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ABSTRACTS

Program and Abstracts of the 13th Transgenic Technology Meeting (TT2016)

Clarion Congress Hotel, Prague, Czech Republic, 20–23 March 2016

The TT2016 meeting is hosted by: the Czech Centre for Phenogenomics (CCP), BIOCEV, Prumyslova 595, 25242, Vestec, Czech Republic

Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec, Prumyslova 595, 252 42, Vestec, Czech Republic

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TT2016 Scientific Program

Sunday 20th March 2016

13:00–20:00 Registration open

Opening of TT2016

17:30–18:00 Welcome address—ISTT president & organizing committee

Session 1: Opening Keynote lecture

18:00–19:00 Andras Nagy (Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Canada)
Utilising transposon-delivered transgenes for understanding reprogramming

19:00– Come together evening

Monday 21st March 2016

08:00–18:00 Registration open

Session 2: Generation of transgenic models I

09:00–09:30 Charles A. Gersbach (Duke Center for Genomic and Computational Biology, USA)
Genome and Epigenome Editing with CRISPR/Cas9 for Gene Therapy and Disease Modeling

09:30–10:00 Ralf Kühn (Max-Delbrück-Center for Molecular Medicine, Germany)
Direct production of mouse mutants using engineered nucleases in one-cell embryos

10:00–10:30 Lluis Montoliu (National Centre for Biotechnology (CNB), Spain)
CRISPR-ing the non-coding genome

10:30–10:50 TEA & COFFEE

Session 3: Generation of transgenic models II (selected presentations)

10:50–11:05 Séverine Ménoret
Increased efficiency of BAC transgenesis by piggyBac transposition but not by CRISPR/Cas9 targeted integration for the generation of human SIRPalpha rats

11:05–11:20 Grzegorz Kreiner
Targeting nucleolus—a new approach in generating transgenic mouse models of neurodegenerative diseases and their exploitation to study possible neuroprotective

11:20–11:35 Melissa A. Larson
Nuclear Transfer between Strains of Inbred Mice

11:35–11:50 Christian Mosimann
Post-genome editing approaches for recombinase genetics in zebrafish

11:50–12:05 Andrei Golovko
10 Years of TIGM: Important Lessons and Future Perspectives

12:05–12:20 Anna Anagnostopoulos
Using the Human–Mouse: Disease Connection to Identify Mouse Models of Human Disease
12:20–13:30 LUNCH & POSTERS

Sponsored session: Applied StemCell, Inc

12:20–13:20 Ruby Chen-Tsai (Applied StemCell Inc.)
Development of integrase-based TARGATTTM method for generating site-specific transgenic rat models

Session 4: Running a Transgenic Service Facility

13:30–15:00 Round table discussion
Marina Gertsenstein (Toronto Center for Phenogenomics (TCP), Canada)
Cord Brakebusch (Biotech Research and Innovation Centre (BRIC), Denmark
Lauryl Nutter (Toronto Center for Phenogenomics (TCP) (Canada)
15:00–15:15 TEA & COFFEE

Session 5: Beyond transgenic model generation

15:15–15:45 Mary Dickinson (Baylor College of Medicine, USA)
Analysis of embryonic lethal mutations in mice using 3D imaging
15:45–16:15 Nicholas Gale (Regeneron Pharmaceuticals, USA)
A large scale mouse phenotyping screen for the discovery of novel biotech targets in oncology and angiogenesis and other therapeutic areas
16:15–16:45 Kent Lloyd (University of California, Davis, USA)
An update on progress by the International Mouse Phenotyping Consortium (IMPC)
16:45–17:00 TEA & COFFEE

Session 6: World of Nucleases: discovery and development

17:00–17:30 Francis Mojica (University of Alicante, Spain)
Taking a look at the CRISPR biology and applications from a historical perspective
17:30–18:00 Bernd Zetsche (Broad Institute of MIT and Harvard Cambridge, USA)
Cpf1 is a single-RNA-guided endonuclease of a Class 2 CRISPR-Cas system
18:00–18:30 Konstantin Severinov (Skolkovo Institute of Science and Technology, Russia)
A pipeline approach for discovery of novel CRISRP-Cas systems

