SYNGEN

SERIES LONDON

Pre-Event Newsletter Sept 2019

FEATURING

Takara Bio – Your Expert Partner for Single Cell Genomics

Recent Advances In Zinc Finger Gene Editing Technology

Seizing The Opportunity Of The Decoded Human Genome

AND MUCH MORE!

NOVOTEL LONDON WEST HOTEL

07 - 08 NOVEMBER 2019 | LONDON, UK

OXFORD GLOBAL CONFERENCES

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Sponsorship Account Executive

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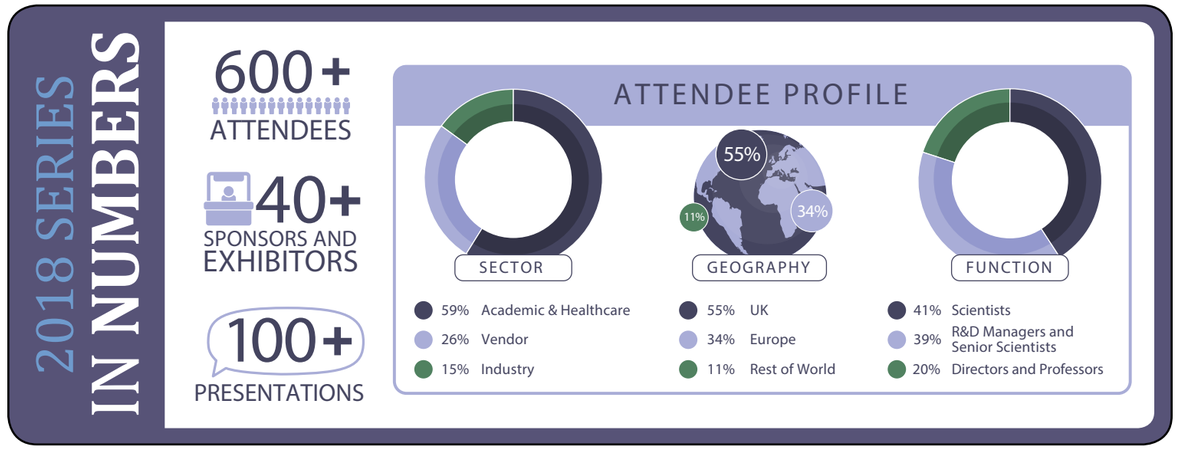
Delegate Sales Executive

Aurelia Iotu
Delegate Sales

We look forward to welcoming you to the event in November!



WELCOME TO OXFORD GLOBAL'S SYNGEN SERIES UK PRE-EVENT NEWSLETTER!

With Oxford Global's SYNGEN London 2019 Series taking place in November, I am delighted to bring you news of key features & exciting additions for this year's congress. The 2018 congress proved incredibly popular, bringing together over 600 attendees in London to discuss collaborative solutions, challenges and the latest developments within the Next Generation Sequencing and Clinical Diagnostics, Single Cell Analysis, Gene Editing and Synthetic Biology research fields. The feedback concerning the quality of scientific talks and seniority & diversity of the attendees was overwhelmingly positive.

This year's congress is celebrating its eleventh year anniversary and building on the exciting talks and extensive networking opportunities from last year, the event will feature the addition of the Digital PCR programme, offering attendees options to benefit from five co-located programmes, each with an impressive line-up of expert speakers.

For 2019, we have partnered with our Diamond Sponsor, Takara Bio to create an exclusive educational opportunity to learn about single cell multi-omics, its applications, challenges and future directions. This focused half a day workshop will kick off at 12.30pm on 6th November with a networking lunch. The best thing is that for delegates registered for the SYNGEN London 2019 Series, it is free to join. However due to limited places registration is required. For more information please email p.franko@oxfordglobal.co.uk.

If that wasn't enough, this year will see even more opportunities to learn from your peers through the addition of in-programme featured workshops available for each conference track. The NGS track will cover **Long and Short Read Sequencing**, Single Cell Analysis will focus on **Single-Cell Sequencing for Immunologist**, Genome Editing will provide updates on **Advancements Of CRISPR In Discovery And Therapeutic Development** and Digital PCR workshop is going to provide insights into **Circulating Tumour DNA Analysis: Moving Towards Precision Oncology**.

I hope you enjoy reading a range of scientific articles and Q&A insights with some of our academic, healthcare and pharma speakers and participating sponsors, and I look forward to welcoming you to the 2019 Congress!

- Peter Franko, Commercial Director

SYNGEN

SERIES UK 2019

NOVOTEL LONDON WEST HOTEL

07 - 08 NOVEMBER 2019 | LONDON, UK

1. 11TH ANNUAL **NEXT GENERATION SEQUENCING & CLINICAL DIAGNOSTICS** CONGRESS
2. 7TH ANNUAL **SINGLE CELL ANALYSIS** CONGRESS
3. 5TH ANNUAL **GENOME EDITING** CONGRESS
4. 2ND ANNUAL **SYNTHETIC BIOLOGY** CONGRESS
5. **DIGITAL PCR** CONGRESS

WHO IS ATTENDING?

For the full attendee list please contact
marketing@oxfordglobal.co.uk

- **600+ senior-level delegates** delegates representing internationally renowned research & academic institutions, clinical research institutions, and healthcare organisations, as well as leading pharmaceutical and biotech companies.
- **Directors, VPs, CEOs, and Heads** working in Next Generation Sequencing, Molecular Diagnostics, Computational Biology, Single Cell Analysis, Single Cell Genomics, Biochemistry, Genetic Modifications, Genome Engineering, Gene Regulation, Viral Vector Engineering, and Gene and Genome Assembly.

These companies and many more:

AstraZeneca · AgenTus Therapeutics · Novartis Institutes for Biomedical Research · UCL · Vanderbilt University Medical Center · NHS · The University of Edinburgh · King's College London · UniversitätsSpital Zürich

Sponsors 2019

DIAMOND

TaKaRa · Clontech · TaKaRa · cellartis

SILVER

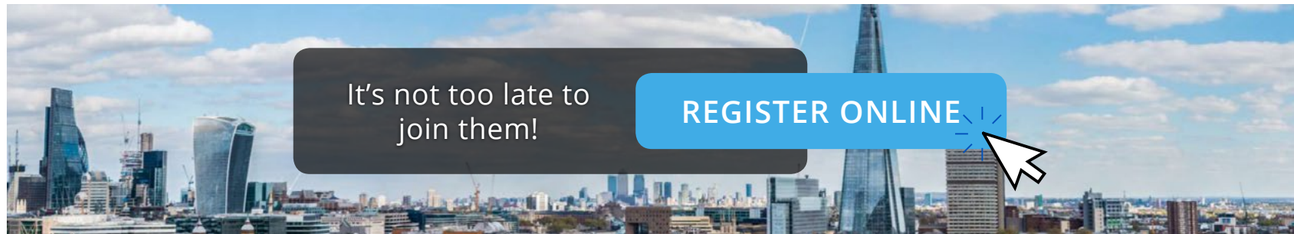
bio-techne · cellenion (Enhancing Life) · charles river · IDT (Integrated DNA Technologies) · STILLA

BRONZE

10x Genomics · Partek (turning data into discovery) · Oxford Genetics (Biology Engineered) · Roche · Celsee · ThermoFisher Scientific · Cytosurge · Samplix · Labcyte · Fluidigm · mission bio · Single Cell Discoveries · PerkinElmer (For the Better) · GenScript (Make Research Easy) · CellSorter · cytena (single cells on demand) · Menarini silicon biosystems · Twist Bioscience

NETWORK AND PROGRAMME

MMI · Synthego · Biolegia · dolomite bio · Source BioScience · BGI · GE Healthcare Life Sciences · CeGaT (Center for Genomics and Transcriptomics) · Covaris · GC biotech · BlueBee · LEXOGEN (Enabling complete transcriptome sequencing) · Bio-Rad · ttplabtech · eppendorf · Formulatrix · Olympus · dispendix · MGI 华大智造 · Novogene · siTOOLs Biotech · Genewiz (A Brooks Life Sciences Company) · SensID · Nepagene · Cardiff University Central Biotechnology Services (Prifysgol Caerdydd Gwasanaethau Biotechnegol Canolog) · VectorBuilder · Millipore Sigma



DISSECTING UVEAL MELANOMA BY QUANTIFYING THE IMMUNE INFILTRATE AND PROGRESSION STEPS WITH DIGITAL PCR

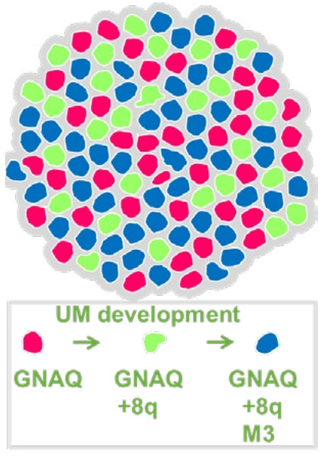
PIETER VAN DER VELDEN

Pieter Van Der Velden,
Principal Investigator,
Leiden University Medical Center

Pieter holds a position at the LUMC in the department of Ophthalmology as PI in the field of uveal melanoma genetics. By applying the most recent technical advances, the heterogeneity of UM is studied. Genetic and cellular heterogeneity provides information on molecular pathogenesis and may ultimately offer chances for treatment. Pieter is part of UM Cure, an European consortium of preclinical researchers that aims to provide treatment for metastatic uveal melanoma

The LUMC Ophthalmology group is involved in the molecular analysis of uveal melanoma. A slow growing tumour in the eye that originates from melanocytes that accumulate a limited number of mutations during tumour development. With digital PCR recurrent mutations were quantified and based on the absolute clonal fractions an order of events could was deduced. Quite surprisingly, irrespective of the seize of the tumour, precursor cells with only driver gene mutations could be detected in most tumours. This was corroborated by driver gene mutations in GNAQ and GNA11 in nevi from the eye that represent a possible precursor for uveal melanoma. This illustrates that digital PCR provides a means to study tumour evolution independent of access to histologically defined precursor lesions.

