DOWNLOAD PDF SINGLE CELL ANALYSIS TECHNOLOGIES AND APPLICATIONS

Chapter 1 : RFA-RM Exceptionally Innovative Tools and Technologies for Single Cell Analysis (R21)

The first-ever comprehensive overview of the methods used in this key technology in modern biology provides the latest working knowledge needed by every scientist entering this growing field. It covers all the current technology and application areas, from microscopy and spectroscopy to proteomics.

Required and Optional Components The forms package associated with this FOA includes all applicable components, required and optional. Please note that some components marked optional in the application package are required for submission of applications for this FOA. All applications should include the following: Describe how the project addresses barriers to single cell analysis in situ that are due to the lack of tools to identify and target cells rigorously, how it provides a major change in practice from current methods for single cell analysis, and to what extent the resulting tool s provide first-in-class, cross-cutting or significantly advanced capacity for single cell analysis of heterogeneity in situ e. Describe how the project will transform the field by generating foundational resources e. Applicants are expected to explain the significance of the specific technical approach with respect to characterizing cell heterogeneity in situ. However, the specific biological assay s to be utilized in the application may be chosen primarily for utility rather than biological novelty in proof-of-concept testing of the exceptionally innovative technique. If using multiplex analysis, the design should indicate how multiple or cross-cutting approaches will be utilized to characterize heterogeneous cellular states in a more integrative manner. If using computational approaches, the design should indicate how these will yield a more comprehensive systems-level view of cell heterogeneity by modeling or dataset analysis. However, a sound rationale should be provided as to why the approach proposed is the most appropriate and likely to generate an exceptionally high impact if successful. In these cases, more emphasis should also be placed in details of the approach, particularly feasibility-testing. In all cases, there should be a particular focus throughout the application on risk management, including alternative strategies if the original plan fails to reach expected performance. These should be tailored to the unique scope of each project and written concretely enough to evaluate what exactly will have been achieved e. Tests should include a comparison against existing benchmark technologies; if a tool is truly first-in-class, comparisons may be done against a nearest neighbor technology. Investigators should briefly note how results will be used to inform future phases of tool development, validation or implementation beyond this R21 project. This section should be included within the page limit of the Research Strategy. If funded, investigators should describe progress toward milestones in the final report of the grant. While it is understood that many tools will be at an early proof-of-concept stage, a central goal of this FOA is to generate transformative tools that will be widely used throughout the research community. Applications that propose to generate such tools are expected to include a detailed plan for sharing these resources and should include the following key elements consistent with achieving the goals of this program: Project management of resource sharing; Description of what specific resources will be shared e. Do not use the Appendix to circumvent page limits. Foreign Institutions Foreign non-U. Submission Dates and Times Part I. Overview Information contains information about Key Dates. Applicants are encouraged to submit applications before the due date to ensure they have time to make any application corrections that might be necessary for successful submission. Organizations must submit applications to Grants. Applicants are responsible for viewing their application before the due date in the eRA Commons to ensure accurate and successful submission. Paper applications will not be accepted. Applicants must complete all required registrations before the application due date. Eligibility Information contains information about registration. For assistance with your electronic application or for more information on the electronic submission process, visit Applying Electronically. See more tips for avoiding common errors. Upon receipt, applications will be evaluated for completeness by the Center for Scientific Review and responsiveness by components of participating organizations , NIH. Applications considered nonresponsive to this FOA include: While the main goal of this FOA is in situ application of technologies, in vitro approaches

