Table of Contents

Program.........................................................................................................................2
  Thursday, September 22, 2016 ..................................................................................2
  Friday, September 23, 2016 .....................................................................................3
Organizers......................................................................................................................4
The venue.......................................................................................................................5
Support and Sponsors .................................................................................................6
Abstracts .......................................................................................................................7
Program

Thursday, September 22, 2016
9.15-9.45: Registration and poster mounting
10.00-10.15: Welcome and opening of the meeting:
Prof. Ulla Wewer, Dean of Health and Medical Sciences, Copenhagen University
10.15-10.20: Organizers welcome and some practicalities:
Tuula Kallunki, Chairperson, Danish Society for Biochemistry and Molecular Biology

Session I: Session chair Tuula Kallunki, Danish Cancer Society Research Center, Copenhagen, Denmark
10.20-10.55: “CRISPR screening of the regulatory genome” Richard Sherwood, Hubrecht Institute and UMC Utrecht, Utrecht, the Netherlands; Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA
10.55-11.30: “CRISPR-based functional genetic screens for enhancer elements” Reuven Agami, Erasmus MC, Rotterdam University, Rotterdam, the Netherlands and the Netherlands Cancer Institute, Amsterdam
11.30-12.05: “CRISPR/Cas9 somatic multiplex mutagenesis for high-throughput functional cancer genomics in mice” Roland Rad, Department of Medicine II, Klinikum Rechts der Isar, Technische Universität München, Munich, Germany

12.05-13.00: Lunch break/exhibition/posters

Session II: Session chair Yonglun Luo, Department of Biomedicine, Aarhus University, Aarhus, Denmark
13.00-14.00: Keynote talk: “Genome editing using CRISPR-Cas systems” Feng Zhang/Jonathan Gootenberg, Broad Institute, Harvard & MIT, Boston, MA, USA
14.00-14.35: “Tune DNA damage repair pathways with recombinant Cas9 proteins”, Yonglun Luo, Department of Biomedicine, Aarhus University, Aarhus, Denmark
14.35-15.10: “Hit and seek: Finding the breaks in a precisely broken genome” Eric Bennett, Departments of Cellular and Molecular Medicine and Dentistry, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

15.10-15.40: Coffee break/exhibition/posters

Session III: Session chair Eric Bennett, Departments of Cellular and Molecular Medicine and Dentistry, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
15.40-16.15: “Chromosome Engineering with Cas9 in vivo” Allan Bradley, Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom
16.15-16.50: “Genome engineering to transform xenotransplantation a clinical reality” Luhan Yang, Department of Genetics, Harvard Medical School, Boston, MA, USA
Friday, September 23, 2016
Session IV: Session chair Cord Brakebusch, Biotech Research and Innovation Center, University of Copenhagen, Copenhagen, Denmark
9.00-9.50: Keynote talk: “Biallelic genome editing of human pluripotent stem cells at scale” Bill Skarnes, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
9.50-10.25: “Lentiviral delivery of genome editing tool kits” Jakob Giehm Mikkelsen, Department of Biomedicine, Aarhus University, Aarhus, Denmark
10.25-11.00: “Genome editing of cancer cell lines” Morten Frödin, BRIC, University of Copenhagen, Copenhagen

11.00-11.30: Coffee break/exhibition/posters

Session V: Session chair Mette Vixø Vistesen, Danish Cancer Society Research Center, Copenhagen, Denmark
11.30-12.05: “Gene editing in mouse zygotes and human iPS cells” Ralf Kühn, Max-Delbrück-Center for Molecular Medicine, 13125, Berlin, Germany.
12.05-12.40: “Applications of CRISPR-mediated gene editing in stem cell research”, Kristine Freude, Group of Stem Cells and Embryology, Dept. of Vet. Science, University of Copenhagen, Copenhagen

12.40-14.00: Lunch break/exhibition/posters

Session VI: Session chair Julian Geiger, Department of Science and Environment, Roskilde University, Roskilde, Denmark
14.00-14.35: “CRISPR/Cas9-induced disruption of gene expression in mouse embryonic brain” Mihail Sarov, Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany
14.35-15.10: “Designer nucleases in modification of large animal primary cells” Nikolai Klymiuk, Chair for Molecular Animal Breeding and Biotechnology (MABB), LMU Munich, Munich, Germany
15.10-15.45: “Beyond knockouts: the International Knockout Mouse Consortium delivers modular and evolving tools for investigating mammalian genes” Wolfgang Wurst, German Research Center for Environmental Health, Institute of Developmental Genetics, Helmholtz Zentrum München, Germany

15.45-16.00: Closing remarks: Yonglun Luo, Department of Biomedicine, Aarhus University, Aarhus, Denmark
Organizers
Yonglun Luo, Cord Brakebusch, Tuula Kallunki, Mette Vixø Vistesen and Julian Geiger (Danish Society for Biochemistry and Molecular Biology (DSBMB))
The venue

**How to get there:**
All lectures will be held in the **Lundsgaard Auditorium** at Panum Institute, **Nørre Allé 20, 2200 København N**. There will be signs leading you from the entrances (green arrows) to the Lundsgaard auditorium (red circle).

From **Nørreport st** you can take busses 150S, 42, 184 or 6A and get off at “**Nørre Campus**” (blue circle). Nørreport st. is connected to the airport by the metro and to the Central Station by most trains.

For **more details** regarding your travels, please consult www.rejseplanen.dk

**At arrival:**
You will find the **registration desk** outside the Lundsgaard Auditorium. Please register during the allocated time (9:15-9:45). If you are **bringing a poster**, please announce it during registration, and you will be informed of where to hang your poster.

**During the conference:**
**Food and coffee** will be provided during the meeting. Unfortunately we don’t have the capacity to embrace specific food allergies or requirements, but there is a canteen at the location.

There is no specific time slot assigned for the **poster session**. Presenters are encouraged to be available during lunch and coffee breaks to answer questions and present their work.

**Recordings or photos** of the presentations (both oral and posters) are not allowed, as the presentations might include unpublished work.

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Abstracts
### Table of abstracts