Though uveal melanoma develops in the void of the eye it rarely presents as a pure mass of tumour cells. Often a variety of immune cells are found within uveal melanoma and thereby immune cells appear to be part of the tumour. Because this varies between tumours with different clinical characteristics, there is an interest to define and to measure the immune infiltrate. In genomics this is commonly inferred from the expression profiles by analysing for immune cell marker expression in uveal melanoma tissue. However, expression of marker genes does not provide an absolute measure for the number of cells and this limitation diffuses the interpretation of expression data. DNA in contrast provides an intrinsic '2N' normalisation and thereby a digital code for the number and types of cells that are present in a tissue sample. Based on this paradigm the LUMC group set out to develop digital PCR assays to count immune cells in the tumour tissue. T cells can be ubiquitously present in uveal melanoma and are defined by VDJ rearrangement of the T cell receptor genes. Based on sequences that are lost during VDJ rearrangement, assays were developed to quantify immune cells in DNA from mixed tissue samples. During uveal melanoma development T cells accumulate and may account for up to 40% of the tumour mass. With absolute T cell numbers the immune infiltrate could be accessed very specifically and this allowed to deconvolute the immune compartment even further. T cell numbers where correlated with the genome wide expression profiles to create immune profiles of all involved cells. This revealed that only a part of the T cell correlated genes are actually derived from T cells while the remainder was expressed by other immune cells. Besides revealing the complexity of the immune infiltrate this also provided an insight into the possible interactions within the immune compartment. CXCL10 for example was expressed by activated macrophages and highly correlated to T cell count and thereby indicated an order of events in UM inflammation in which macrophage activation precedes T cell infiltration.



Combined these studies showed that molecular dissection of UM is very much assisted by absolute quantification of tumour clones and immune cells and that this only requires access to tumour DNA.

Pieter will be expanding on this topic on Day 2 of our Digital PCR Congress, with his talk '*Digital PCR For The Molecular Analysis Of Uveal Melanoma.*'

RESOURCES AND ASSISTANCE FOR CREATING GENETICALLY MODIFIED RODENTS

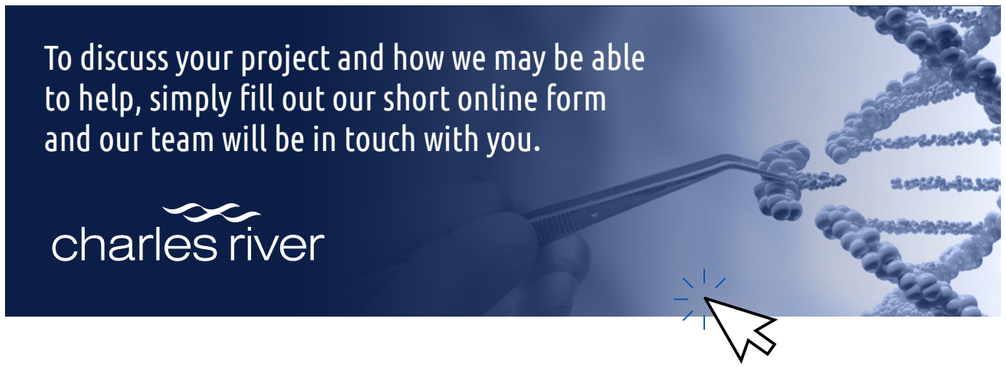
Many would agree that the advent of genome editing technologies such as CRISPR/Cas9 are revolutionising the way new transgenic models are created. However, their impact on the rodent genome requires careful verification, as erroneous modifications can lead to non-functional alleles or unwanted mutations that can seriously affect your research programme.

With this in mind, below are some resources, which we trust you will find useful:

- **Webinar** series on **CRISPR/Cas9** and generating transgenic mice and rats
- **Publication** on methods to quickly generate large genomic variants
- **Web page** listing considerations on where/how to source your models

Together with our industry partner **PHENOMIN-ICS**, Charles River has a solid track record for creating new mouse and rat lines on behalf of our clients. You may be concerned regarding the cost implications of outsourcing your projects. Our specialists can help you determine a true, full-cost comparison.

We wish you all the best in your scientific endeavors, and look forward to meeting up with you at the 5th Annual Genome Editing Congress.



SPEAKER Q&A: **TIINA PALOMÄKI**

Tiina Palomäki,
Senior Researcher,
Finnish Medicines Agency

Dr Tiina Palomäki has been working as a senior researcher at the Finnish medicines agency responsible for preclinical aspects of advanced therapy medicinal products and biotechnological products since 2006. She has been a member of the EMA Committee for advanced therapies (CAT) 2014-2017, a member of the Cell-based product working party 2008-2012 and an alternate member of the Gene therapy working party 2008-2010. Prior to joining the Finnish medicines agency she worked 13 years in academic research. She graduated from the University of Helsinki 1993, and made her PhD and a post doc in the Dept of Biosciences in the University of Helsinki. 2004-2006 she worked in Biomedicum Helsinki as a senior investigator in basic research aiming at development of human embryonic stem cell–based therapy.

What are you working on The Finnish Medicines agency and/or the CAT?

I am working as a senior researcher in the Finnish medicines agency as a responsible person for preclinical aspects of advanced therapy medicinal products i.e. somatic cell therapies, tissue engineered products and gene therapy medicinal products as well as biotechnological products. My principal activities include review of marketing authorisation applications and clinical trial applications as wells as scientific advice both at the national and at the EMA (European Medicines Agency) level. I have had a significant contribution to the regulation of advanced therapies. I have been actively involved in development of the guidelines for advanced therapies in the Committee for advanced therapies (CAT) of the EMA and its predecessors Cell-based product working party (CPWP) and Gene therapy working party (GTWP) with the aim to harmonise the regulatory requirements in the EU and to facilitate clinical development of advanced therapies.

What are the key challenges in regulating gene-edited medicine?

Genome editing, as all new technologies, is loaded with high expectations but at the same time, it is faced with fear of what it might bring to us. Genome editing can provide means to permanently change human genome in ways we have not seen before. The possibility of creating inheritable changes in the germ line is concerning, and if unregulated may lead to questionable and unethical experimentation, which upon realization has created worldwide turmoil in the scientific and medical community. Therefore, mutual understanding between scientists, legislators, regulators, health care professionals and ethicists on the boundaries for application of genome editing in humans is paramount to ensure safe and ethical use of genome editing in medicine.

At present, there are perceived risks such as off-target effects, and the yet unknown long-term effects that can affect the attitudes and the speed of clinical application of gene edited medicinal products. It is important that all gene edited medicinal products are regulated similarly to ensure that they are all subject to same level of scrutiny in order to ensure patient safety. Therefore, harmonized view on the regulation of such products is important.

What potential do you think genome editing has for healthcare as a whole?

Genome editing has the potential to provide treatment in the areas of unmet medical need and possibly provide cure for some of the previously untreatable diseases. With genome editing it is possible to develop more effective targeted and personalized therapies to patients that would most likely benefit from the treatment. Genome editing has entered into clinical development phase and a few clinical trials are underway. The technology is under intense research to develop more specific, accurate and thus, safer tools for genome editing. Once more information and insight of safety of gene editing from patients in the early clinical trials has accumulated, it is likely that the number of clinical trials utilizing genome edited products will rapidly increase.

Similar to some other personalized therapies it is likely that the introduction of genome edited medicinal products into healthcare would face the issues of high cost and reimbursement but would eventually provide much needed therapies for those that currently lack effective treatments.

What do you intend to get out of attending the Genome Editing Congress in London in November?