may be appropriate as an initial step in support of some studies. These costs may include salary for staff to be specifically hired under a temporary appointment for the project, consultant costs, equipment, supplies, travel, and other items typically listed under Other Expenses. Applicants should indicate the number of person-months devoted to the project, even if no funds are requested for salary and fringe benefits. Should an extramural application include the collaboration with an intramural scientist, no funds for the support of the intramural scientist may be requested in the application. The intramural scientist may submit a separate request for intramural funding as described above. Application Review Information 1. Criteria Only the review criteria described below will be considered in the review process. As part of the NIH mission , all applications submitted to the NIH in support of biomedical and behavioral research are evaluated for scientific and technical merit through the NIH peer review system. For this particular announcement, note the following: An R21 grant application need not have extensive background material or preliminary information. Accordingly, reviewers will focus their evaluation on the conceptual framework, the level of innovation, and the potential to significantly advance our knowledge or understanding. Appropriate justification for the proposed work can be provided through literature citations, data from other sources, or, when available, from investigator-generated data. Preliminary data are not required for R21 applications; however, they may be included if available. Reviewers should balance the risk with the likelihood of the new tools having an exceptionally high impact on the field, if successful. However, research designs should focus on innovative tool development for in situ analysis of single cell heterogeneity, rather than innovative biological concepts about cellular states or heterogeneity. Any proposed biological assay s should be chosen strictly for utility rather than biological novelty in proof-of-concept testing of the innovative technique. Overall Impact Reviewers will provide an overall impact score to reflect their assessment of the likelihood for the project to exert a sustained, powerful influence on the research field s involved, in consideration of the following review criteria and additional review criteria as applicable for the project proposed. Scored Review Criteria Reviewers will consider each of the review criteria below in the determination of scientific merit, and give a separate score for each. An application does not need to be strong in all categories to be judged likely to have major scientific impact. For example, a project that by its nature is not innovative may be essential to advance a field. Significance Does the project address an important problem or a critical barrier to progress in the field? How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field? How will the project address barriers to single cell analysis in situ e. How do the technical barriers relate to currently intractable scientific questions about cell heterogeneity, such that the application will transform the field by generating foundational resources e. If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field s? Innovation Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed? How does developing the tool s provide a major change in practice from current methods for single cell analysis? To what extent do the tool s provide first-in-class, cross-cutting or significantly advanced capacity for single cell analysis of heterogeneity in situ e. Approach Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed? How will the tools be made sufficiently sensitive, selective or appropriate for the intended use in single cell analysis in situ? If using multiplex analysis, how effectively will multiple or cross-cutting approaches be utilized to characterize heterogeneous cellular states in a more integrative manner? If using

computational approaches, how will they yield a more comprehensive systems-level view of cell heterogeneity by modeling or dataset analysis? In all applications, how well has a current benchmark technology been used to assess the relative utility of the new tool s? How rigorous is the proof-of-concept test of the tool s in a complex biological tissue or living organism? How useful are the proposed milestones e. Environment Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements? Additional Review Criteria As applicable for the project proposed, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an overall impact score, but will not give separate scores for these items. Protections for Human Subjects For research that involves human subjects but does not involve one of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation according to the following five review criteria: For research that involves human subjects and meets the criteria for one or more of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate: For additional information on review of the Human Subjects section, please refer to the Guidelines for the Review of Human Subjects. For additional information on review of the Inclusion section, please refer to the Guidelines for the Review of Inclusion in Clinical Research. Vertebrate Animals The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following five points:

Chapter 2 : SELECTBIO - Single Cell Analysis Summit

Here, with single cell RNA-seq emphasized, an overview of the discipline, progresses, and prospects of single-cell analysis and its applications in biology and medicine are given with a series of logic and theoretical considerations.