1. Efficient CRISPR/Cas9 genome engineering using zygotes and embryos derived from transgenic mice overexpressing Cas9
2. Generation of a novel CRISPR interference library for identifying epigenetic vulnerabilities in cancer cells
3. Lineage tracing of mouse intestinal stem cells with sequential modified genes using CRISPR-targeted genome editing
4. Comparative analysis of NHEJ and HDR repair pathways for genomic editing using CRISPR/Cas9 technology in ES cells
5. Role of PSEN1 in neuronal differentiation and Alzheimer’s disease – modeling human neurodegeneration in a dish
6. Scalable CRISPR-Cas9 tools for knock out genomic elements including long noncoding RNAs
7. The EMBL-EBI CRISPR Archive
8. Investigating drug tolerance in malignant B-cells with CRISPR/Cas9 mediated genome editing
9. Inquiring the genome: the use of CRISPR-Cas technique in a transient transgenic strategy to interrogate regulatory element function
10. Mouse model generation using CRISPR/Cas9 at the Transgenic Core Facility of the University of Copenhagen
11. Development of an *in vitro* experimental set-up for clinical translation of *KCNQ-1* gene functionality on insulin regulation
12. Generation of human ES lines with EIF2B4 and EIF2B5 gene mutations for modelling of EIF2B-related leukodystrophies
13. Engineering the mouse genome using CRISPR/Cas9 technology
14. Golden Gate assembly of synergistic activation mediator CRISPR gRNA expression array for simultaneous activation of four EMT Transcription Factors
15. Designing transcriptional roadblocks to elucidate the non-coding genome
16. Understanding the biogenesis of cIrS-7
17. The secondary structure of guide sequences and chromatin accessibility influence CRISPR/Cas9-mediated gene editing efficiency
18. Identification and characterization of a few components of NER machinery in malaria parasite *Plasmodium falciparum*
19. The CRISPR-Cas9 Minipig
20. Establishment of a MODY3 disease model in induced pluripotent stem cells using CRISPR
21. Unveiling the mechanism underlying prostate cancer susceptibility variant rs11672691
22. CRISPR-Cas9 optimized delivery systems for human primary cells
23. Landing transgenes at “safe harbor” loci in human cell lines
<table>
<thead>
<tr>
<th>Abstract</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic edition by CRISPR / Cas9 system for guided insertion of the GCDH gene, responsible for Glutaric Aciduria type I</td>
<td>24</td>
</tr>
<tr>
<td>Manipulation of potassium channels in tumor specific T cells for improved functionality in the suppressive tumor microenvironment</td>
<td>25</td>
</tr>
<tr>
<td>Investigation of the disease mechanisms of ShortChain-acylCoA Dehydrogenase deficiency by biallelic knockout using CRISPR/Cas9</td>
<td>26</td>
</tr>
<tr>
<td>Editing the livestock genome</td>
<td>27</td>
</tr>
<tr>
<td>miRNA profiling in plasma from patients with sleep disorders reveals dysregulation of miRNAs in narcolepsy and other central hypersomnias</td>
<td>28</td>
</tr>
<tr>
<td>Albumin transcytosis in kidney proximal tubule cells – the role of megalin, cubilin and FcRn receptors</td>
<td>29</td>
</tr>
<tr>
<td>Defining the earliest step of cardiovascular development using CRISPR genome editing technology</td>
<td>30</td>
</tr>
</tbody>
</table>
Efficient CRISPR/Cas9 genome engineering using zygotes and embryos derived from transgenic mice overexpressing Cas9

**Alberto Cebrian-Serrano, Chris Preece, Daniel Biggs and Benjamin Davies**

*Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX4 3AS, UK.*

Genome manipulation in the mouse via microinjection of CRISPR/Cas9 site specific nucleases has allowed the production time for mouse model development to be significantly reduced and the technique is now being widely adopted as the method of choice by research and core service laboratories. The method is of particular interest with respect to modelling human genetic disease, since pathogenic genetic variants can be efficiently introduced into the mouse genome.

For laboratories establishing these techniques, the exact mode of delivery of the CRISPR/Cas9 components needs to be optimized. Successful genome manipulation in the mouse has already been reported using Cas9 supplied by microinjection of a DNA construct, purified *in vitro* transcribed mRNA and recombinant protein. As an alternative to these options, we have investigated the feasibility of supplying Cas9 genetically and for this purpose have generated a line of transgenic mice which overexpresses wild-type Cas9 ubiquitously, through the use of a CAG-Cas9 transgene, targeted to the *ROSA26* locus via recombinase mediated cassette exchange.

Microinjection of fertilized zygotes prepared from transgenic Cas9 overexpressing females with guide-RNAs resulted in high numbers of mutant mice and whole embryo analysis revealed that the level of mutagenesis was found to be significantly higher when Cas9 was supplied genetically relative to exogenous supply. Genetic supply of Cas9 was able to mediate loss-of-function alleles by indel mutation, point mutation changes by homology directed repair and small deletions using two gRNAs. These preliminary results suggest that generation of a mutant mouse can now be reliably achieved in a single microinjection session, vastly reducing the animal cost and the timing of transgenic production.
Generation of a novel CRISPR interference library for identifying epigenetic vulnerabilities in cancer cells

Aliaksandra Radzisheuskaya

Biotech Research and Innovation Center, Copenhagen, Denmark

CRISPR interference (CRISPRi) represents a newly developed tool for targeted gene repression. It has great application potential for studying gene function and mapping gene regulatory elements. However, the optimal parameters for efficient single guide RNA (sgRNA) design for CRISPRi are not fully defined. In this study, we systematically assessed how sgRNA position affects the efficiency of CRISPRi in human cells. We analyzed 155 sgRNAs targeting 41 genes and found that CRISPRi efficiency relies heavily on the precise recruitment of the effector complex to the target gene transcription start site (TSS). Importantly, we demonstrate that the FANTOM5/CAGE promoter atlas represents the most reliable source of TSS annotations for this purpose. We also show that the proximity to the FANTOM5/CAGE-defined TSS predicts sgRNA functionality on a genome-wide scale. Moreover, we found that once the correct TSS is identified, CRISPRi efficiency can be further improved by considering sgRNA sequence preferences. Using the discovered sgRNA parameters, we generated a novel CRISPRi library targeting all major chromatin-associated proteins, which we apply for identifying epigenetic vulnerabilities of cancer cells.
Lineage tracing of mouse intestinal stem cells with sequential modified genes using CRISPR-targeted genome editing

Ann-Sofie Thorsen, Richard Kemp, Xiangang Zou and Doug Winton

Cancer Research UK Cambridge Institute, Robinson Way, CB2 0RE Cambridge, UK

The mouse intestinal crypt contains on average 5-7 functional stem cells. Over time one stem cell will outcompete and replace the neighbouring stem cells to populate the entire crypt in a process of monoclonal conversion. Intestinal cancer develops from accumulated tumorigenic mutations in intestinal crypt stem cells. Lineage tracing studies have shown that stem cells with oncogenic KrasG12D mutations more frequently replace their neighbours by biased stem cell competition compared to wild type stem cells. Investigation of how consecutive mutations, following a KrasG12D mutation, affects stem cell behaviour and proliferation will help explain intestinal cancer initiation and progression. The new method of CRISPR-induced genome editing has made it easier and faster to create new mouse models. Expression of a tamoxifen-activatable Cre recombinase (CreER) from the KrasG12D transcript in an fsf (frt STOP frt) KrasG12D mouse model makes it possible to induce secondary genetic changes in the original KrasG12D mutated stem cell. The new mouse-model can be crossed on to a variety of already available floxed mouse models (e.g. p53flox). This makes it a useful tool to study stem cell dynamics in multiple and diverse consecutive mutational environments. This study utilizes CRISPR targeting in mouse embryonic stem (ES) cells to insert CreER before the STOP codon in the last exon of KrasG12D. After clonal verification the ES cells are made into mouse-models so that the study of single stem cells with defined sequential mutations can begin.
Comparative analysis of NHEJ and HDR repair pathways for genomic editing using CRISPR/Cas9 technology in ES cells

Astrid Jensen¹, Jeroen De Groot¹, Uma Saha², Steve Festin², Anne-Marie Zuurmond¹

¹Charles River Leiden, Darwinweg 24, 2333 CR Leiden, Netherlands ²Charles River Wilmington, 251 Ballardvale Street, Wilmington MA 01887, USA

The error prone NHEJ repair pathway is often the pathway of choice when utilizing CRISPR/Cas9 to generate a genetic knockout. Despite the high efficiency of creating indel mutations, the pathway has its drawbacks. Indels can easily span from 1-200 bp, without any control on their exact length or location (upstream/downstream). Deletions are often in-frame, which might result in semi-functional proteins.

The HDR allows for precise editing of the genome by including a repair template in the process. Using a single stranded template ensures that this template is not randomly inserted elsewhere in the genome or is targeted by the Cas9 enzyme.