My expectations for the meeting reflect the rapid and intense development of the area. My work as a regulator necessitates keeping myself updated with the new technologies and more specific and safer tools for genome editing, and I expect to get an up-to-date snapshot of the field. Equally importantly, innovations aiming at improved safety and the information on the preclinical safety of various gene editing approaches are in my focus, as well as information on the gene edited products entering into clinical development. I am hoping to get a good coverage of the field in terms of genome edited medicinal products, and to identify the players in the field.

BIO-TECHNE ANNOUNCES COMMERCIAL RELEASE OF RNASCOPE® HIPLEX ASSAY: A MULTIPLEX IN SITU HYBRIDIZATION ASSAY FOR TISSUES

Expanding research tools for Spatial Genomics by combining molecular information with spatial context in one simple assay.

MINNEAPOLIS, July 11, 2019 /PRNewswire/ -- Bio-Techne Corporation (NASDAQ:TECH) today announced the expansion of the Advanced Cell Diagnostics™-branded RNAscope platform with release of the RNAscope HiPlex Assay. The RNAscope platform is an advanced in situ hybridization assay that enables visualization of single-molecule gene expression with single-cell resolution directly in intact tissues.

> *"Our aim is to provide researchers with a platform to precisely characterize cells and gain greater understanding of the transcriptome in a complex and heterogeneous tissue environment through higher plexing"*

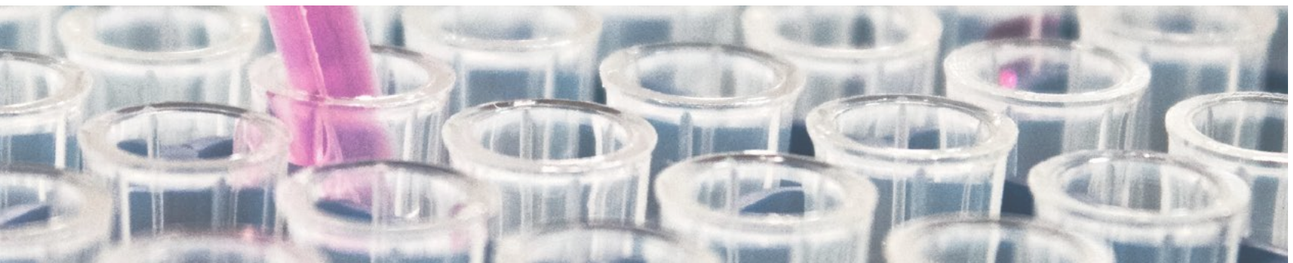
The RNAscope HiPlex Assay enables researchers to gain greater insights into cellular mechanisms and functions by combining a simplistic workflow with the capability of simultaneously detecting up to twelve RNA targets. Comprehensive spatial studies require tools that permit higher multiplexing capabilities with minimal time, simple processing, and quality performance, while conserving precious samples. Such multiplexing cannot be achieved with traditional in situ hybridization techniques. The RNAscope HiPlex Assay utilizes Bio-Techne's patented signal amplification and background suppression technology to deliver unrivalled specificity and sensitivity with optimal signal generation. Using this assay, researchers are able to perform experiments that generate more data per sample with better characterization of the samples, such as identifying specific cell types with known cellular markers, without compromising the morphological features of the tissue in question.

Jacob Swanson at the Hospital for Special Surgery in New York City, NY, commented, "The RNAscope HiPlex Assay allows a researcher to add histological context to scRNAseq data, which is important for fully understanding the biology of distinct cell populations. Being able to stain a single section of tissue with up to twelve mRNA probes makes the RNAscope HiPlex Assay a powerful tool for distinguishing cell types in situ. I would recommend the RNAscope HiPlex Assay to anyone looking to visualize distinct populations of cells from scRNAseq data in situ."

Kim Kelderman, President of Bio-Techne's Diagnostics and Genomics Segment, commented, "We are pleased to expand our in situ RNA tissue analysis leadership position with the release of the RNAscope HiPlex Assay. This is a new platform that is built on our proprietary RNAscope core technology. Our aim is to provide researchers with a platform to precisely characterize cells and gain greater understanding of the transcriptome in a complex and heterogeneous tissue environment through higher plexing."

bio-techne®

The RNAscope HiPlex Assay kits from Bio-Techne is intended for research use only.



REGULATING NOVEL BIOTECHNOLOGIES
CHRISTIANE NIEDERLAENDER

Christiane Niederlaender,
Senior Quality Assessor
and Unit Manager,
Biologicals, MHRA

Christiane Niederlaender is a Senior Quality Assessor and Unit Manager, Biologicals with the Medicines and Healthcare Products Regulatory Agency (MHRA) in the UK, where she has worked since 2011. She is also the Acting Unit Manager of the Biologicals Unit there. Christiane has experience in the assessment and regulation of all classes of biological medicinal products and has been responsible for the regulatory review of many new biological products including recombinant proteins, blood products and several Advanced Therapy Medicinal Products. She is the current UK representative at the Committee for Advanced Therapies (CAT) at the EMA. Prior to joining the MHRA, Christiane has worked for several years at the UK Human Tissue Authority (HTA) in the regulation of Tissue and Cell Therapies under the European Tissues and Cells Directive. Christiane received a Ph.D. in molecular developmental neurobiology from Kings College London and has spent several years researching and publishing in the area of cancer, development and neurobiology before taking a law degree and joining the regulatory profession.

What have you been working on at the MHRA?

As a Senior Quality Assessor in the Biologicals Unit of the MHRA, I have been dealing with the licensing and assessment of all types of biological medicinal products. At the same time, I frequently provide scientific advice during the development phase of biological medicines. I have specialist expertise in advanced therapy medicinal products (ATMPs) and have been a member of the Committee for Advanced Therapies (CAT) at the EMA. I have also been regularly involved with the EMA's Biologics Working Party (BWP).

ATMPs consist of cell based and gene therapy products which represent cutting edge science, from this stems my general interest in novel biotherapeutic products. I have been actively involved in the MHRA's Innovation Office (IO) service which provides regulatory advice and guidance to organisations developing innovative medicines, medical devices or novel manufacturing processes. I have also been involved in providing advice via the Innovation Task Force (ITF) at the EMA which provides a similar service for very early stage products and technologies.

In your experience, what have been the key challenges in the regulation of novel biotechnologies?

Although biotechnological medicines, such as enzymes and antibodies manufactured using recombinant DNA technology, are now regarded as established, it is important to bear in mind that they represent comparatively new technologies in the field of medicines. Hence regulators of biological medicines have already gained experience in adapting the regulatory environment to the challenges that new developments in biotechnology pose.

Developers of new biotechnologies are not normally familiar with the regulatory landscape and one of the first hurdles to overcome is to identify where in the framework a product fits. It could be a medicine, a device, a diagnostic or none of these and be simply a manufacturing aid. The precise application of a product may not be known during early development and at that point many technologies may fit potentially under a variety of different regulatory frameworks, so developers need to bear in mind the different scenarios that could eventually apply.

For the regulators themselves it is important to be kept abreast of new developments by companies which helps identify potential problem areas early on.

What potential do you think synthetic biology has for healthcare as a whole?

Synthetic Biology is such a wide field that it is impossible to underestimate the promise for healthcare and medicines, the limits are only the creativity of researchers in the field.

There are exciting developments in almost every aspect of them. Therapies that are closer to the clinic at this point are the resurgence of engineered therapeutic microbes, where there is potential to address a very wide range of diseases from cancer to infections and inherited disorders. In an era of perpetual donor shortage for organ transplants, bioprinting and the growth of organs in the laboratory also has to be ranked as on of the key hopes associated with the field. Of course there is great promise from synthetic biology for the development of regenerative medicines in general.

Apart from directly applicable approaches, the expansion of biology-based tools that can be employed in research will speed up the discovery of new treatments immensely.

What did you intend to get out of attending the Synthetic Biology Congress in London in 2018?

I saw this meeting as a great opportunity to learn about the latest developments in biotechnology and related areas, and there were plenty of opportunities to learn something new. I also wanted to get a more in-depth understanding of what researchers see as the key issues for new products in this field, when it comes to bringing their technologies into clinical use.

I was particularly excited to learn about the latest trends in using CRISPR gene editing, which has shown such promise in recent years. I also have a special interest in leaning about applications for computational approaches and AI in biotech. As regulators, we have identified that in respect of these there is a need to consider how we integrate them into the regulatory framework as they can be used in a way that has significant impact on the quality of manufacture of biological medicines.