Background[edit] A typical human cell consists of about 2 x 3. It is more challenging to perform single cell sequencing in comparison with sequencing from cells in bulk. The minimal amount of starting materials from a single cell make degradation, sample loss and contamination exert pronounced effects on quality of sequencing data. In addition, due to the picogram level of the amount of nucleic acids used, [2] heavy amplification is often needed during sample preparation of single cell sequencing, resulting in the uneven coverage, noise and inaccurate quantification of sequencing data. Recent technical improvements make single cell sequencing a promising tool for approaching a set of seemingly inaccessible problems. For example, heterogeneous samples, rare cell types, cell lineage relationships, mosaicism of somatic tissues, analyses of microbes that cannot be cultured, and disease evolution can all be elucidated through single cell sequencing. A genome constructed in this fashion is commonly referred to as a single amplified genome or SAG. It can be used in microbiome studies, in order to obtain genomic data from uncultured microorganisms. In addition, it can be united with high throughput cell sorting of microorganisms and cancer. One popular method used for single cell genome sequencing is multiple displacement amplification and this enables research into various areas such as microbial genetics, ecology and infectious diseases. Furthermore, data obtained from microorganisms might establish processes for culturing in the future. MDA stands for multiple displacement amplification. Multiple displacement amplification MDA is a widely used technique, enabling amplifying femtograms of DNA from bacterium to micrograms for the use of sequencing. Reagents required for MDA reactions include: In 30 degree isothermal reaction, DNA is amplified with included reagents. As the polymerases manufacture new strands, a strand displacement reaction takes place, synthesizing multiple copies from each template DNA. At the same time, the strands that were extended antecedently will be displaced. MDA products result in a length of about 12 kb and ranges up to around kb, enabling its use in DNA sequencing. There are two components to this process: Due to scant amounts of DNA, accurate analysis of DNA poses problems even after amplification since coverage is low and susceptible to errors. In addition, MDA shows a high ratio of allele dropout, not detecting alleles from heterozygous samples. Various SNP algorithms are currently in use but none are specific to single cell sequencing. To solve this, when patterns can be generated from false CNVs, algorithms can detect and eradicate this noise to produce true variants. Single cell genomics is a powerful way to obtain microbial genome sequences without cultivation. This approach has been widely applied on marine, soil, subsurface, organismal, and other types of microbiomes in order to address a wide array of questions related to microbial ecology, evolution, public health and biotechnology potential. Such overlap may provide redundancy of pathway activation and tumor cell resistance. This is similar to single cell genome sequencing, but with the addition of a bisulfite treatment before sequencing. Forms include whole genome bisulfite sequencing, [21] [22] and reduced representation bisulfite sequencing [23] [24] Comparison of single cell methylation sequencing methods in terms of coverage as at on Mus musculus Single-cell RNA sequencing scRNA-seq [edit] Current methods for quantifying molecular states of cells, from microarray to standard RNA-seq analysis, mostly depend on estimating the mean value from millions of cells by averaging the signal of individual cells. Given the heterogeneity of cell population, measurement of the mean values of signals overlooks the internal interactions and differences within a cell population that may be crucial for maintaining normal tissue functions and facilitating disease progression. Thus the cell-averaging experiments provide only partial information of the molecular state of the system. Through gene clustering analyses , rare cell types within a cell population can be identified, thereby making characterization of the subpopulation structure of a heterogeneous cell population possible. While tumor heterogeneity can be attributed to accumulated mutations , even genetically identical cells, under the same

environment, display high variability of gene and protein expression levels. Single-cell RNA sequencing on a large number of single cells can identify such uncommon RNA and also reveal the copy-number distribution of the whole mRNA population in individual cells. Principally, the current scRNA-seq methods contain the following steps: The ideal scRNA-seq preserves and accurately quantifies the initial relative abundance of mRNA in a cell, covers the entire transcript lengths with equal representation at each position, and retains strand information. However, different PCR efficiency on particular sequences for instance, GC content and snapback structure will also be exponentially amplified, producing libraries with uneven coverage. On the other hand, while libraries generated by IVT can avoid PCR-induced sequence bias, specific sequences may be transcribed inefficiently, thus causing sequence drop-out or generating incomplete sequences. UMIs or the ability to process pooled samples. Single cell RNA-seq could be applied to differentiate cancer cells from normal blood cells and obtain the expression profiles of tumor cells at the same time. Similarly, single cell RNA-seq can also be used to analyze rare cell types in early human embryo and adult stem cells, both of which exist transiently and difficult to be characterized with current technologies. Finally, single cell analysis can be applied to the study of infectious diseases. Fluorescence-activated cell sorting FACS is the most widely used approach and is employed by several high-throughput core facilities, such as Bigelow Laboratory Single Cell Genomics Center. Laser-capture microdissection LCM can also be used for collecting single cells. Although LCM preserves the knowledge of the spatial location of a sampled cell within a tissue, it is hard to capture a whole single cell without also collecting the materials from neighboring cells. Both of FACS and microfluidics are accurate, automatic and capable of isolating unbiased samples. However, both methods require detaching cells from their microenvironments first, thereby causing perturbation to the transcriptional profiles in RNA expression analysis. For a typical mammalian cell containing , mRNA, sequencing data from at least 50 single cells need to be pooled in order to achieve this minimum CV value. However, due to the efficiency of reverse transcription and other noise introduced in the experiments, more cells are required for accurate expression analyses and cell type identification.