In this study we performed a comparative analysis of the two repair pathways following a CRISPR/Cas9 mediated double-stranded cleavage to generate gene knockouts in C57BL/6NCrl mouse embryonic stem cells. The NHEJ pathway resulted in a gene editing efficiency of 73% using a two-vector CRISPR/Cas9 system with 3 out 4 indels being small in-frame mutations. Frequency of homozygosity for confirmed indels was found to be 14%.

For the HDR pathway, we first demonstrated that a small 50 nt ss template with a SNP still had a gene editing efficiency of 25%, which could be improved to 57% when the template was enlarged to 100 nt. Next, we designed a 120 nt ss template with a small 10 nt tag, to introduce a premature stop codon as well as a frameshift mutation upon integration in the gene of interest to generate knockouts by HDR. PCR validation and sequencing revealed a 44% gene editing efficiency of which around 12% turned out to be homozygous. As a conclusion a controlled gene knockout can be obtained, with relative high efficiency, using the HDR pathway in the C57BL/6NCrl ESC. This is a great advantage over the NHEJ pathway which returns a high frequency of undesired in-frame mutations.

Abbreviations: NHEJ: non-homologous end joining, HDR: homology driven repair, nt: nucleotide, ESC: embryonic stem cells, ss: single stranded, SNP: single nucleotide polymorphism
Role of PSEN1 in neuronal differentiation and Alzheimer’s disease – modeling human neurodegeneration in a dish

Carlota Pires1*, Anna Poon1, Benjamin Schmid2, Troels T. Nielsen3, Lena E. Hjermind3, Jørgen Nielsen1, Christian Clausen2, Poul Hyttel1, Bjørn Holst2 and Kristine Freude1

1 Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. 2 Bioneer A/S, Hoersholm, Denmark. 3 Danish Dementia Research Center, Rigshospitalet, Copenhagen University Hospital, Denmark

Keywords: PSEN1, iPSC, CRISPR, neuronal differentiation

Alzheimer’s disease (AD) is the most common type of dementia and targets the cerebral cortex and certain subcortical regions. The familial (FAD) form shows early-onset symptoms and it is attributed to mutations in presenilin (PSEN) 1 and 2 and amyloid precursor protein (APP) genes. PSENs are constituents of the gamma-secretase complex and it correlates with increased toxic Aβ42 and senile plaques in AD brain. Sporadic (SAD) type has a late-onset and no causative genetic background has been identified except for apolipoprotein E (APOE) risk factor.

Human induced Pluripotent Stem Cells (hiPSC) allow for the generation of patient-specific neurons, providing in vitro tools for studying neurodegeneration. We have generated patient-derived PSEN1-hiPSCs carrying one of five different PSEN1 mutations (A79V, L150P, M146I, H214R and L282F). These PSEN1-hiPSCs were carefully characterized in regards to pluripotency by quantitative PCR and immunocytochemistry for key pluripotency genes/proteins. Furthermore, their differentiation potential was explored by embryoid-body formation and differentiation into all three germ layers. To assess even subtle disease phenotypes we have generated isogenic controls for each line via genome editing, by using the CRISPR-Cas9 system combined with ssODNs carrying the corrected nucleotide and inserted silent mutations. We are currently assessing disease phenotypes caused by different PSEN1 mutations including apoptosis (Caspase 3/7), cell ROS and autophagy assays, ELISAs to determine the relative increase in Aβ42 over Aβ40, spontaneous action potential and mitochondrial dysfunction, which is related to the “Ca2+ hypothesis” in AD. Moreover, terminally differentiated neurons from PSEN1, isogenic and sex/age-matched control lines are being analyzed via RNAseq to evaluate possible biomarker candidates, which will then be assessed/validated in the respective patients’ CSF and blood samples, and also to evaluate if different mutation sites lead to distinct pathway alterations. Proteomics and metabolomics will also be studied so that a broad and detailed idea of the mutations’ impact is constructed.
Scalable CRISPR-Cas9 tools for knock out genomic elements including long noncoding RNAs

Carme Arnan¹,²,³, Estel Aparicio-Prat¹,²,³, Carlos Pulido-Quetglas¹,²,³, Taisia Polidori¹,²,³, Toni Hermoso¹,², Emilio Palumbo¹,²,³, Julia Ponomarenko¹,², Roderic Guigó¹,²,³ and Rory Johnson¹,²,³

¹ Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain, ² Universitat Pompeu Fabra (UPF), Dr. Aiguader 88, 08003 Barcelona, Spain, ³ Institut Hospital del Mar d’Investigacions Mèdiques (IMIM), Dr. Aiguader 88, 08003 Barcelona, Spain

CRISPR/Cas9 makes it possible to investigate the function of diverse genomic elements in their endogenous context. Pairs of single guide RNAs (sgRNAs) can be used to delete regulatory elements and small RNA genes, while longer RNAs can be silenced through promoter deletion. We present two tools for knockout of noncoding elements: a vector system adapted for this purpose called DECKO* (Double Excision CRISPR Knockout) and, a bioinformatic pipeline for paired sgRNA design called CRISPETa*. Both are fully scalable, from single-gene studies to complex pooled libraries. DECKO applies a simple two-step cloning to generate lentiviral vectors expressing two sgRNAs simultaneously. The key feature of DECKO is its use of a single 155 bp starting oligonucleotide carrying the variable sequences of both sgRNAs. DECKO plasmids also contain fluorescent and antibiotic markers for convenient selection and flow cytometry monitoring.

CRISPETa is a bioinformatic pipeline for flexible and scalable paired sgRNA design based on an empirical scoring model. Multiple sgRNA pairs are returned for each target. Any number of targets can be analyzed in parallel. Fast run-times are achieved using a precomputed off-target database. sgRNA pair designs are output in a convenient format for visualisation and oligonucleotide ordering.

DECKO and CRISPETa will be useful for researchers seeking to harness CRISPR for targeted genomic deletion, from single-target to high-throughput scales.

References:


*CRISPETa is available at: http://crispeta.crg.eu/
The EMBL-EBI CRISPR Archive

Daniel Zerbino

European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, CB10 1SD, United Kingdom

EMBL-EBI has closely accompanied molecular biologists for more than 20 years, collecting all forms of experimental results: nucleotide sequences, protein structures, GWAS results... As the world of biology is about to be flooded with exciting new results produced with CRISPR, it naturally behoves EMBL-EBI to collect and distribute this wealth of information.

EMBL-EBI is therefore creating an archival service that will encourage the open sharing and reproducibility of CRISPR results. In a first step, we aim to determine, in collaboration with the leading teams, the minimum information required to ensure the reproducibility of knock out screen data. This specification will lay out a template for our archive of screen results.

