GEMs using the 10X Genomics Chromium platform. More specifically, the skin cell suspensions were mixed with a reverse transcription master mix and loaded onto the 10X Genomics Single Cell 3' Chip. Following this, the sample was then split into GEMs that contained gel beads precoated with oligonucleotides. These were used to capture any mRNAs that had been released to be reverse transcribed. The gel beads also contained a 16-bp 10 × barcode for cell indexing and a 10-bp unique molecular identifier (UMI) to enable the researchers to differentiate among the different transcripts. The barcoded cDNA was amplified to obtain enough material for library construction. The libraries were then sequenced using 150-bp paired-end sequencing on Illumina's NovaSeq 6000 system. From this, the researchers could identify 13 different immune cell types and examine how they changed throughout development. In addition, the researchers identified transcription factors that could potentially regulate the transitions of these cells, providing information on the development of the immune system in human skin during the gestational period.

The second study examined fibroblast heterogeneity in fibrotic skin disease, using keloid as a paradigm[6]. The researchers used scRNA-seq to examine and compare individual cells, providing a depth of information that would not be accessible using other techniques. Samples were processed using the Chromium Single Cell 3' Library & Gel Bead Kit v2, Chromium Single Cell 3' Chip kit v2, and Chromium i7 Multiplex Kit. The cell suspension was then loaded onto the 10x Genomics Chromium Controller machine to produce GEMs which contained gel beads coated with oligos containing poly-dT sequences. These sequences capture any mRNAs released following cell lysis and contain barcodes that identify the transcript. Following RT-PCR, the cDNA was recovered, purified, and amplified to create a 3' -end single-cell RNA library that was then sequenced using Illumina short reads sequencing platform (HiseqX & Novaseq).

All Sequences were processed using the 10X Cell Ranger Package. Results showed that there are four types of subpopulations of keloid fibroblast. Mesenchymal fibroblasts play an important role in keloids and are involved in collagen overexpression. The results from scRNA-seq further revealed the role mesenchymal fibroblasts have in skin fibrosis development, demonstrating that these cells were heavily interacting with all other cells. These findings provide important information for understanding the development of skin fibrosis and could be used to identify potential targets for the treatment of the disease.

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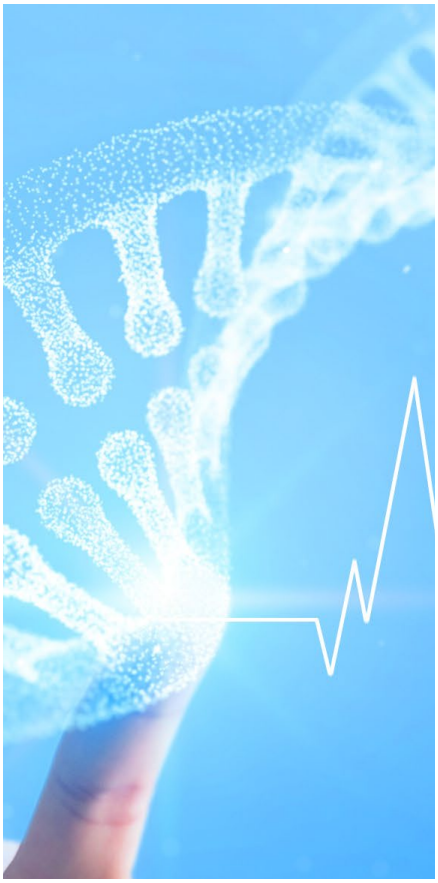
Conclusions

Single-cell RNA sequencing is a useful tool that can be used to answer a wide range of questions, from looking at the development of the immune system in human skin to examining the interactions between cancer cells and their microenvironment. This tool goes beyond what bulk cell analysis techniques offer as it enables researchers to examine the heterogeneity between cells and discover mechanisms that could have been missed using more traditional techniques.

Novogene offers easily accessible single-cell technologies that can be used to support your research. We offer high-quality single-cell sequencing using the 10x Genomics Chromium system and Illumina NGS platform. Our services include Single Cell Gene Expression, Single Cell Immune Profiling, and Single Cell ATAC. We also have a team of experts who can assist with bioinformatics and interpretation of your results, providing an efficient and accurate service that produces publication-ready results.



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