Tuesday 22nd March 2016

Session 7: Advances in animal biotechnology

08:30–09:00 Eckhard Wolf (LMU Munich, Germany)
COST Action BM1308 "Sharing Advances on Large Animal Models—SALAAM"
09:00–09:30 Yonglun Luo (Aarhus University, Denmark)
Efficient precision gene editing in pigs: Towards a new era in generating genetically designed pigs of human diseases and regenerative medicine
09:30–10:00 Chris Proudfoot (The Roslin Institute of the University of Edinburgh, UK)
Genome engineering livestock
10:00–10:15 *TEA & COFFEE*

Session 8: Advances in animal biotechnology (selected presentations)

10:15–10:30 Soo Young Yum
Production and Analysis of a multi-copy integrated transgenic cattle via *transposon*
10:30–10:45 Amy Kaucher
Production of germline ablated male pigs via *Crispr/Cas editing of the NANOS2 gene*
10:45–11:00 Sean Stevens
Pig Genome Engineering for Xenotransplantation
11:00–11:15 Wiebke Garrels
Multiplex Transgenesis in Cattle via *the Sleeping Beauty Transposon System*

Parallel sessions

Session 9: Animal Ethics

11:20–11:40 Henriette Bout (University of Amsterdam, the Netherlands)
Ethical aspects of the Crispr/Cas9 technology
11:40–12:00 Aurora Brønstad (University of Bergen, Norway)
The AALAS-FELASA Working Group on Harm-Benefit analysis of animal studies
12:00–12:20 Michelle Stewart (Mary Lyon Centre, Medical Research Council UK)
The Four R's of phenotyping GA mice—Robustness, Reproducibility, Rigorousness, and Randomisation

Session 10: Technology development II

11:20–11:40 Toru Takeo (Center for Animal Resources and Development (CARD), Japan)
Efficient production of mouse oocytes using superovulation by immunization against inhibin

11:40–12:00 Jeff Batton (GeneSearch, Inc., USA)
New Cost-Effective Methods for Stem Cell Procedures on Pre-Implantation Embryos

12:00–12:20 Sandra Hope (Brigham Young University, USA)
Microfabricated Lance Array Nanoinjection system delivers CRISPR-Cas9 to Hundreds of Thousands of Cells Simultaneously

12:20–14:00 LUNCH & POSTERS

Session 11: Gene manipulation and genome editing and (disease) models I

14:00–14:30 Ethan Bier (University of California, USA)
The implications of active genetics

14:30–15:00 Didier Stainier (Max Planck Institute for Heart and Lung Research, Germany)
Genetic compensation induced by deleterious mutations but not gene knockdowns

Session 12: Gene manipulation and genome editing and (disease) models II (selected presentations)

15:00–15:15 Marie-Christine Birling
Generation of genomic structural variants by CRISPR/Cas9 genome editing in rodents

15:15–15:30 Katharina Boroviak
The possibilities and limitations of CRISPR/Cas9 in mouse zygotes

15:30–15:45 Kevin A. Peterson
CRISPR/Cas9 mediated gene modification provides a robust platform for modeling developmental disorders

15:45–16:00 TEA & COFFEE

Session 13: Orbis pictus lecture

16:00–16:45 Richard Behringer (University of Texas MD Anderson Cancer Center, USA)
Transgenic approaches in diverse animals species

16:45–18:00 ISTT GENERAL ASSEMBLY

19:15– Gala dinner (Zofin Palace)

<u>Wednesday 23rd March 2016</u>

Session 14: Technology development

08:30–09:00 Masato Ohtsuka (Tokai University School of Medicine, Japan)
GONAD and Easy (Isi)-CRISPR: novel mouse genome engineering tools