Chapter 3 : Single cell sequencing - Wikipedia

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Single-cell isolation[edit] Many single-cell analysis techniques require the isolation of individual cells. Methods currently used for single cell isolation include: Cell identification is ensured by the combination of fluorescent markers with image observation. Precision delivery is ensured by the semiconductor controlled motion of DEP cages in the flow cell. The development of hydrodynamic-based microfluidic biochips has been increasing over the years. In this technique, the cells or particles are trapped in a particular region for single cell analysis SCA usually without any application of external force fields such as optical, electrical, magnetic or acoustic. Hydrodynamic microfluidics facilitates the development of passive lab-on-chip applications. A latest review gives an account of the recent advances in this field, along with their mechanisms, methods and applications. This has led to the development of strategies for whole genome amplification WGA. Currently WGA strategies can be grouped into three categories: Controlled priming and PCR Amplification: This method uses the well established DNA amplification method PCR to try and amplify the entire genome using a large set of primers. Although simple, this method has been shown to have very low genome coverage. Despite these improvement MDA still has a sequence dependent bias certain parts of the genome are amplified more than others because of their sequence. Bias in this system is reduced by only copying off the original DNA strand instead of making copies of copies. Purpose[edit] There are two major applications to studying the genome at the single cell level. One application is to track the changes that occur in bacterial populations, where phenotypic differences are often seen. These differences are missed by bulk sequencing of a population, but can be observed in single cell sequencing. Since cancer cells are constantly mutating it is of great interest to see how cancers evolve at the genetic level. These patterns of somatic mutations and copy number aberration can be observed using single cell sequencing. The first step in quantifying the transcriptome is to convert RNA to cDNA using reverse transcriptase so that the contents of the cell can be sequenced using NGS methods as was done in genomics. Once converted, there is not enough cDNA to be sequenced so the same DNA amplification techniques discussed in single cell genomics are applied to the cDNA to make sequencing possible. The transcriptome is often used to quantify the gene expression instead of the proteome because of the difficulty currently associated with amplifying protein levels. Gene dynamics are usually studied to determine what changes in gene expression effect different cell characteristics. For example, this type of transcriptomic analysis has often been used to study embryonic development. RNA splicing studies are focused on understanding the regulation of different transcript isoforms. Single cell transcriptomics has also been used for cell typing, where the genes expressed in a cell are used to identify types of cells. Antibodyâ€"based methods[edit] The antibody based methods use designed antibodies to bind to proteins of interest. These antibodies can be bound to fluorescent molecules such as quantum dots or isotopes that can be resolved by mass spectrometry. Since different colored quantum dots or different isotopes are attached to different antibodies it is possible to identify multiple different proteins in a single cell. Quantum dots to be washed off of the antibodies without damaging the sample, making it possible to do multiple rounds of protein quantification using this method on the same sample. To do this, two antibodies are designed for each protein needed to be quantified. The two antibodies are then modified to have single stranded DNA connected to them that are complimentary. When the two antibodies bind to a protein the complimentary strands will anneal and produce a double stranded piece of DNA that can then be amplified using PCR. Each pair of antibodies designed for one protein is tagged with a different DNA sequence. Several groups have focused on oocytes or very early cleavage-stage cells since these cells are unusually large and provide enough material for analysis. These include using filter aided sample preparation , the use of magnetic beads , or using a series of reagents and centrifuging steps. The major difference between quantification

methods is some use labels on the peptides such as tandem mass tags TMT or dimethyl labels which are used to identify which cell a certain protein came from proteins coming from each cell have a different label while others use not labels quantify cells individually. The mass spectroscopy data is then analyzed by running data through databases that convert the information about peptides identified to quantification of protein levels. Since proteins are responsible for determining how the cell acts, understanding the proteome of single cell gives the best understanding of how a cell operates, and how gene expression changes in a cell due to different environmental stimuli. Although transcriptomics has the same purpose as proteomics it is not as accurate at determining gene expression in cells as it does not take into account post-transcriptional regulation. Techniques[edit] There are four major methods used to quantify the metabolome of single cells, they are: The first three methods listed use fluorescence microscopy to detect molecules in a cell. Usually these assays use small fluorescent tags attached to molecules of interest, however this has been shown be too invasive for single cell metabolomics, and alters the activity of the metabolites. The current solution to this problem is to use fluorescent proteins which will act as metabolite detectors, fluorescing when ever they bind to a metabolite of interest. Its advantages are that there is no need to develop fluorescent proteins for all molecules of interest, and is capable of detecting metabolites in the femtomole range. This method is also capable of detecting metabolites present in femtomole concentrations. In general the focus of metabolomics is mostly on understanding how cells deal with environmental stresses at the molecular level, and to give a more dynamic understanding of cellular functions.

Chapter 4 : Single-cell Analysis Market by Product, Application & End User - | MarketsandMarkets

This chapter "Single Cell Proteomics" has previously been published in: Proteomics for Biological Discovery, edited by Timothy D. Veenstra and John R. Yates; John Wiley & Sons, Inc.