Speaker Insight

HIGH-THROUGHPUT IMMUNE RECEPTOR SEQUENCING

WILFRED VAN IJCKEN

Wilfred van IJcken, Dr. ir, COO,
Center for Biomics, Erasmus MC

Wilfred van IJcken received his PhD in Molecular Sciences from the Wageningen University and Research Center, The Netherlands in 2001. During his PhD he sequenced and annotated the SeMNPV virus genome, resulting in the identification of a novel structural virus protein and a novel baculovirus envelope fusion protein. In 2002, he joined as a post-doc the group of Prof. Frank Grosveld at Erasmus MC in Rotterdam, where he initiated and started the genomics core facility. He implemented a wide range of genomics techniques including microarrays, imaging and high throughput automation. In 2007 he was one of the first offering Next Generation Sequencing to the Dutch research community. He was appointed as assistant professor in 2008 to boost innovation on next generation sequencing technology. Implementation of next generation sequencing in clinical diagnostics was achieved in 2012 and further professionalized by achieving ISO15189 accreditation in 2016. He is co-founder of a startup company focusing on methylation sequencing. Currently, he is responsible for the daily management (both financial and operational) of the genomics core facility with 10 full-time direct reporting technicians and bioinformaticians. He is also actively involved in medical student teaching and trainings. He is participating in several national programs and organizer of many NGS data analysis courses. He performs consultancy for several life science companies in and outside The Netherlands. Dr. ir. van IJcken is a regular speaker at international conferences and co-applicant of numerous successful grants. Teaming up with national and international research groups has yielded over 200 publications in international peer-reviewed journals.

The human immune system is a fine network consisted of the innumerable numbers of functional cells that balance the immunity and tolerance against various endogenous and environmental challenges. Although advances in modern immunology have revealed a role of many unique immune cell subsets, technologies that enable us to capture the whole landscape of immune responses against specific antigens have been not available to date. Over the past several years, high-throughput next-generation sequencing has been developed as a powerful tool to profile T- and B-cell receptor repertoires in a given individual at the single-cell level. Sophisticated immuno-bioinformatic analyses by use of this innovative methodology have already been implemented in clinical development of antibody engineering, vaccine design, and cellular immunotherapy.

Q1: What is your role at the Erasmus Center for Biomics?

The work of my team is focused on pushing the boundaries of current genomics technologies and developing new innovative genomic assays using next generation sequencing. T2C and Med-Seq are recent examples of such new genomics assays that we have invented. Targeted Chromatin Capture is a novel high resolution high throughput method to detect genomic interactions and regulatory elements in genomes. Med-Seq is a new and affordable method for methylated DNA sequencing. It generates highly reproducible genome-wide CpG methylation profiles for >50% of all potentially methylated CpGs, at a sequencing depth less than one-tenth required for whole-genome bisulfite sequencing. We also strive to develop new bioinformatic tools, such as Nimbus, which is one of the few design-driven analyses suites for amplicon-based NGS data.

Q2: What work have you done in Immune Repertoire Sequencing? What is your team involved in?

Together with colleagues from Internal Medicine and Immunology we are interested to apply recent advances in single cell sequencing to the great diversity of the T-cell receptor (TCR) repertoire. T cells express a TCR alpha/beta receptor, consisting of a TCRα and TCRβ chain. These TCR chains are highly diverse in their variable domains, which are uniquely formed through recombination processes involving V and J genes (TCRA locus) or V, D, and J genes (TCRB locus). This V(D)J recombination process generates high T cell diversity, which allows for recognition of a broad range of antigens. Single cell transcriptomics is a powerful tool for in-depth characterization of populations of cells by providing detailed information of individual cells. Single cell transcriptomics can be combined with TCR αβ repertoire analysis at the single cell level. Little is known as to what the potential of this combined approach comprises of. Therefore, the aim of this study was to evaluate the range of research questions that can be addressed by this combined approach, in particular with respect to clinical samples.

Q3: What are the current findings? Briefly describe the results.

We show that combined single-cell transcriptomics and TCR ab repertoire analysis can be applied to low-abundant populations of cells increasing the possibilities to analyse mRNA expression profiles and TCR ab repertoire of clinically relevant samples. We performed experiments to validate the approach and analysed combined TCR and transcriptomic profiles.

Q4: What are the opportunities and challenges involved?

This enables us to look both at the bulk as well as the single cell level. So using this approach we can now look at the total TCR diversity but also zoom in to the single cell level and determine single cell transcriptomic profiles and changes in patient samples e.g. after intervention or in time. A major challenge of clinical samples is the low cell number. To tackle this we use a combination of two systems (iCell8 and cellenONE) that enables us to work with small volumes as well as low cell numbers.

Q5: What is your aim in attending the congress?

I would like to meet other scientist that are working in the single cell sequencing field and learn from their experiences. Moreover, I would like to get updated on the latest advances in assays, instruments and bioinformatic analyses in the field of single cell sequencing.

BAM

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Application Note:

Arraying of single cells for quantitative high throughput Laser Ablation ICP-TOF-MS

Konrad Löhr[1,2,], Olga Borovinskaya[3], Guilhem Tourniaire[4], Ulrich Panne[1,2], Norbert Jakubowski[1], Alba Simón[5]*

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[5] SCIENION AG, Volmerstrasse 7b, 12489 Berlin, Germany

Abstract

With the development of ever more sensitive mass spectrometry systems, leading researchers are currently pushing boundaries to allow proteomics studies at the single cell level. Researchers at BAM successfully developed a novel method combining single cell isolation and arraying by cellenONE® and subsequent quantitative analysis and isotopic fingerprinting of these single cells by laser ablation ICP-TOF mass spectrometry.

Materials and method

Human monocytic leukemia cells (from cell line THP-1) were fixed and dyed with mDOTA-Ho and Ir-DNA-intercalator prior to arraying.

20 µL of cell suspension were aspirated using PDC70, coating type 3 with a sciFLEXARRAYER S3 (SCIENION AG) and single cells were arrayed using cellenONE® software (cellenion SASU) as fields of 10 x 10 positions (500 µm center-to-center distances) on glass slides (Superfrost+, Thermo Scientific).

Cells were ablated with 10 laser pulses per printed single spot (d=150µm) with 100 Hz repetition rate and a fluence of 0.36 J cm^{-2}. All measurements were carried out using the Analyte G2 ArF Excimer LA system (193 nm, Teledyne CETAC Technologies) with aerosol rapid introduction system (ARIS) coupled to an ICP-TOF-MS (icpTOF, TOFWERK).

Signals from cells were identified using the Originlab® peak finder function which searched for the peak centers of nucleated cells using the 193Ir-DNA-intercalator signal.

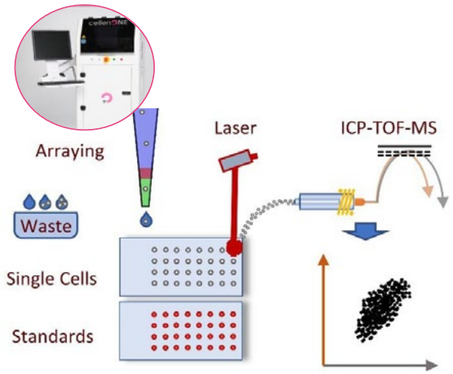

Figure 1. Scheme describing the method for quantitative analysis and isotopic fingerprinting by laser ablation ICP-TOF mass spectrometry with cellenONE®.

Results and Discussions

Using optimized cellenONE parameters, high single cell accuracy (>99%), and high cell recovery (>90%) were achieved. But more importantly, the LA-ICP-TOF-MS method could be fully automated to undertake ablation and measurement at a rate ~1 cell per second. In total, 562 single cells were measured to provide sufficient statistical significance.

Thirty calibration series with four different concentrations of mDOTA Ho and Ir-DNA-intercalator (10 spots per calibration series) were also spotted. These were used to calculate the content of both elements per cell and Figure 2 shows the Ir and Ho mass distributions in THP-1 cells. The Ir mass in cells was well above the LOQ (dashed vertical line), while for Ho mass some cells were close to the LOQ. The histogram of Ho content per cell appears to follow a simple normal distribution, while the Ir distribution had two maxima. It was also demonstrated (not shown here) that the two maxima of the Ir-DNA-intercalator were linked to the amount of DNA, which varies according to cell-cycle phases.

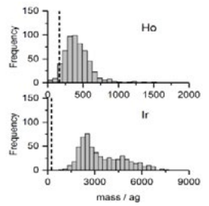

Figure 2. Histograms of Ho and Ir mass per single cell. Dashed lines represent LOQs of 102 ag for Ho and 236 ag for Ir, respectively.

LA-ICP-TOF-MS was also employed to detect naturally occurring isotopes in the whole mass range as fingerprints of individual cells (not shown here) and precise quantitative determination of metal-containing cell dyes was possible down to contents as low as ~100 ag.

Adapted from the article:
Löhr, K et al. - Arraying of single cells for quantitative high throughput Laser Ablation ICP-TOF-MS – Anal. Chem. 2019.
https://doi.org/10.1021/acs.analchem.9b00198

Conclusions

A novel single cell arraying approach was tested as preparation method for high throughput analyses of single cells by LA-ICP-TOF-MS. This automated method was proven to allow quantitative determination of isotopes (both endogenic and exogenic) which could be used as fingerprints of individual cells with detection limits as low as one hundred attogram per single cell.