Naturally, the full extent of the experimental possibilities opened up by CRISPR has yet to be determined. We aim to progressively extend our archive to other assays, starting with targeted mutagenesis. This future database is currently being designed and we welcome all suggestions and requests from the specialists.
Investigating drug tolerance in malignant B-cells with CRISPR/Cas9 mediated genome editing

Emil Aagaard Thomsen\textsuperscript{1}, Louise B. Krogh\textsuperscript{1}, Benyamin Ranjbar\textsuperscript{1}, Karen Dybkær\textsuperscript{2}, and Jacob Giehm Mikkelsen\textsuperscript{1}

\textsuperscript{1}Department of Biomedicine, Aarhus University, Aarhus; \textsuperscript{2}Department of Hematology, Aalborg University Hospital, Aalborg

The capacity of malignant cells to develop resistance to active anti-cancer compounds is a continuous challenge in cancer treatment. Diffuse Large B-cell Lymphoma (DLBCL) represents the largest sub-group of Non-Hodgkin lymphomas (NHL). Treatment of DLBCL with the R-CHOP treatment is often affected by acquired or inherent tolerance towards one or more of the compounds in the R-CHOP regimen. In this project, we focus on genetic determinants for tolerance of B-cells to the monoclonal antibody rituximab (the ‘R’ in R-CHOP). Rituximab targets the surface protein CD20, which is expressed on B-cells throughout most of their development. To generate cancerous B-cells lacking expression of CD20, we utilized the CRISPR/Cas9 system delivered to cells by lentiviral transduction. Among a set of single guide RNAs (sgRNAs), we identified sgRNAs causing robust indel formation in the B-cells, as measured by TIDE analysis and flow cytometry using a CD20-specific antibody. Notably, treating the cells with Rituximab could further enrich the population of cells carrying CD20 indels, indicating that CD20 knockout cells were resistant to Rituximab. We are currently exploiting these findings for identification of other gene variants that affect Rituxumab tolerance using lentivirus-based CRISPR/Cas9 libraries.
Inquiring the genome: the use of CRISPR-Cas technique in a transient transgenic strategy to interrogate regulatory element function

I. Rollán, M. Gómez, T. Rayón, C. Badía and M. Manzanares

Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain

The main question in Developmental Biology is to understand how a single cell becomes a complex multicellular organism. For this purpose, gene expression must be tightly controlled in time and space. This control can be carried out by regulatory elements lying throughout the genome.

Regulatory elements can be clustered with the genes they regulate and remain relatively near from them, but sometimes they are located few megabases away. In such case, it is necessary for the genome to adapt a three-dimensional form in order to get both elements into contact. The functioning of these regulatory elements is complex and strictly dependent on the interactions that each element has with other proximal and distal sequences within a genomic locus. For this reason, targeting the endogenous locus is the tool of choice to study such elements in their original chromosomal context.

We use the CRISPR-Cas9 technique in our laboratory in order to unravel the tangle of this genomic organization. To do this, we interrogated two different locus: Cdx2 and IrxA. We performed our screening at three different levels by:

1) Generating Cdx2 KO blastocyst, targeting the first exon of the gene. In this case, we reached a deletion efficiency of almost 90%.
2) Deleting a CTCF binding site between Irx2 and Irx4 genes. This deletion gave rise to same phenotype obtained in the CTCF KO in the embryonic heart: downregulation of Irx4.
3) Directing targeted integration of a “regulatory domain reader” cassette (RDR-cassette), in order to elucidate the regulatory landscape, which is acting in a particular region of the genome.

The goal of this approach is to apply these questions in a transient transgenic manner.
Mouse model generation using CRISPR/Cas9 at the Transgenic Core Facility of the University of Copenhagen


Transgenic Core Facility, Department of Experimental Medicine, Health Science Faculty, University of Copenhagen.

The Transgenic Core Facility of the University of Copenhagen has the mission of providing the scientific community with a wide range of services comprising embryonic stem cell derivation and manipulation, generation of new genetically modified mouse strains by both transgene random integration and gene targeting techniques, as well as mouse line re-derivation by embryo transfer and mouse line archiving by sperm and embryo cryopreservation. In the last three years, we have participated in the CRISPR/Cas9 revolution implementing the use of this system to generate new mouse models for several research groups. Our facility has optimized protocols to produce knockout mice in just one step by guide RNA and Cas9 mRNA injection into the pronucleus of the mouse zygote. Moreover, we have used CRISPR/Cas9 to generate a number of more complex mouse models such as point mutants, conditional knockouts and larger knockins via modification of embryonic stem cells and production of germ-line-competent chimeric mice with high efficiency. The facility advises on the best way to achieve the desired mouse model and provides all the necessary technical support to complete each new project. Finally, the Transgenic Core Facility has signed a partnership with Sigma-Merc that gives our users cheaper access to CRISPR-related products as well as to project consultation and troubleshooting with professionals from Sigma. We are looking forward to helping you establishing the new mouse models you need for your research, please visit us at http://transgenicmice.ku.dk and contact us at Transgenics@sund.ku.dk.
Development of an *in vitro* experimental set-up for clinical translation of *KCNQ-1* gene functionality on insulin regulation

Jinyi Zhang, Morten Lundh, Torben Hansen, Jens J. Holst, Jørgen K. Kanters, Brice Emanuelli and Signe S. Torekov

1Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; 2The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; 3Gentofte, Aalborg and Herlev University Hospitals, Hellerup, Denmark; 4Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

**Aim**

Investigate the cellular effect of disease-causing mutations in the Voltage-gated potassium channel *KCNQ-1* gene in modulating β cell function. Background *KCNQ-1* long QT syndrome patients have hyperinsulinemia and symptomatic hypoglycemia. *KCNQ-1* gene encodes the voltage-gated potassium channels on both cardiomyocytes and pancreatic β cells, the *KCNQ-1* mutations related to LQT or SQT syndrome change the repolarization time in cardiomyocytes, leading to disordered heart rhythm. In our study, mutant models are developed to verify if these mutations influence on the regulation of insulin secretion in β cells.

**Material and methods**

The *KCNQ-1* human mutations were identified from Long QT or Short QT patients. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 mediated gene editing technology was applied to introduce these mutations into INS-1 cells. The paired gRNAs were cloned into pSpCas9n(BB)-2A-GFP and pSpCas9n(BB)-2A-Puro respectively, and then co-transfected with repair templates into INS-1 cells by electroporation. The mutants were determined by genotyping and sequence analysis. The functionalities of these newly generated mutant β cells were evaluated by measuring insulin secretion in response to glucose stimulation by Rat Insulin ELISA assay.

**Results**

In our initial insulin secretion tests, the selected INS-1 832/3 cells in which we achieved deletion in *KCNQ-1* gene displayed high basal insulin secretion and a lack of response under glucose stimulation. INS-1E cells are now being used in our further studies because of its better insulin response. The functional test results showed that our Cas9/sgRNA expression vectors efficiently introduce indel mutations to the targeting sequence in INS-1 cell lines. However, the desire point mutations have not yet been obtained with these conditions. We are trying to introduce the desired mutation in INS-1E cells with various approaches (increasing template concentration or adding chemical inhibitors) to increase the HDR efficiency.

**Conclusion**

The INS-1 cell models developed in our study will be used to trace the hormone secretion variation in cellular level, and to explore the cell autonomous effects of *KCNQ-1* mutations in this cell-type.
Generation of human ES lines with EIF2B4 and EIF2B5 gene mutations for modelling of EIF2B-related leukodystrophies

Osorio J, Schmid B, Goldman SA.

Center for Basic and Translational Neuroscience, University of Copenhagen

Mutations in the eukaryotic translation initiation factor 2B (EIF2B) gene cause one of the major childhood disorders of the forebrain white matter called Vanishing White Matter Disease (also known as Childhood Ataxia with Central Hypomyelination, VWMD/CACH). The disease is characterized by extensive white matter destruction, often triggered by a stressful event such as head trauma or fever, leading to stepwise and irreversible neurological deterioration with early disability and death. The effects by which the disease affects predominantly glial cells and the nature of such defect remain unclear.

To study the pathophysiology of this disorder, we have used CRISPR/Cas9 to generate human embryonic stem cell lines with three different single point mutations, each reflecting a distinct severity phenotype. We will subsequently direct the resultant diseased and isogenic control cells to a glial progenitor fate and then study their maturation capacity in vivo, in normal conditions and in response to a stress paradigm. We will additionally analyze expressed mRNAs via RNA sequencing in oligodendrocytes, astrocytes and their glial progenitors in vitro, with the aim to identify major pathway distinctions between affected and control cells in specific glial cell types.