 See other articles in PMC that cite the published article. It enables the identification of the minor subpopulations that may play a critical role in a biological process of a population of cells, which conventionally are regarded as homogeneous. It provides an ultra-sensitive tool to clarify specific molecular mechanisms and pathways and reveal the nature of cell heterogeneity. It also facilitates the clinical investigation of patients when a very low quantity or a single cell is available for analysis, such as noninvasive prenatal diagnosis and cancer screening, and genetic evaluation for in vitro fertilization. Within a few short years, single-cell analysis, especially whole genomic sequencing and transcriptomic sequencing, is becoming robust and broadly accessible, although not yet a routine practice. Here, with single cell RNA-seq emphasized, an overview of the discipline, progresses, and prospects of single-cell analysis and its applications in biology and medicine are given with a series of logic and theoretical considerations. The new generation of methodologies, especially the next and the third and so on generation of sequencing technology, plays a critical, leading role in genomics related fields. A massive analysis of cell populations would not be complete and representative without an extensive examination of a significant number of individual cells. Single-cell analysis or single-cell biology, as a new frontier, seeks to study a number of individual cells directly isolated from multicellular organisms, or collected in culture, providing unprecedented resolution for the understanding of the structure and function of an organ or tissue or system, and the interaction of single cells on a global scale [3 , 6 , 7]. This ability greatly promotes the understanding of life at a fundamental level and has vast applications in medicine [8 - 11]. The genomic sequencing of a single microorganism, taking advantage of the small genome size of a microorganism, was the first successful effort in next generation sequencing of a single cell genome, encouraging the birth of single-cell analysis [12]. The sequencing of a single microorganism enables the discovery and investigation of unknown microorganisms, from the human body to the deep sea, which would otherwise be impossible to sequence using standard cell numbers because they cannot be efficiently and faithfully cultured in the laboratory and because almost all microorganism populations are highly heterogenetic [2 , 12]. More powerful and less expensive high-throughput sequencing coupled with Multiple Displacement Amplification MDA and its derivations has achieved great progress in uncovering somatic mutations in the human genome [13]; another new method, Multiple Annealing and Looping-Based Amplification Cycles MALBAC showed faithful copy-number variation detection [14 , 15]. It is no doubt that human single-cell genomics â€” Whole Genome Sequencing or Whole Exome-Sequencing WGS or WES-seq has great potential in clinical applications, especially screening, diagnosis and monitoring [9 - 11]. Notably, the sequencing of the transcriptome of single-cells or single-cell RNA-seq has become the dominant technology in academic research because mRNA bridges genome structure and epigenomic modifications with the phenotype, revealing gene function and regulatory networks, and is relatively easier to perform than proteomics. A variety of versions of PCR-based methods have been developed for exponential amplification and sequencing of the transcriptome [17]. A widely used method, called Smart-seq and its updated versions Smart-seq2 and Smarter-seq, promises near full-length coverage of transcripts [18]. Smart-seq and its variants are very popular probably due to their successful optimization and commercialization, and because with these methods, mRNAs are highly, selectively amplified from total RNA and gDNA, and so no pre-purification of RNA is required. Other versions of PCR-based methods with different features have also been developed for different purposes. However, it is recognized that exponential PCR amplification, although highly efficient, may cause the distortion of the original ratio between transcripts,