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Application Note #5
September 2019

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MENARINI SILICON BIOSYSTEMS LAUNCHES VRNxT VOLUME REDUCTION INSTRUMENT

Automated volume reduction increases precision and accuracy in rare cell workflows

Bologna, Italy and Huntingdon Valley, Pa. -- Menarini Silicon Biosystems, the pioneer of liquid biopsy and rare cell technologies, has launched the new VRNxT™, a volume reduction instrument optimized to remove manual sample volume reduction steps in cellular biology workflows.

By standardizing sample volume reduction, the VRNxT adds precision and accuracy to rare-cell and single-cell analysis, minimizing the loss of precious samples and increasing workflow throughput. While the VRNxT has been developed to standardize the DEPArray workflow*, broader applications in volume reduction are possible.

"The VRNxT was created in response to direct customer feedback and is the must-have addition to DEPArray, or any other workflow," said Gianni Medoro, Chief Technology Officer at Menarini Silicon Biosystems. "This new device removes manual volume reduction steps and streamlines the overall process, adding precision and accuracy while reducing hands-on time."

The VRNxT automated volume reduction instrument removes manual pipetting steps, reducing hands-on time up to 90% and eliminating operator variability. No specific skill sets or training are required for operation, thereby delivering user-independent results with high reproducibility and success, with 99% of single cells retained after volume reduction.

"The instrument helped us to reduce our error rate and processing time," said Matija Snuderl, M.D., Department of Pathology Director, Molecular Pathology NYU Langone Health, who tested the new instrument in his laboratory. "We handle rare cell types isolated from FFPE specimens. With the VRNxT, we were able to lower the chance of error in the volume reduction step, which is especially important when single-cell samples are collected, and decrease the time required to process these samples."

The VRNxT uses rotational motion for sample volume reduction and can process up to four samples at once, decreasing the time required for sample preparation. Starting from any volume in a PCR tube, the VRNxT consistently reduces sample volume to approximately 12.5 µL in 25 seconds and approximately 2 µL in about eight seconds.

The DEPArray System is the only image-based sorting and isolation platform that combines microfluidics with microelectronics and microscopy to isolate and manipulate individual rare cells with 100% precision.

**The workflow described is for research use only. Not for use in diagnostic procedures. The performance characteristics, safety, and effectiveness of the workflow have not been established and are not cleared or approved by the FDA.*

About Menarini Silicon Biosystems

Menarini Silicon Biosystems offers unique rare cell technologies and solutions that provide clinical researchers with access to unparalleled resolution in the study of cells and their molecular characterization. The company's CELLSEARCH® and DEPArray™ technologies together provide an end-to-end solution for enumeration and sorting of rare cells with single-cell precision.

Menarini Silicon Biosystems, based in Bologna, Italy, and Huntingdon Valley, PA, US, is a wholly owned subsidiary of the Menarini Group, a multinational pharmaceutical, biotechnology and diagnostics company headquartered in Florence, Italy, with 17,640 employees in 136 countries.

For more information, visit www.siliconbiosystems.com

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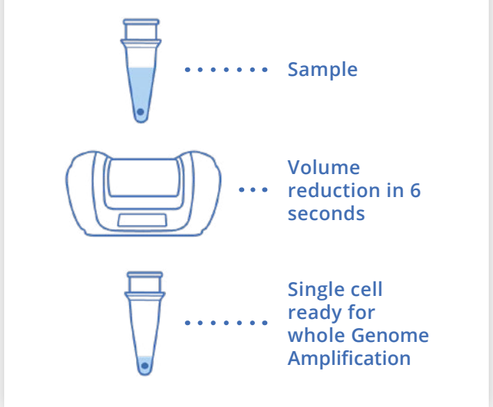
VRNxT: VOLUME REDUCTION INSTRUMENT



RECENT ADVANCES IN ZINC FINGER GENE EDITING TECHNOLOGY

Zinc finger proteins (ZFPs) are naturally occurring proteins that regulate gene expression by recognizing and binding to specific sequences of DNA. Sangamo Therapeutics, Inc., a genomic medicine company, uses a library of more than 3,200 ZFPs to engineer potential therapeutic tools for targeting a specific location in the human genome. By attaching different functional domains to those ZFPs, Sangamo is able to edit DNA or adjust the expression level of a particular gene to treat serious diseases. Zinc finger nucleases (ZFNs) are engineered by attaching a nuclease to a pair of ZFPs. ZFNs enable genome editing by cutting DNA at a precise location.

The ability to engineer highly specific gene editing nucleases with little or no detectable activity at unintended genomic sequences is a key safety factor for therapeutic applications. In August 2019, Sangamo's scientists described in *Nature Biotechnology* two new strategies for optimizing the specificity of ZFN genome editing[1]. When attempting to improve the specificity of genome editing tools, on-target editing efficiency is often sacrificed. However, with Sangamo's new strategies, the high efficiency which had been observed previously with ZFN-mediated genome editing was preserved, while off-target activity was reduced by approximately 1000-fold, to below the level of detectability.

The strategies to achieve these results entail engineering the two key functional regions within the ZFN structure, namely adjusting the binding affinity of the zinc finger array which recognizes DNA, and slowing the catalytic rate of the Fok1 cleavage domain. The two approaches, which are complementary, may be combined to enable near 100% on-target modification with no detectable off-targets in select cell types.

About Sangamo Therapeutics, Inc.

Sangamo Therapeutics is committed to translating ground-breaking science into genomic medicines with the potential to transform patients' lives using gene therapy, *ex vivo* gene-edited cell therapy, and *in vivo* genome editing and gene regulation.

For more information, visit www.sangamo.com

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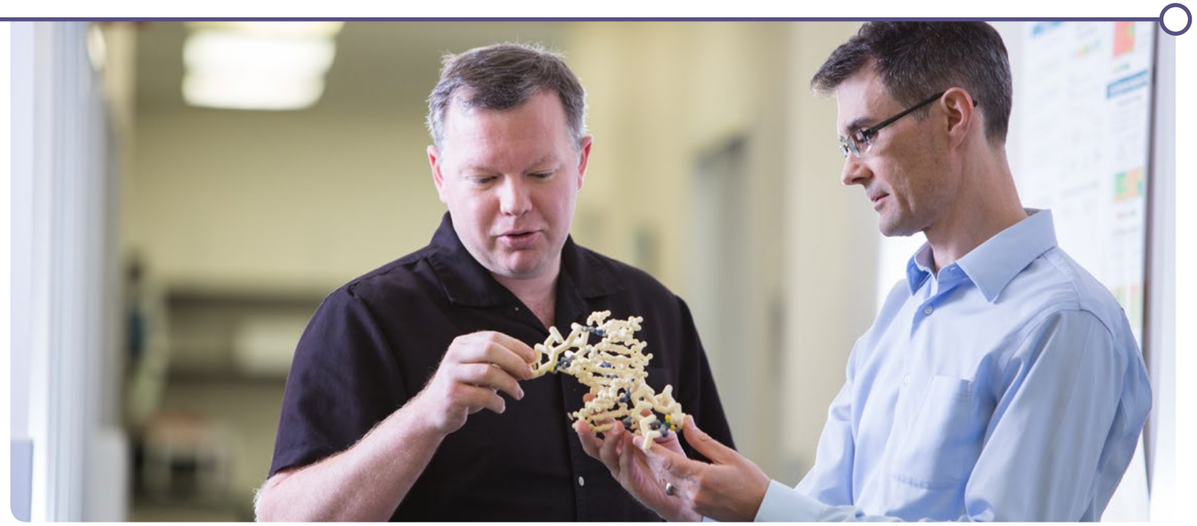
In order to adjust the binding affinity of the zinc finger recognition domain, the authors substituted a discrete, positively charged residue in the zinc finger framework to eliminate a nonspecific contact with the negatively charged phosphate backbone of the DNA. By varying the number of fingers bearing this substitution, the authors showed in cellular studies that they could effectively tune ZFN affinity into an optimally specific range, with no loss of on-target efficiency.

In a related series of cellular studies, the authors screened single-amino acid substitutions in the Fok1 nuclease domain in order to identify those able to improve specificity by slowing down catalysis. The goal of the study was to observe whether mutations would provide more time for the ZFNs to selectively dissociate from off-target sites prior to a cleavage event, which would improve global specificity. These studies yielded single-residue substitutions that could increase specificity by more than 1000-fold.

In a final study detailed in the manuscript, Sangamo scientists applied these two strategies in a therapeutically relevant setting by designing ZFNs that targeted the endogenous TCR-alpha gene in T-cells. Treatment of these



T-cells with optimized ZFNs resulted in a greater than 98% on-target knockout efficiency of the TCR-alpha gene with undetectable off-target activity at a median assay background level of 0.01%. Sangamo believes these engineered improvements to the specificity of its ZFN genome editing platform have the potential to enable the routine generation of designed nucleases capable of high efficiency editing with minimal or no detectable off-target activity.