By the end of this project, I aim to have collected critical information regarding the role of human glial populations in the pathology of VWM/CACH. Working with human glial cells in this disease is a novel approach and will provide new relevant information to develop treatment strategies for this fatal and incurable disorder.
Engineering the mouse genome using CRISPR/Cas9 technology

Joffrey Mianné¹, Adam Caulder¹, Gemma Codner¹, Ruairidh King¹, Rachel Fell¹, Marina Maritati¹, Alasdair Allan¹, MLC Microinjection team¹, Maximilian Haeussler², Jean-Paul Concordet³, Sara Wells¹ and Lydia Teboul¹

¹The Mary Lyon Centre, MRC Harwell Institute, Didcot, United Kingdom, ²Santa Cruz Genomics Institute, MS CBSE, University of California, 1156 High Street, Santa Cruz, CA 95064, USA, ³INSERM U1154, CNRS UMR 7196, Muséum National d’Histoire Naturelle, Paris, France

Mouse models are valuable tools to understand genes functions, genetic diseases and to develop and test new therapeutic treatments in vivo. The ability to introduce tailored modifications within the mouse genome is essential to generate these models for the study of Human diseases. The recently developed CRISPR/Cas system as genome engineering tool has brought new perspectives for the generation of mouse models in a more efficient and precise fashion, at reduced price, all within a shorter time scale.

Here we will report the use of the CRISPR/Cas9 technology at the Mary Lyon Centre, MRC Harwell Institute, to introduce a wide range of modifications within the mouse genome through different methods.

We will first present our high throughput mouse production pipeline allowing us to generate alleles containing indels, tailored deletions or point mutations through direct injection into zygotes. We will then report the use of the CRISPR/Cas9 technology to engineer and enhance the genetic background of the C57BL/6N mouse strain by correcting mutations in the Cdh23 and Crb1 genes.

Also, we will present our data obtained for enhancing the homologous recombination rate in mouse embryonic stem cell through co-electroporation of IKMC targeting vector and CRISPR reagents.

Finally, we will present an article introducing the CRISPOR site (http://crispor.org), a freely available webtool allowing for selection of guide sequences according to potential off-targets and on-target efficiency prediction. We will show results from a study evaluating sgRNA activity prediction algorithms against datasets from large-scale CRISPR mutant production efforts.

Developing these methods and tools for genome engineering will enable the generation of a wide range of increasingly complex alleles in mice, both in custom and high throughput context.

Keywords: mouse, CRISPR
Golden Gate assembly of synergistic activation mediator CRISPR gRNA expression array for simultaneous activation of four EMT Transcription Factors

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Epithelial-mesenchymal transition (EMT) has been widely accepted as the early stage of tumor metastasis, and has received increasing attention as a mechanism of acquired resistance to chemotherapeutics in e.g. lung cancer. The EMT program involves the disruption of cell-cell adherence and tight junctions, as well as loss of cell polarity of the epithelial cancer cell for the acquisition of a mesenchymal-like phenotype. EMT is molecularly characterized by the loss of the epithelial marker E-cadherin, and a subsequent upregulation of the mesenchymal markers vimentin and N-cadherin, termed the cadherin-switch. In particular, E-cadherin transcriptional repressors of three major families: SNAIL, ZEB, and TWIST, also known as EMT-inducing transcription factors (EMT-TFs), play a crucial role in this process. Individual EMT-TFs are shown to induce a partial EMT phenotype as evidenced by a direct or indirect downregulation of E-cadherin. This suggests that full EMT will only be achieved when all necessary component pathways of the network are activated. Intriguingly, there is no thorough understanding of the hierarchy of the different EMT-TFs, because they often are studied individually. Moreover, EMT-TF studies often rely on transgene overexpression resulting in unnatural expression levels which calls for caution for correct interpretation of the results.

In this study, we modified our recently developed Golden Gate Assembly of CRISPR gRNA expression array to include MS2 stem loops to work with the CRISPR/dCas9 Synergistic Activation Mediator (SAM) system to simultaneously activate expression of multiple genes. As a proof of principle, we used this approach to activate EMT-TFs (TWIST, SNAIL1, SNAIL2, and ZEB1) either individually or simultaneously.

The epithelial lung cancer cell line HCC827 was co-transfected with gRNA expression array vector, MS2-p65-HSF1 expression vector and dCas9-VP64 expression vector in a molar ratio of 1:2:2 for 72 hours. Transfected cells were harvested for RNA and subjected for RT-qPCR analysis. We observed upregulation for all the gRNA targeted EMT-TF genes. The highest activation was observed for TWIST with ~120 and ~130-fold upregulation by multiple and individual targeting. The lowest activation was observed for ZEB1 with ~3.5 and ~2.5-fold upregulation by multiple and individual targeting. Interestingly, activating SNAIL expression with specific gRNAs also resulted in ~20-fold upregulation of SLUG and ~2.5-fold upregulation of ZEB1 expression indicating a regulatory hierarchy.

Despite successful activation of all EMT-TFs no definitive EMT was observed given only minor changes in E-cadherin and N-cadherin expression were observed. This could be a reflection of the only 72h time interval examined which could be insufficient for a complete EMT phenotypic shift. The presented EMT-TF approach is under use to investigate the regulatory hierarchy between EMT-TFs and their ability to collectively induce a full EMT phenotype using longer time frames.
Designing transcriptional roadblocks to elucidate the non-coding genome

Joseph Paul Parker

ncRNA (non-coding RNA) constitutes a vast majority of transcripts in many organisms. Despite selective pressure to eliminate unnecessary use of resources, this transcription occurs across species and suggests an evolutionary purpose. Explanations have been found for selected ncRNA but they do not account for the magnitude of transcription activity in genomes.

To further examine the purpose and prominence of ncRNAs, we intend to design the optimal transcriptional roadblock using a catalytically-deactivated Cas9 (dCas9). This will deactivate the act of ncRNA transcription to examine the effect of its loss. The act of non-coding transcription, rather than the ncRNA product, is often the functionally significant molecular event. Intriguing examples include ncRNA-based transcriptional interference (TI).

TI is defined by ncRNA transcription overlapping with protein-coding transcript units, interfering with gene expression. TI of a coding gene can occur in tandem configuration or, if the ncRNA overlaps on the antisense strand, convergent or divergent configuration. Specifically, we will target a locus in *S. cerevisiae* where a non-coding RNA extends into a protein coding sequence necessary for viability, resulting in an abnormally long RNA transcript and phenotypic growth defect.