causing the loss of relatively lowly expressed transcripts and the magnification of the original ratio â€” it tends to make the original small difference much larger. One advantage of PCR-based mRNA transcriptome amplification bias is that it makes the expression difference between samples more visible, but on the other hand, it may distort the difference when the original difference is marginal. IVT-based methods are characterized by linear amplification, which was originally developed for the RNA amplification from a low quantity of cells in by Dr. James Eberwine [3 , 23]. The early version suffered from low efficiency usually less than fold amplification and a laborious procedure, but now it is greatly improved. Several of the recent versions are very efficient, including the method dubbed Cell Expression by Linear amplification and sequencing CEL-seq , which is a highly multiplexed method [24] and quantitative single-cell RNA-seq Quartz-seq , which although not high-throughput, promises high reproducibility and sensitivity [25]. This type of method uses the highly efficient, low bias and uniform nature of amplification by RCA on circularized cDNA regardless the sizes of the original transcripts [22 , 26]. These features facilitate amplification of cDNAs on a microfluidic platform with very small volumes, and most notably, full-length sequence coverage. This pitfall has been resolved in our recently updated protocol. We have also developed a new strategy basing on PMA, which enables the separation of the gDNA from the RNA in the cytoplasm while simultaneously amplifying the whole transcriptome and the whole genome from the same single cell manuscript in preparation. This is a promising new direction that could potentially lead to the simultaneous, genome-wide scale analyses of the transcriptome, genome or even epigenome and proteome of an individual cell. New Efforts in Single Cell Analysis Bringing New Breakthroughs Single-cell analysis need not just involve standard RNA and DNA sequence examination, telomeres at the ends of all chromosomes [27], or more complex features that are encoded in DNA, such as CpG methylation and chromatin structure epigenome [28 - 31] and even the proteome [32 - 36] could be globally interrogated, which however currently are all suffered from a low coverage and low resolution particularly chromatin analysis. Single-cell proteomics is currently challenging in a conventional laboratory setting with limitations in the multiplicity and coverage of proteins, measured by candidate antibody probes and mass spectrometry, but significant progresses have been achieved [37 - 40]. A new method relying on the translation ratio provides a more practical tool to analyze proteomics at single-cell level [41]. Specifically, this method uses a reporter system that conjugates translation regulatory motifs to sequences encoding a nuclear-targeted fluorescent protein and a controllable destabilization domain [41]. Analysis of histone modifications, which involves a measurement of the various modified histones, is attractive to many scientists; however, the development of the technology at single cell level is in the early stages with only a limited number of factors detected in an assay [30 , 42]. It is worth noting that new methods or new applications lead to surprising new biological discoveries. Transcriptome In Vivo Analysis TIVA , enables the transcriptomic profiling of single cells resident in their natural microenvironment [43], although laborious yet with a direct procedure. This method uses a RNA capture procedure that is both noninvasive and spatially precise with potential applications in embryo, neuron and cancer cell studies where the understanding of the very specific spatiotemporal gene expression pattern is important [3]. These technologies are extremely exciting allowing us to study individual cells for their transcriptomics in their natural microenvironment. This is critical since once a cell is harvested, the relationship of the cell with its microenvironment, and probably the original molecular profile of the cell, usually will no longer be available. Other impressive efforts have been aimed at avoiding any bias possibly introduced during amplification by barcoding and decoding the original transcripts before and after amplification [41 , 42]. Recently, a new application that analyzed the gene expression profiles of single nuclei found an unexpectedly large number of transcripts: Another recent report revealed dynamic, random mono allelic gene expression in mammalian cells [48]. A large scale of single cell analysis for a complex tissue, spleen, showed the special power of this technology in deciphering the heterogeneity, and the dynamic composition and the functional mechanism of the tissue in responding to pathogen stimulation [49]. All these studies will have a great potential to be extended Figure 1.

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Chapter 5 : Single Cell Genomics: Technology and Application | Frontiers Research Topic

We offer analysis tools that can be used throughout various single-cell workflows for cellular biologists and researchers performing a wide spectrum of basic, translational, and clinical research projects.