These recent results add to Sangamo's body of research demonstrating the high degree of precision, efficiency, and specificity of ZFNs for genome editing. In March 2019, *Nature Communications* published data demonstrating new ZFN architectures enabling high-precision genome editing. These new architectures yielded a 64-fold increase in the diversity of ZFNs available for targeting any DNA segment[2].

Recent preclinical results using gene regulation approaches for CNS disorders

In addition to ZFNs for *ex vivo* gene editing and in vivo genome editing, Sangamo is also developing zinc finger protein transcription factors (ZFP-TFs) for gene regulation, which are engineered by attaching a transcription factor to a ZFP. ZFP-TFs are used to regulate gene expression by either activating or repressing the activity of a gene. Sangamo is evaluating ZFP-TFs as a novel therapeutic approach for diseases of the central nervous system.

In July 2019, Sangamo's scientists and their collaborators at the CHDI Foundation published a manuscript in Nature Medicine describing the activity of allele-selective ZFP-TFs in preclinical models of Huntington's disease (HD)[3]. In this work, ZFP-TFs were engineered to selectively target the mutant form of the huntingtin gene (HTT) and repress its transcription, selectively lowering production of the mutant Huntingtin protein (mHTT). Preclinical data from HD patient-derived fibroblasts and neurons demonstrated that a single administration of ZFP-TFs resulted in the selective repression of over 99% of HD-causing HTT disease alleles over a wide dose range, while preserving the expression of at least 86% of healthy wild-type HTT alleles.

Sangamo also presented preclinical data at the 14th International Conference on Alzheimer's & Parkinson's Diseases (ADPD) held in Lisbon, Portugal, in March 2019, and at the 22nd Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT) held in Washington, United States, in April 2019. These data notably described the effects of tau-targeted ZFP-TFs delivered with adeno-associated viruses (AAVs) in the mouse and nonhuman primate (NHP) brain, and demonstrated significant (>80%) reduction of tau expression in the NHP brain following administration of ZFP-TFs. Tau pathology is strongly linked to the progression of several neurodegenerative diseases, called tauopathies, including Alzheimer's disease.

Sangamo's Director of Cell Engineering, David Fenard, will be speaking at our 5th Annual Genome Editing Congress on 8th of November

1. *Miller JC, Patil DP, Xia DF, Paine CB, Fauser F, Richards HW, Shivak DA, Bendaña YR, Hinkley SJ, Scarlott NA, Lam SC, Reik A, Zhou Y, Paschon DE, Li P, Wangzor T, Lee G, Zhang L, Rebar EJ, Enhancing gene editing specificity by attenuating DNA cleavage kinetics, Nature Biotechnology 2019, 37: 945-952.*
2. *Paschon DE, Lussier S, Wangzor T, Xia DF, Li PW, Hinkley SJ, Scarlott NA, Lam SC, Waite AJ, Truong LN, Gandhi N, Kadam BN, Patil DP, Shivak DA, Lee GK, Holmes MC, Zhang L, Miller JC, EJ Rebar, Diversifying the structure of zinc finger nucleases for high-precision genome editing, Nature Communications 2019, 10: 1133.*
3. *Zeitler B, Froelich S, Marlen K, Shivak DA, Yu Q, Li D, Pearl JR, Miller JC, Zhang L, Paschon DE, Hinkley SJ, Ankoudinova I, Lam S, Guschin D, Kopan L, Cherone JM, Nguyen HOB, Qiao G, Ataei Y, Mendel MC, Amora R, Surosky R, Laganiere J, Vu BJ, Narayanan A, Sedaghat Y, Tillack K, Thiede C, Gärtner A, Kwak S, Bard J, Mrzljak L, Park L, Heikkinen T, Lehtimäki KK, Svedberg MM, Häggkvist J, Tari L, Tóth M, Varrone A, Halldin C, Kudwa AE, Ramboz S, Day M, Kondapalli J, Surmeier DJ, Urnov FD, Gregory PD, Rebar EJ, Muñoz-Sanjuán I, Zhang HS. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington's disease, Nature Medicine 2019, 25:1131-1142.*

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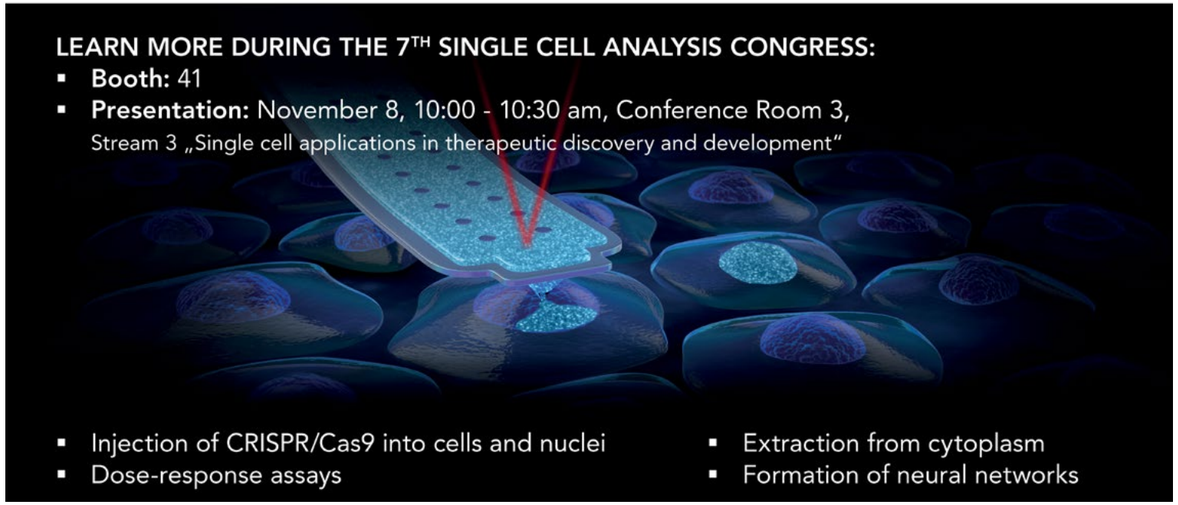

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APPLYING SINGLE CELL ANALYSIS IN THERAPEUTIC DISCOVERY & DEVELOPMENT

CHRISTOS PROUKAKIS, MICKAËL MÉNAGER

QUEEN SQUARE INSTITUTE OF NEUROLOGY

What are the important developments in single cell analysis research?

CP: The field is advancing dramatically, with different types of data possible at the single cell level. There is still a divide between those who are more focused on the genome or on the transcriptome of single cells. Analysis of one may inform the other, and both are needed to obtain a clear genetic picture. Combining genome and transcriptome sequencing has been reported, and interestingly given the title "G&T" by Thierry Voet's group. Further application of this in disease samples will be crucial to elucidate mechanisms at the single cell level.

MM: Important developments in single cell analysis research are coming from the development of tools leading to the encapsidation and analysis of up to several thousands of cells in the same experiment, allowing now to ask more and more precise questions regarding the heterogeneity of a given sample. In parallel of the increased capacity of single-cell libraries, we are also seeing a development of tools allowing now to extract more and more complexes analyses from single-cell experiment, such as pseudo-time correlation with the reconstitution of single-cell lineage, and more importantly comparisons of cluster of cells coming from different single-cell RNA-seq libraries and samples with data integration analyses with methods using machine learning algorithms to establish molecular and cellular networks based on single-cell analyses.

What are the application strategies for single cell in therapeutic discovery and development?

CP: This is already becoming a vital tool in cancer. In my own field of neurodegeneration, we first have to understand the extent of genomic heterogeneity. Most single cell genomic data from human brain is from healthy tissues. Disease-related discoveries from targeted single-cell DNA analysis include the report of somatic copy number gains of the amyloid precursor protein gene in Alzheimer's from Jerold Chun's lab, and our report of the same phenomenon in the alpha-synuclein gene in Parkinson's disease. At the genome-wide level, Chris Walsh's lab has reported excess somatic SNVs in rare DNA repair disorders that cause neurodegeneration. Ongoing work should help clarify how "unique" the genome of each cell is in the diseased brain.

MM: Several applications are conceivable regarding analysis at the single-cell level in therapeutic discovery and development, as it seems reasonable now to try to use single-cell transcriptomic analyses to explore and compare the heterogeneity of cell clusters among different samples coming from different patients:

1. Identification of so-called "pathological cell clusters" enriched in patients vs controls, followed with identification of biomarkers and potentially pathways deregulated based on genes differentially expressed and defining "pathological cell clusters".
2. A better stratification of patients relying not only on clinical signs but also on comparisons performed at the single-cell molecular level.
3. A comparison of the evolution of cell clusters coming from patients before and at different time during and after a given treatment could allow to precisely identify the cells targeted by a successful treatment and/or to understand the different responses/efficiency of a given treatment on different patients.

Overall, we can expect based on single-cell transcriptomic experiments to get better stratification of patients, identify new molecular pathways potentially targetable by new therapeutics and get into personalized medicine by adjusting treatments / therapeutics at a very fine tune level for each individual.