While similar constructs have been previously developed, this project aims to fuse dCas9 with several proteins and protein domains known to have transcriptional termination capabilities and to screen several gRNA candidates to optimize its activity in yeast, before attempting to transfer it into higher organisms. This tool’s applications would be widespread, but particularly useful to labs investigating ncRNA.
Understanding the biogenesis of ciRS-7

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Circular RNAs (circRNAs) are a novel class of RNA demarcated by a covalently closed-loop structure. CircRNAs are often produced from internal exons of protein-coding genes in a cell-type, tissue and differentiation stage specific manner, are highly stable and display conservation across species. The biogenesis of circRNAs has not yet been fully elucidated, but requires the spliceosome and can be modulated by both cis and trans acting factors. For ciRS-7, a functionally well-characterized circRNA that acts as a miR-7 sponge, only two mature ciRS-7 splice variants but no precursor or mature linear transcript that overlaps with the ciRS-7 exons has been identified, limiting inferences as to the biogenesis pathway of this circRNA. Therefore, the purpose of this project was to characterize the pre-ciRS-7 transcript, determine the promoter driving its expression as well as to examine the circularization mechanism. Initial bioinformatic analysis disclosed putative promoter regions situated ~ 75 kb and ~ 20 kb upstream of the site to which the exons incorporated into ciRS-7 map. RT-PCR analysis showed a positive correlation between expression of ciRS-7 and upstream LINC00632 transcript variants located between putative promoter regions and ciRS-7 and disclosed that the ciRS-7 exons could be incorporated into linear processed transcripts that also contained LINC00632 exons. CRISPR-Cas9 technology will be applied to delete putative promoter regions in the genome using a two-sgRNA approach to determine the effect of this on ciRS-7 expression. Additionally, putative promoters will be inactivated using dCas9-KRAB and stimulated using dCas9-VP64. Furthermore, as regions that flank exons subject to backsplicing have previously been shown to contain functional elements of importance for circularization, it will also be investigated how the removal of these regions from the genome using CRISPR/Cas9 technology affects ciRS-7 expression.
The secondary structure of guide sequences and chromatin accessibility influence CRISPR/Cas9-mediated gene editing efficiency

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CRISPR-Cas9 systems have emerged as the method of choice for genome editing in a wide range of organisms, but despite the increasing popularity of these systems, factors such as off-target target cleavage and large variations in on-target efficiencies continue to limit their applicability. In this study, we compared SpCas9 with two SpCas9 variants (SpCas9-HF and eSpCas9(1.1)) designed to reduce off-target cleavage using both reporter system and targeting endogenous genomic loci, and identified that eSpCas9(1.1) is superior over SpCas9-HF in terms of specificity while retain similar on-target efficiency as SpCas9. We then investigated the sequence determinants of CRISPR-Cas9 mediated gene targeting efficiency using a chromatin-free surrogate reporter system (C-Check) targeting 14 genomic loci and 27 guide RNAs (gRNA). We found that the efficiency of Cas9-mediated gene editing is largely dependent on the secondary structure of the gRNA guide sequences. Furthermore, we investigated the effect of chromatin accessibility on CRISPR-Cas9 mediated gene editing efficiency in the same loci in HEK293T and Hela cells, and found that CRISPR-Cas9 gene editing is more efficient in euchromatin than heterochromatin regions. In addition, we proved that Cas9-mediated cleavage can therefore be affected by modifying local chromatin architecture using small-molecule HDAC inhibitors (Sodium Butyrate and VPA) or fusing Cas9 to chromatin-remodelling protein domains (VP64 and P300-Core). Our study uncover the factors influencing CRISPR-Cas9-based gene editing efficiency, which will facilitate the future design of gRNA and improvement of Cas9 systems for genome editing.
Identification and characterization of a few components of NER machinery in malaria parasite Plasmodium falciparum

Leila Tajedin, Renu Tuteja

The global anti-malarial therapy against Plasmodium falciparum remains a challenge owing to drug resistance. Plasmodium lives in an oxidative stressful environment and it must have defense mechanisms including DNA repair system to control the redox balance. It has been suggested that impaired/defective DNA repair pathways including UV induced DNA damage repair might aid the emergence and persistence of drug resistance in P. falciparum strains. The fact that NER is the principle pathway involved in repair of UV induced DNA lesions motivated us to perform a comprehensive in silico search and comparative analysis of the putative NER components including the TFIIH complex in P. falciparum with orthologues of human and yeast. It was observed that almost all the major components of NER machinery are present in P. falciparum’s genome albeit with poor similarity in some of them. The presence of most of the genes involved in NER pathway in P. falciparum’s genome suggesting that this machinery could be potentially active in the parasite. Moreover, none of the putative components have been functionally characterized so far.

Subsequently, among NER components, we performed molecular studies on XPD and its interacting partner, p44 from P. falciparum 3D7 strain. Our in vitro results confirmed that PfXPD displays the characteristic ssDNA dependent ATPase and 5’–3’ DNA helicase activities that were modulated by Pfp44. We report the existence of two high molecular weight expressed protein forms of Pfp44 in intraerythrocytic stages. Furthermore, cellular localization studies found both these proteins to exhibit a distinct cytoplasmic distribution pattern apart from nuclear localization (observed for first time in case of Pfp44 which was found to be localized even in parasitophorous vacuole). This implies that PfXPD and Pfp44 might also be involved in other TFIIH independent functions which will be scrutinized in future studies. We also propose to perform correlation studies between SNPs found in DNA repair genes and drug resistance in collaboration with other research centers.

Keywords: Plasmodium falciparum, DNA repair pathway, NER machinery, TFIIH, Helicase activity, Drug resistance
The CRISPR-Cas9 Minipig

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The pig is a preferred model for many diseases but studies have been hindered by time and budget limitations. We have cloned a transgene minipig expressing Cas9 under a ubiquitin promoter, that substantially will reduce the cost and time to develop new pig models with unique gene alterations. This was carried out by inserting a transposon harboring the CRISPR-Cas9 gene into the genome of minipig fibroblasts. Fibroblasts containing one copy of the Cas9 transgene were selected and used for cloning by somatic cell nuclear transfer, which gave rise to seven founder pigs. The genetic design has been validated in vitro in fibroblasts isolated from the cloned Cas9 minipigs, where Sanger sequencing of DNA from fibroblasts transfected with guide RNAs against TP53 and PTEN revealed knockout of the genes. Moreover, transfection with guide RNA against KRAS in combination with a homology directed repair templated revealed the desired G12D point-mutation leading to constitutive activation of KRAS.

IVIS scanning and IHC staining of tissues sections from one of the founder pigs verified transgene expression in major organs including heart, lung, liver, colon, and prostate. However, expression in hematopoietic cells could not be confirmed. The transgene expression varied from 25 to 100 percent positive cells among different organs and cell types.

A virus-based technology will be implemented for delivery of guide RNAs to induce gene alteration(s) in vivo. First, a pilot study in Danish Landrace pigs has validated that our adeno- associated virus particles indeed are capable of infecting the lung epithelium of pigs. The porcine Cas9 model is currently being validated in vivo by induction of lung cancer through mutation of TP53, PTEN, and KRAS. Here it is expected that a few cells (25-200) will be mutated in all three genes and that these will clonally expand to develop adenomas in the lung.

In conclusion, the development of the Cas9 minipig will open a new research area in minipigs, where gene alterations can be induced rapidly at a reduced cost. This will provide new models to study human diseases in a larger animal.

The project is financed by AU NOVA and a list of smaller Danish funds.
Establishment of a MODY3 disease model in induced pluripotent stem cells using CRISPR

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Maturity onset diabetes of the young (MODY) is a rare inherited form of diabetes leading to impairment of beta cell function before the age of 25. The most common form of MODY is caused by the duplication of a cytosine at position 872 in the HNF1A gene (MODY3; HNF1A P291fsinsC), which is believed to lead to nonsense mediated decay (NMD) and haploinsufficiency. In this study, the HNF1A P291fsinsC mutation was introduced into induced pluripotent stem cells (iPSCs) derived from a healthy individual by use of CRISPR-Cas9 technology. Restriction fragment length polymorphism and sequencing was used to identify an apparent heterozygous (cl 54-5) and homozygous (cl. 66-1) HNF1A P291fsinsC line, which both displayed normal karyotypes. To investigate the disease etiology in more detail, the gene edited and the wild-type iPSC lines were differentiated toward pancreatic endoderm (PE). Flow cytometry analysis showed that 65-80% co-expressed the PE markers PDX1 and NKX6.1 at this stage, which was confirmed by immunocytochemistry. Western blotting with a HNF1A antibody showed that HNF1A protein was not produced by either of the gene-edited lines, suggesting the possibility of a detrimental knockout in the HNF1A wt allele of cl 54-5. qPCR analysis showed that HNF1A transcripts were significantly downregulated four-fold in cl 54-5 (25%) and cl 66-1 (26%) compared with the wild-type line, however, treatment with the NMD inhibitor Cycloheximide rescued the HNF1A transcript levels to 82% and 69%, respectively. In contrast, transcript levels of HNF1B, PDX1 and NKX6.1 remained unchanged w/wo cycloheximide treatment. In conclusion, the gene-edited HNF1A iPSC models are useful for elucidating disease mechanisms related to early-onset diabetes. It would be interesting to extend these analyses to iPSC lines from patients with HNF1A P291fsinsC mutations and their isogenic controls.