The automated workflow, ready-to-use reagents and optimized IFCs integrated fluidic circuits eliminate tedious pipetting steps and sample mixing to increase productivity. The single-cell gene expression workflow using C1 and Biomark lets you: These small, noncoding RNAs regulate thousands of human protein-coding genes that are involved in biological processesâ€"such as differentiation, development and cell cycle managementâ€"as well as illnesses including cancer and cardiovascular disease. A single miRNA may regulate several biological systems, introducing cell-to-cell variability and making it difficult to reliably interpret and predict miRNA targets. Studying miRNA expression at the single-cell level offers greater insight into the differences between cells in the same population. Using the Fluidigm C1 and Biomark HD systems, the workflow for single-cell miRNA expression profiling provides an easy and reliable way to automatically capture and process individual live cells for routine miRNA analysis. Single-cell miRNA expression profiling allows you to: Use reliable chemistry and proprietary microfluidic technology to enable cell isolation and detection of miRNAs at the single-cell level Automate cell capture, staining, lysis, reverse transcription and miRNA amplification to simplify cell handling and eliminate systematic error Visualize and verify single cells with a variety of surface stains Generate and store miRNA libraries and interrogate single cells later for a diverse range of miRNAs Go from cell to analyzed data in less than 13 hours Process 96 single cells across more than miRNAs in a single run Whole Genome Sequencing Researchers are profiling genetic signatures of tumors and other complex diseases to identify causal and regulatory mutations. These diseases are often driven by somatic mutations that originate in individual cells and create genetically diverse clonal populations. As mutated cells proliferate, they derive complex polyclonal networks with low-abundant populations that cannot be fully interrogated by deep sequencing of bulk samples. In order to associate particular variants with specific cell populations, each cell must be measured independently. With whole genome sequencing, you can get the whole picture. The C1 single-cell whole genome sequencing workflow is the only approach that enables you to comprehensively and reliably identify mutations in both regulatory and protein-coding regions of the genome and directly associate these variants to clonal populations. Single-cell whole genome sequencing on the C1 offers: Bulk methods are unable to provide the clonal context of infrequent somatic mutations, making it impossible to define genetic signatures for particular cell populations. By sequencing the exome of individual cells, you can reveal the true heterogeneity and cell lineages present in a sample. The C1 single-cell whole exome sequencing workflow is the easiest method for discovering putative variants at the first stages of your research. One integrated workflow lets users isolate, stain, lyse, amplify and prepare NGS libraries from 96 individual cells in under 24 hours. The C1 single-cell whole exome sequencing workflow: Identifies novel or low-frequency variants at the first stage of experimental approach Provides a sensitive assay and an easy workflow to individualize and process low quantities of genomic DNA from single cells Collects data three times faster Offers optimized protocols that reduce standard reagent costs by 6x Targeted DNA Sequencing The Fluidigm single-cell targeted DNA sequencing workflow gives you the power to identify somatic mutations in known genes or loci from cell populations that are undetectable in bulk samples. This process enables new insights into intertumor and intratumor heterogeneity, cell lineage and clonal architecture, mosaicism involved in autoimmune disorders and disease prediction and progression. One integrated workflow lets users isolate, stain, lyse, amplify and prepare NGS libraries from 96 individual cells in less than 24 hours. The C1 single-cell targeted DNA sequencing workflow: Get access to scripts and other applications developed by Fluidigm and the C1 community, including epigenetics, mRNA sequencing, cell staining and more. Single-Cell Proteomics Protein Analysis Understanding complex processes in heterogeneous tissues lies at the heart of modern biology. The ability to identify cell subpopulations and their state and to characterize

their function is leading to breakthroughs in basic analysis, translational research and drug development. Single-cell protein analysis by mass cytometry enables the most comprehensive profiling of cell surface and intracellular protein markers.

Chapter 6 : Single-Cell Analysis Using Digital PCR | LSR | Bio-Rad

Luncheon Presentation: NGS Barcoding Technologies, CRISPR and Expression Profiling for Single Cell Genetic Analysis. Alex Chenchik, President and CSO, Cellecta. Clonal barcodes, or UMIs, can be combined with Perturb-Seq and single cell analysis techniques to generate important insights.

Article Source Historically cell populations were considered homogeneous and conventional cell-based assays utilized the average responses from a population of many thousands of cells. The results obtained from such analyses represent an amalgam of the biological status of each cell within the population analysed without considering individual cell phenotypes. To better understand the variations from cell to cell, scientists need to study single cells. Recent advances of single cell analysis techniques have facilitated the ability to discern biological insights within individual cells and provided the means to revealing previously hidden relationships between individual cells within a population or to detect subpopulations. Minority, rare cell events, and small changes between individual cells may hold the key to answering hitherto unresolved questions in cancer, stem cell biology, immunology, developmental biology and neurology, and facilitate therapeutic decision making in precision medicine. The system is Ideal for a wide range of applications including profiling natively paired TCRs, isolating antibody coding sequences, encapsulating cells in hydrogels or encapsulating expression libraries for FACS sorting. Cell isolation technologies are characterized by 3 performance criteria: Current cell isolation techniques broadly fall into 2 categories: These comprise affinity methods that typically involve labelling, such as affinity matrices, fluorescence-activated cell sorting and magnetic-activated cell sorting, many are based on microfluidic platforms. The most wanted single cells are however those that are isolated unlabelled and retain full viability. New miniaturized all-in-one single cell platforms that enable both the isolation and analysis are proving of increasing interest to researchers, particularly in the area of single cell genomics. In December HTStec undertook a market survey on single cell isolation mainly among research labs in academia, pharma and biotech. The objectives were to understand current interest, progress made in implementation and requirements for single cell isolation. It is intended to provide the reader with a brief insight into recent market trends. It covers only 11 out of the 31 original questions detailed in the full report. The full published report should be consulted to view the entire dataset, details of the breakdown of the response to each question, its segmentation and the estimates for the future. Please contact info htstec. Sources used to isolate single cells: The sources most used by survey respondents to isolate single cells are presented in Figure 1. The main cell types used for single cell applications are reported in Figure 2. The cell type from which respondents most want to isolate single cells was primary cells. There was limited interest in non mammalian cell types. The starting population from which survey respondents most want to isolate single cells are given in Figure 3. The main technologies currently used to isolate single cells are presented in Figure 4. This showed that cells with desired phenotype were ranked most relevant. This was followed in relevance by cells that are singlet i. Least relevant was cells at cell cycle specific stage of development. The importance of isolating individual cells for downstream experiments are given in Figure 6. This revealed it was of critical importance to most survey respondents research i. This was followed by desirable i. The importance of isolating viable single cells to survey respondents planned research is detailed in Figure 7. The main downstream analysis methods used or planned to be used with isolated single cells are presented in Figure 8. Please note this should NOT be regarded as a true market share projection. It is based on the supplier of single cell isolation technologies that first comes into the mind of survey respondents, which may not be the same as those who they purchase most consumables or instruments from. The downstream application most likely to be impacted by single cell isolation technologies are given in Figure The biggest limitations of currently available single cell isolation technologies were rated in Figure All other factors were rated moderately limiting, suggesting they all represent significant problems in single cell isolation. The selected findings reported above revealed suspension cell cultures were still most used to isolate single cells. The main cell