Single-cell analyses will be successfully applied to therapeutic discovery and development depending on the capacity of existing/new tools/software to compare samples coming from different libraries and experiments

What are the most important tools and technologies impacting the single cell market?

CP: Better ways to amplify single cell genomes are being developed, with new versions of the Picoplex (now from Takara) and Qiagen MDA kits as commercially available examples. As the number of cells with these methods is small, and the amplification cost per cell high, I welcome the higher throughput DNA amplification for CNV detection by 10X Genomics, which has just become available. Miniaturisation of reactions is also possible with specific equipment and helps reduce reagent cost. Before one worries about how to amplify a cell genome, the technical challenge of how to get it in the first place needs to be met. Additional new ways to isolate single cells or nuclei, in addition to the more traditional FACS and laser capture microdissections, include systems on inverted microscopes (by Cell Microsystems and Qiagen), dielectrophoresis (DEParray by Menarini), and the Icell8 by Takara.

MM: The democratization of automated DROP-seq systems, such as 10X genomics, MARS-seq technology and others, allowing encapsidation of thousands of cells and the generation of a common NGS library are among the most important tools and technologies that have led in the last 2 years in an explosion of publication focused on single-cell transcriptomic analyses.

Coupled with those technologies, kit are now proposed to not only performed single-cell analyses at the transcriptomic level but also to look at T Cell Repertoire (TCR) based on VDJ recombination, and also at the DNA level with Common Natural Variants (CNV).

We should also mention now the possibility to couple single-cell transcriptomic experiments with cell surface antibody detection (CITE-seq/ REAP-Seq) and the barcoding of samples (cell hashing) allowing to process several samples in the same experiment.

What is the future for single cell in 2019?

CP: Detection of structural variants can be challenging with short reads. Recent evidence has shown the additional insight provided by long reads from the Oxford Nanopore Technology and PacBio systems. Long reads from single cell genomes will hopefully be possible, together with advanced bioinformatic tools to remove amplification chimeras. Combining such data with Illumina-based read-depth analysis should provide more robust detection of single cell large-scale genomic changes. More widespread use of combined DNA and RNA sequencing will also be helpful.

MM: In 2019, we will see the appearance of commercially available methods to perform chromatin accessibility experiments at the single-cell level (CHIP-seq; ATAC-seq) and maybe an increased focus of single-cell experiments at the protein level. We will also see emergence of technics to perform In situ single-cell RNA-seq experiment in tissues and to analyze both DNA and RNA content coming from the same cell.

Hopefully, the cost of library generation and sequencing will be decreasing and the coverage/number of transcripts detectable will increase leading to an even more important democratization of single-cell use in clinical research and therapeutic discovery and developments. A standardization somehow of the method of analyses of different samples will be welcome as more and more tools are now generated, based on different technics leading too often in a different interpretation of the same single-cell data.

Christos Proukakis, Associate Professor and Honorary Consultant Neurologist, University College London Institute of Neurology

Christos Proukakis is a clinical academic neurologist, currently an Associate Professor in the University College London Institute of Neurology. Neurogenetics has been his focus since his PhD in hereditary spastic paraplegia. In his present appointment, he has worked on the genetics and cell biology of Parkinson's disease (PD). He has combined this with his role as Honorary Consultant Neurologist at the Royal Free London NHS foundation trust, where he holds clinics in PD / movement disorders, and general neurology. He is investigating the possible role of somatic mutations in PD and related disorders. His team recently published the first evidence of somatic copy number variants (gains) in the crucial alpha-synuclein gene in substantia nigra. He is looking forward to harnessing the technological advances which allow sequencing of single cells to study not just how the genome we inherit shapes the healthy and diseased brain, but also how the genome of individual brain cells may lead to, or be altered by, disease.

Mickaël Ménager, Lab Director, Team Atip-Avenir, Inserm

As laureate of the 2016 ATIP-Avenir program, Mickaël created his laboratory "Inflammatory responses and transcriptomic networks in diseases" which was integrated to the Imagine Institute in Paris, last June. He obtained a PhD in Immunology in 2008 in the Unit of Pr Alain Fischer (Necker Hospital, Paris) and completed a postdoctoral training in Pr Dan Littman (New York University School of Medicine, USA) by 2017, where he was studying the interaction between HIV-1 and dendritic cells. Now, by combining two state of the art methods, such as single-cell transcriptomic and network inference with machine learning algorithms, he is aiming to better stratify, diagnose and treat patients suffering from autoinflammatory diseases.

Christos Proukakis will be speaking on Day 2 of the Single Cell Analysis congress with his talk *'Somatic CNV Detection In The Multiple System Atrophy Brain By Single Cell Sequencing'*

SEIZING THE OPPORTUNITY OF THE DECODED HUMAN GENOME

JANE WILKINSON

Jane Wilkinson,
Senior Director, Broad Institute

Jane is a Senior Director at the Broad Institute where she leads the Broad Genomics Alliance Management team.

Jane has over 20 years of high-throughput genomics experience from the Wellcome Trust Sanger Genome Center, UK where she was a key leader on the Human Genome Project and at Monsanto Company, USA where she led a new directive in plant genomics. Jane has been at the Broad Institute for fifteen years and has worked on various initiatives including Cancer, Mendelian, Infectious and Common Diseases.

Tell me a little about the Broad Genomics team and their mission

The Broad Institute was founded to seize the opportunity that arose from the Human Genome Project -- the international effort that successfully deciphered the entire human genetic code. Despite that accomplishment, scientists knew they still lacked a clear understanding of the genetic basis of disease, and how to translate that understanding into more effective prevention, diagnosis, and treatment.

Since the Human Genome Project, the Broad Genomics Platform has played a leadership role in the design, data generation, and methods development in support of major genomic resource projects including: the HapMap, the 1000 Genomes Project, The Cancer Genome Atlas (TCGA), Comparative Reference Genomes, ENCODE, Genotype-tissue Expression Project (GTEx), Human Microbiome Project, Exome Sequencing Program Center for Mendelian Genomics, Human Cell Atlas (HCA) Exome Aggregation Consortium (ExAC) and Genome Aggregation Database (gnomAD) projects and All Of Us.

Over the past ten years, we have been one of the largest producers of human genomic information in the world. Currently, the group produces approximately 500 terabases of genomic data per month - a rate equivalent to a 30x human whole genome every 10 minutes. The group has processed more than 3 million samples from more than 1,400 groups in more than 50 countries.

Thinking about the challenges of genomics - what have been the key developments of the Broad Genomics within the past few years?

Over the past 20 years, the wave of genomic innovation and development has been incredible. The introduction of the first 'next-generation' sequencing machines (454 and Solexa) in 2005 and 2006 led the way to whole exome sequencing and the <$1000 whole genome. Applying lean manufacturing work design has allowed us to scale-up to handling over 10,000 samples per week - we completed our 100,000th whole human genome on the 15th anniversary of the completion of the Human Genome in 2018. And recently we have launched half a dozen new services, including Clinical Whole Genome Sequencing, Liquid Biopsy, and Single Cell Sequencing. We have also redesigned our Whole Exome which has resulted in an increase in capacity and more importantly - a significant drop in price.

You mentioned the new Whole Exome - can you tell me more how this was developed and the biggest impact it has made?

As sequencing costs continue to drop, the utility of whole genome sequencing for common disease research is beginning to be realized. However, even with cheaper whole genomes, complex common disease studies still often require larger case-control cohorts in the 10s of thousands and greater. Even with a $1000 genome, achieving the necessary power to find meaningful association is expensive. This realization has driven us to redevelop our germline whole exome product and workflow to reduce cost and simultaneously increase quality. The new Broad Custom Exome developed with Twist BioSciences leverages our custom hyb capture workflow, extremely even coverage, and a high on target percentage (~90%) to allow us to offer a high quality exome at ~USD$200. In addition to a high quality exome and lower price, we have re-engineered our automated workflow to enable a 3-fold increase in capacity in the past year, and are now capable of processing >300,000 exomes per year to better support the internal research needs of the Broad Community as well as external customers.

There's a lot of interest in Liquid Biopsy in the community - tell me more about how Broad Genomics has implemented this and how it's being used.

We have developed a custom unified target enrichment workflow to support a broad suite of somatic studies that require high sensitivity and specificity for low allele fraction variants. This method incorporates extremely stringent error correction using duplex UMIs and ultra deep targeted sequencing. Using array-synthesized custom panels from Twist BioScience, we can now rapidly and in a cost effective manner target disease and study-specific genes to high depth. With Twist custom panels and our workflow, panel sizes can range from 10's of kilobases to multiple megabases in size and routinely achieve greater than 90% of sequenced bases on or near target, maximally utilizing gigabases of sequencing purchased. With cheaper cost of entry into a custom panel synthesis, investigators can now sequence only their regions of interest to extreme depth (20,000-50,000X raw coverage) to maximize the likelihood of detecting variants of importance at very low allele fractions. This allows the investigator to decide whether a larger fixed pan-cancer panel or a more targeted small panel is best suited to their study regardless of sample/ patient number. This method and workflow is particularly well suited for blood biopsy cfDNA as well as bone marrow aspirate clonal hematopoiesis studies.