This work was funded by the European Bank for induced pluripotent Stem Cells (EBiSC) supported by the Innovative Medicines Initiative (IMI) under grant agreement 115582.
Unveiling the mechanism underlying prostate cancer susceptibility variant rs11672691

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Vast majority of prostate cancer susceptibility SNPs are located at non-coding regions, among which some can transcribe into non-coding RNA including long non-coding RNAs (lncRNA). Many lncRNAs have been found to be important in cancerogenesis. Yet the causal relationship between prostate cancer SNPs and lncRNAs remain totally unknown. Here we find that the prostate cancer risk and aggressiveness-associated SNP rs11672691 at 19q13 locus directly affect the expression of an lncRNA, PCAT19 through disrupting the binding affinity of HOXA2. We validate the observation by single nucleotide mutation using the genome editing tool CRISPR/Cas9 in prostate cancer cells. The G allele of rs11672691 increases the transcription of PCAT19 resulting in upregulation of the genes including CEACAM21, an eQTL gene of rs11672691. Suppression of PCAT19 or CEACAM21 inhibits prostate cancer cell proliferation. Clinical data show that the transcripts from intron2 and exon3 of PCAT19 are specially overexpressed in malignant prostate tumors. Together, we demonstrate that rs11672691 impacts genetic predisposition to prostate cancer through disrupting the chromatin binding of HOXA2 in misregulating the expression of PCAT19 or CEACAM21.
CRISPR-Cas9 optimized delivery systems for human primary cells

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The use of the CRISPR-Cas9 system has facilitated the generation of isogenic cell lines. Regarding these issue, non-integrative delivery systems must be optimized to easily generate or repair human primary cells. In this context, we are developing new strategies as well as implemented established ones in order to find the best approach to edit the human genome of primary cells, specially hMSC and hiPSC. To that aim we are exploring the use of:

- Non-integrative viruses (IDLVs)
- Expression plasmids: optimization of the number and location of NLSs
- IVT sgRNAs and RNA-Cas9
- RNPs (sgRNAs-Cas9 protein)
Landing transgenes at “safe harbor” loci in human cell lines

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Ectopic expression of transgenes in human cell lines is an important approach to perform rescue experiments with wild-type and mutant alleles, to integrate reporter constructs, and to study overexpression phenotypes of gene products. For most reproducible and comparable expression of the transgenes, their integration should be stable and at a well defined genomic locus. For this purpose I will present a toolbox for integrating landing platforms (FRT and attP sites) at loci of “safe harbor genes” using CRISPR. Your desired gene-of-interest can be combined with different tags and promoters using MultiSite Gateway cloning, and afterwards integrated into the genome via the landing platform.
Genomic edition by CRISPR / Cas9 system for guided insertion of the GCDH gene, responsible for Glutaric Aciduria type I

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Glutaric aciduria type 1 (GA-1) is an autosomal recessive disorder in the common catabolic pathway of lysine, hydroxylysine, and tryptophan. It is caused by deficiency of the glutaryl-CoA dehydrogenase (GCDH) that leads to the neurotoxic accumulation of 3-hydroxyglutaric and glutaric acid. Affected patients develop brain damage resulting in dystonia. Current therapy consists in carnitine supplementation and a diet restricted in aminoacids. However, this is not totally effective and some patients develop acute encephalopathic crisis with severe neurological sequelae. The aim of this work is to correct the genetic defect in AG-I patients using gene targeting strategies. We have developed a proof of concept approach to restore GCDH deficiency in AG-I fibroblasts patients, through the targeted insertion of the GCDH gene into the AAVS1 genomic locus by genetic surgery with the CRISPR / Cas9 system. For this purpose, fibroblasts from GA-1 patients were transduced with lentiviral particles containing specific guide sequences against intron 1 of the PPP1R12C gene in the AAVS1 locus and expressing the Cas9 nuclease (pLentiCRISPR-T2). We could observe that a specific cut in the desired locus was achieved. In parallel, we generated a recombination cassette with two homology arms for the AAVS1 locus expressing the GCDH gene under the control of the phosphoglycerate kinase promoter (pHA-PGK-GCDH).

In this cassette a promoterless EGFP cDNA preceded by a splice acceptor site and a translational self-cleaving 2A sequence was also included. AG-I fibroblasts transduction with both (pLentiCRISPR-T2 and pHA-PGK-GCDH), revealed a limited number of GFP positive cells that contained the GCDH cassette. Currently, we are studying the ability of the strategy to restore functional defects in the gene-corrected GCDH deficient fibroblasts.
Manipulation of potassium channels in tumor specific T cells for improved functionality in the suppressive tumor microenvironment

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Treatment of metastatic melanoma with adoptive T cell transfer has shown promising results. Unfortunately, T cells are often not able to fully control and eliminate the cancer, and this is very likely due to a number of tumor suppressor mechanisms. We believe that the expression of matrix metalloproteinase 23 (MMP-23) may very well be a novel mechanism by which tumors suppress anti-tumor T cell responses. It has been shown that MMP-23 can block the voltage-gated potassium channel Kv1.3. Together with Kca3.1, Kv1.3 is important for Ca2+ homeostasis during activation of T cells. By blocking of Kv1.3, MMP-23 can inhibit T cell activation, and a correlation has been found between expression of MMP-23 in melanoma and a poor response to immunotherapy as well as fewer tumor infiltrating lymphocytes.

In the present study, we have shown inhibition of T cell proliferation in the presence of synthetic blockers of Kv1.3. We have furthermore used the new CRISPR/Cas9 technology to create MMP-23 knock-out cancer cell lines, and we have used lentiviral transduction to introduce the expression of MMP-23 in several cancer cell lines. The next steps will be to genetically engineer T cells to express upregulated levels of Kca3.1 using lentiviral transduction as well as to knock-out Kv1.3 by CRISPR/Cas9. This way, we hope to create T cells which are unaffected by MMP-23 and which will depend on Kca3.1 for Ca2+ homeostasis during activation.