types used for single cell applications were primary cells. The preferred starting population was an abundant representative sample i. The importance of isolating individual cells for downstream experiments was of critical importance to most survey respondents research i. The majority of respondents reported that it was critical to maintain the viability of isolated single cells. The main downstream analysis method used or planned to be used with isolated single cells was RT-qPCR. Although much progress has been made during the recent years in single cell isolation there are still major challenges with isolation methodologies. Several new and emerging technologies now available to isolate viable single cells address some of these issues: When a DEP cage is moved by a change in the electric field pattern, the trapped cell moves with it. Cytena Single Cell Printer enables fully automated isolation of single cells into standard microwell plate formats. The instrument uses an inkjet-like principle featuring a disposable, one-way printing cartridge. The cell sample is pipetted into the cartridge and an external actuator is used to eject droplets out of it. The integrated optical sensor allows for determination of cell number in each droplet. A fast shutter mechanism sorts the droplets containing exactly one single-cell into the substrate. Unwanted droplets are deflected into waste. Within the QIAscout array, the microrafts serve as releasable culture sites for individual cells or colonies. QIAscout arrays are mounted on inverted microscopes and can be imaged by brightfield, fluorescence and confocal microscopy. A motorized release device is mounted onto a 4x, 5x or 10x objective of common inverted lab microscopes to allow for selection and picking of individual microrafts. To isolate target cells, simply press the button on the controller which makes the release needle pierce through the lower surface of the QIAscout array and dislodge an individual microraft with your cell of interest attached. The released microraft is then recovered using a magnetic wand and transported to a secondary vessel e. This semi-automated system encapsulates single cells or biomolecules into picodroplets, ready for downstream screening and analysis. It does this at a rapid rate of up to 70, picodroplets per second, and the flow rate can be highly controlled. These picodroplets can be stabilised using novel surfactants and cells can be grown in them, and even incubated or stored for many days. Over the coming years we can expect single cell techniques will become a powerful tool in unravelling longstanding questions in our understanding and treatment of human disease. HTStec Limited has exercised due care in compiling and preparing these Selected Findings from its Report, which is based on information submitted by individuals in respondent companies. No warranty is either expressed or implied with respect to the use of these Selected Findings. Under no circumstances shall HTStec Limited be liable for incidental, special, indirect, direct or consequential damages or loss of profits, interruption of business, or related expenses that may arise from use of these Selected Findings, including but not limited to those resulting from inaccuracy of the data therein.

Chapter 7 : Single-Cell Microgels: Technology, Challenges, and Applications. | Single Cell Analysis

single cell analysis is the new frontier in omics, and single cell omics has the potential to transform systems biology through new discoveries derived from cellular.

Chapter 8 : Single Cell Analysis: From Technology to Biology and Medicine

As described in this review, single cell analysis is the new frontier in Omics, and single cell Omics has the potential to transform systems biology through new discoveries derived from cellular heterogeneity.

Chapter 9 : Single Cell Isolation Trends: Technologies, Limitations & Applications | Single Cell Analysis

Single cell analysis is a rapidly evolving field with applications in cancer, PGD, immune response, and others. This method is enabling researchers to more deeply understand basic biology and disease development.