Just recently The Multiple Myeloma Research Foundation announced that it is launching the MMRF CureCloud™, a data hub that generates, aggregates and visualizes data to accelerate the delivery of precision medicine to multiple myeloma patients. Broad Gennomics will be providing our liquid biopsy capabilities as part of this initiative.

Connecting this large scale genomic data generation to translational analysis can be a pinch point for many researchers - can you tell me more about how Broad Genomics has tackled this?

We have tackled this issue in two specific ways. 1. Through the creation of a cloud-platform, Terra, we have enabled researchers to retrieve, analyse, and share their data in a scalable, accessible, and secure environment. We have further created repositories of open-source, best-practice methods in that platform so that researchers can use the same tools as we do to analyze these datasets. 2. For groups who do not have a fleet of bioinformaticians sitting around, we have created the Translational Analysis Group or TAG. TAG is comprised of 3 PhD-level computational scientists, an associate computational biologist, and a variant curation scientist. This group uses best-practice pipelines developed at Broad and beyond, to analyse our users data in a fee-for-service model. The group is fully cloud-native and all data and analytical outputs are made available through our cloud-platform. The team has run >15,000 analyses on samples ranging from whole genome sequencing for germline variant calling to somatic analysis of tumor variants from liquid biopsy. The team can validate analytical pipelines for use in clinical trials, clinical diagnostics, or therapeutic development.

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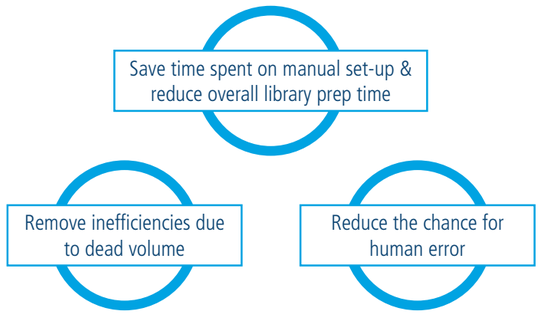

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VectorBuilder's web platform also offers unified data management that stores all the vectors, orders, and technical inquiries generated by customers in their accounts. All vectors feature a unique identifier and permanent URL, allowing researchers to retrieve and share any vectors they designed in an easy and secure manner.

Shortly after its launch in 2015, VectorBuilder has quickly become the world's largest provider of custom vector cloning and virus packaging services. VectorBuilder has won many awards for its highly innovative platform, which was acclaimed for combining sophisticated vector design capability with streamlined data management and extreme ease of use. To date, VectorBuilder has served tens of thousands of researchers in thousands of universities and companies worldwide, and has been cited in a plethora of scientific papers.

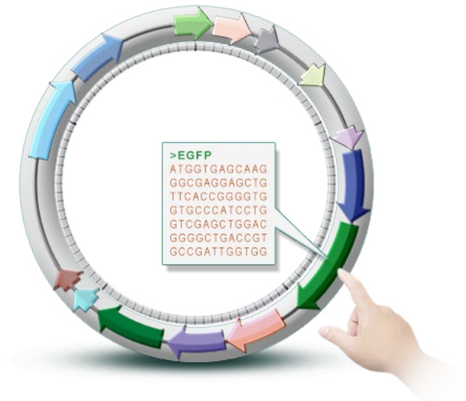
The creators of VectorBuilder believe that a vector is just a reagent, not a research project. Yet, we see too often that biologists, especially budding trainees, spend countless hours toiling in the lab trying to clone a few vectors, sometimes unsuccessfully after months of hard work. We believe this to be a colossal waste of human potential. We therefore decided to create VectorBuilder, a platform that makes vector construction easy, fast, affordable – and yes, fun – for researchers around the world.

If you are still not ready to outsource your cloning and virus packaging to VectorBuilder, consider this: About 40 years ago, labs made their own restriction enzymes. About 30 years ago, labs synthesized their own primers. About 20 years ago, labs made their own antibodies. Does anyone still do any of these in their own labs? No, because researchers have increasingly realized the value of outsourcing the making of research tools to more cost-effective commercial entities, so they can focus on the use of these tools to study their biological questions of interest. So, make the smart move, outsource to VectorBuilder!

Besides cloning and virus packaging, VectorBuilder offers many other molecular biology services such as **library construction (CRISPR/shRNA), BAC modification (recombineering), mutagenesis**, and many more.

VectorBuilder prides itself as a community resource for all things related to vectors. As such, it provides comprehensive educational materials about vectors and vector components, which are placed under Learning Center on the menu bar. With these materials, an apprentice of the trade can quickly learn the pros and cons of different vector system (e.g. lentivirus versus adenovirus) and what components to choose for their vectors (e.g. what fluorescent proteins to use). VectorBuilder also offers many free bioinformatics tools for DNA sequence analysis, such as sequence alignment, shRNA target design, and gRNA off-target analysis, which are placed under Tools on the menu bar.

Stop the headaches of building your own vectors and viruses, and outsource to **VectorBuilder** today!



OXFORD GLOBAL CONFERENCES

FORTHCOMING EVENTS

OXFORD GLOBAL CONFERENCES

Biologics Series

UK

13th Annual Proteins & Antibodies Congress
27 - 29 April 2020 | London, UK

7th Annual Peptides Congress
27 - 29 April 2020 | London, UK

2nd Annual Bispecifics in Discovery & Development Congress
27 - 29 April 2020 | London, UK

Co-located Events

Biomarkers Series

UK

15th Annual Biomarkers Congress
18 - 20 February 2020 | Manchester, UK

Genomic Markers Congress
18 - 20 February 2020 | Manchester, UK

Co-located Events

US

4th Annual Biomarkers & Precision Medicine USA Congress
08 - 09 October 2019 | San Diego, USA

Cell Series

UK

8th Annual Cell Culture & Bioprocessing Congress
29 - 30 October 2019 | London, UK

6th Annual Stem Cell & Regenerative Medicine Congress
29 - 30 October 2019 | London, UK

5th Annual Cell & Gene Therapy Congress
29 - 30 October 2019 | London, UK

Co-located Events

Formulation & Delivery Series

UK

6th Annual Formulation & Drug Delivery Congress
22 - 23 April 2020 | London, UK

5th Annual Inhalation & Respiratory Drug Delivery Congress
22 - 23 April 2020 | London, UK

Biomanufacturing Congress
22 - 23 April 2020 | London, UK

Co-located Events

US

3rd Annual Formulation & Drug Delivery USA Congress
17 - 18 March 2020 | San Diego, USA

3rd Annual Inhalation & Respiratory Drug Delivery USA Congress
17 - 18 March 2020 | San Diego, USA

Co-located Events

Immuno Series

UK

5th Annual Advances in Immuno-Oncology Congress
21 - 22 May 2020 | London, UK

Autoimmunity & Immunology Congress
21 - 22 May 2020 | London, UK

Co-located Events

US

2nd Annual Advances in Immuno-Oncology USA Congress
08 - 09 October 2019 | San Diego, USA

PharmaTec Series

UK

17th Annual Pharmaceutical IT & Data Congress
24 - 25 September 2020 | London, UK

3rd Annual Artificial Intelligence in Drug Development Congress
24 - 25 September 2020 | London, UK

Cyber Security & Data Protection in Pharma & Healthcare Congress
24 - 25 September 2020 | London, UK

SmartLabs & Laboratory Informatics Congress
24 - 25 September 2020 | London, UK

Co-located Events

R&D Series

EU

21st Annual Drug Discovery Summit
26 - 27 May 2020 | Berlin, Germany

8th Annual Drug Design and Medicinal Chemistry Congress
26 - 27 May 2020 | Berlin, Germany

2nd Annual Neuroscience Drug Discovery & Development Congress
26 - 27 May 2020 | Berlin, Germany

Co-located Events

SynGen Series

UK

11th Annual Next Generation Sequencing & Clinical Diagnostics Congress
07 - 08 November 2019 | London, UK

7th Annual Single Cell Analysis Congress
07 - 08 November 2019 | London, UK

5th Annual Genome Editing Congress
07 - 08 November 2019 | London, UK

2nd Annual Synthetic Biology Congress
07 - 08 November 2019 | London, UK

Digital PCR Congress
07 - 08 November 2019 | London, UK

Co-located Events

US

6th Annual Next Generation Sequencing USA Congress
07 - 08 April 2020 | Boston, USA

6th Annual Single Cell Analysis USA Congress
07 - 08 April 2020 | Boston, USA

4th Annual Genome Editing USA Congress
07 - 08 April 2020 | Boston, USA

Co-located Events

Register your interest, e-mail us:

info@oxfordglobal.co.uk