With this study, we hope to highlight a new mechanism by which tumor cells suppress T cells, namely by blocking of Kv1.3 by MMP-23. Furthermore, we hope to find a way to circumvent this inhibition by genetically changing the potassium channels of the T cells, which may provide important knowledge to future T cell therapies against cancer.
Investigation of the disease mechanisms of ShortChain-acylCoA Dehydrogenase deficiency by biallelic knockout using CRISPR/Cas9

**Fogh S, Olsen RK, Gregersen N, Aagaard L.**

Mitochondria are involved in numerous processes essential for normal function and homeostasis of the cell. They are the location of ATP synthesis and harbor the sites for the citric acid cycle and fatty acid oxidation. The initiating enzyme for shortchain fatty acids to enter the citric acid cycle is ShortChain acylCoA Dehydrogenase (SCAD) encoded by the ACADS gene. So far, around 50 different variations in ACADS have been identified resulting in misfolding and reduced activity of SCAD. In our patient database of around 500 patients with variation in ACADS there is not registered any patients with a double nullmutation. In addition, two common ACADS variants, c.625G>A (p.Gly209Ser) and c.511C>T (p.Arg171Trp), are found in the healthy population with an allele frequency of 21% and 8%, respectively. In patients, this frequency rises to 67% for c.625G>A, but remains the same for c.511C>T. This combination made us speculate that maybe the accumulation of the SCAD substrate butyrate is toxic at high levels but beneficial at low levels.

Using CRISPR/Cas9 we want to investigate the impact of biallelic knockout of the ACADS gene on cell fitness and survival. The knockout is carried out in Normal Human Dermal Fibroblast (NHDF) cell lines with gRNA targeting the first and last exon in ACADS followed by clonal expansion.

We will use the cells to investigate mitochondrial function, comparing knockout with control cells. We will compare oxygen consumption and extracellular release as a measure of the capacity of the mitochondria to produce energy from mitochondrial respiration and glycolysis. This in addition with metabolomics focusing on mitochondrial energy metabolism, protein processing, antioxidant defense, morphology, and apoptosis will help us understand the disease mechanism of SCAD.
Editing the livestock genome

Dr Simon Lillico

Recent advances in genome engineering have produced a range of tools that allow the precise modification of livestock genomes. Microinjected directly into the newly fertilised zygote, genome editor reagents are proving to be a very effective methodology for inducing changes in the DNA sequence at their target sites, opening up a multitude of options for genome manipulation. This ability to modify the genome, without the necessity of transgene insertion, allows the production of animals with designer traits such as improved disease resistance, enhanced production values or as models of human disease. We present data detailing our experiences with TALENs, ZFNs and CRISPR/Cas9 to induce NHEJ, HDR or deletion events in either sheep or pigs.
miRNA profiling in plasma from patients with sleep disorders reveals dysregulation of miRNAs in narcolepsy and other central hypersomnias

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Study Objectives: miRNAs have been implicated in the pathogenesis of human diseases including neurological disorders. The aim is to address the involvement of miRNAs in the pathophysiology of central hypersomnias including narcolepsy with cataplexy and hypocretin deficiency (NC), narcolepsy without cataplexy (NwC) and idiopathic hypersomnia (IH).

Design: We conducted high-throughput analysis of miRNA in plasma from patients with NC, NwC and IH in comparison with healthy controls (HC) using quantitative real-time polymerase chain reaction (qRT-PCR) panels.

Setting: University hospital based sleep clinic and research laboratories.

Patients: Twelve patients with NC, 12 patients with NwC, 12 patients with IH and 12 HC.

Measurements and Results: Using analysis of miRNA in plasma with qRT-PCR we identified 50, 24 and 6 miRNAs that were changed in patients with NC, NwC, IH, respectively, compared to HC. Twenty miRNA candidates which fulfilled the criteria of two-fold change and p-value < 0.05 were selected for validation of miRNA changes in an independent cohort of patients. Four miRNAs were significantly changed between NC patients and HC. Levels of miR-30c, let-7f and miR-26a were increased, whereas the level of miR-130a was decreased in NC compared to HC. The miRNAs changes were not specific for NC, since the levels of the four miRNAs were also altered in patients with NwC and IH compared with HC.

Conclusion: The levels of four miRNAs are changed in plasma from patients with NC, NwC and IH suggesting that alterations of miRNAs can be involved in the pathophysiology of central hypersomnias.
Albumin transcytosis in kidney proximal tubule cells – the role of megalin, cubilin and FcRn receptors

Søren Thorup Scheuer

Background: The renal handling and metabolism of albumin involves glomerular filtration followed by endocytic uptake in the proximal tubule. Endocytosis is mediated by the megalin/cubilin receptor complex located in the kidney proximal tubule after which it is targeted for lysosomal degradation. Recent evidence suggests the existence of an alternative, transcellular salvage-pathway, which allows for intact albumin to be returned to the bloodstream by intracellular translocation to the neonatal IgG receptor, FcRn. The existence, as well as the relevance of this pathway remains controversial.

This study aims to determine if FcRn is involved in transcellular transport of albumin, using an in vitro model of kidney proximal tubule cells.

Methods: Transcellular transport of FITC-tagged albumin is studied using a confluent monolayer of a proximal tubule cell line (LLC-PK1) growing on transwell membranes. To investigate the role of the FcRn receptor and the megalin/cubilin receptor complex we will establish stable cell lines with targeted up- or downregulation of FcRn. Upregulation is achieved by transfection with cDNA, while knock down of the receptors is obtained by the CRISPR-Cas9 technology. This will allows us to compare the transcellular transport of albumin in a controlled environment where the only variable being the expression of the FcRn receptor.

Perspectives: The results from these experiments should allow us to identify as proof of concept if such FcRn dependent transcellular albumin transport mechanism exists. This is important for the understanding of albuminuria in kidney diseases and may provide a potential new pathway for modulating the renal pharmacokinetics of albumin bound drugs and other substrates in health as well as in albuminuric kidney diseases.
Defining the earliest step of cardiovascular development using CRISPR genome editing technology

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We hypothesize that key vascular genes play significant roles in the decision of stem cell fate of iPSCs into vascular progenitor cells.

**Aim:** Understanding of cardiovascular development is of great importance in order to facilitate tissue regeneration e.g. in heart failure, by promoting new vessels in the damaged area of the heart.

**Background:** To understand the cardiovascular development it is crucial to investigate how stem cells adopt specific cell fates and subsequently assemble into functional vessels or heart tissue. Novel techniques have emerged that enables genome editing in a variety of cell types, including human induced pluripotent stem cells (iPSCs). These techniques are powerful tools to study how specific genes influence cell fate decisions in the early differentiation stages and throughout development. We study two well established and one uninvestigated cardiovascular key-factors and their effect on stem cell decision and cardiovascular development.

**Methods:** To investigate the impact of each of the chosen key-factors during vascular cell lineage differentiation, we use the CRISPRi technology. This technique facilitates inducible and sequence-specific repression and re-expression of the genes of interest. The modified iPSC lines are characterized during differentiation and analyzed for preferences of cell fate; favoring pluripotency, mesodermal or any of the cardiovascular cell types. The analysis is performed using flow cytometry and immunocytochemistry to quantify the number of pluripotent cells (SOX2+ and OCT4+), mesodermal cells (FLK1+), and each of the terminally differentiated vascular cell types; endothelial cells (ECs) (CD31+), including arterial (Cn40+) and venous (EPH4+) endothelial cells, vascular smooth muscle cells (αSMA+) and cardiomyocytes (TnT+). In addition, beating of cardiomyocytes and tube formation assay/LDL uptake assay of Endothelial cells are used to assess functionality of the cardiovascular cells.

**Results:** We have generated several highly specific and efficient iP - CRISPRi cell lines using guideRNAs targeting each of the promoters of the chosen key-factors. Novel data show that the individual cell lines with repression of each of the chosen key-factors demonstrate abnormal cardiomyocyte differentiation and delayed beating capacity.

**Perspective:** Results obtained in the present project are expected to add significant knowledge to the regulation of stem cell fate in general, and this may on a long term be used in advancing novel stem cell therapeutics.

**Keywords:** induced Pluripotent Stem Cells, vasculature, CRISPR