09:00–09:30 Haoyi Wang (Institute of Zoology, Chinese Academy of Sciences, China)
CRISPR-Cas9 Application in Mouse Model Creation and Transcription Regulation

09:30–10:00 Tomomi Aida (Medical Research Institute (MRI) of Tokyo Medical and Dental University (TMDU), Japan)
Gene cassette knock-in in mice with cloning-free CRISPR/Cas system

10:00–10:15 TEA & COFFEE

Session 15: Genetics, Epigenetics, Stem cell manipulation

10:15–10:45 Robin Lovell-Badge (Francis Crick Institute, UK)
Regulation of Sox9 in the gonad during sex determination

10:45–11:15 F. Kent Hamra (University of Texas Southwestern Medical Center, USA)
Rat Germline Editing in Donor Spermatogonial Stem Cells

11:15–11:45 John Schimenti (Cornell University, USA)
GWIS: Genetics with Interrogation of SNPs

11:45–13:30 LUNCH & POSTERS

Session 16: Generation of transgenic models I: Models of diseases and applications (selected presentations)

13:30–13:45 Yann Herault
Modelling and understanding rare genetic diseases with intellectual disabilities for tomorrow's treatment

13:45–14:00 Petr Kasparek
TALEN-mediated inactivation of Klk5 and Klk7 rescues lethal phenotype of Netherton syndrome mouse model

14:00–14:15 Javier Martín-González
A powerful new tool to improve immune-compromised mouse models: derivation of NRG embryonic stem cell lines.

14:15–14:30 Prem Premsrirut
RNAi and CRISPR/Cas9 based In Vivo Models for Drug Discovery

14:30–14:45 TEA & COFFEE

Session 17: Orbis pictus lecture II

14:45–15:30 Thomas Boehm (Max Planck Institute of Immunology and Epigenetics, Germany)
Genetic basis of lymphoid organ formation

Session 18: Genetics & models pluripotency/iPS cells

15:30–16:00 Rene Maehr (UMass Medical School, USA)
Towards using pluripotent stem cell-based disease models to study immune syndromes

16:00–16:30 Ron Weiss (Massachusetts Institute of Technology, USA)
Mammalian Synthetic Biology: From Parts to Modules to Therapeutic Systems

16:30–16:45 TEA & COFFEE

Session 19: Award session

16:45–16:50 3rd ISTT Young Investigator Award
16:50–17:10 Pablo Ross
Embryonic stem cells and interspecies blastocyst complementation in farm animals

Session 20: Closing Keynote Lecture

17:10–17:55 Denis Duboule (University of Geneva, Switzerland)
A Genetic Approach of Long-range Gene Regulation During Development and Evolution
17:55–18:10 Close of Meeting

TT2016 Hands-on Workshops

Workshop organization committee

Radislav Sedlacek (CCP, Prague, Czech Republic)
Martin Fray (MRC, Harwell, UK)
Petr Bartunek (IMG, Prague, Czech Republic)
Bjoern Schuster (CCP, Prague, Czech Republic)
Inken M. Beck (CCP, Prague, Czech Republic)
Nicole Chambers (CCP, Prague, Czech Republic)

INFRAFRONTIER-I3—Mouse cryopreservation workshop

Day 1—Wednesday 16th March 2016

08:30–09:00 Arrival and registration
09.00–09:15 Welcome and introduction to the IMG and CCP
Radislav Sedlacek, IMG, Prague, Czech Republic
09:15–09:25 Introduction to the course and the teaching material
09:25–10:45 Principles and benefits of cryobiology
Martin Fray, Medical Research Council, Harwell, UK
10:45–11:00 ***TEA and COFFEE***
11:00–12:00 New approaches to exchanging mouse strains
Lluis Montoliu, National Centre of Biotechnology, Madrid, Spain
12:00–13:00 ***LUNCH***
13:00–15:00 Sperm Harvesting/Freezing Introduction
A. Sperm freezing—(Practical)
B. Preparing epididymides held for refrigerated transportation in Lifor (Practical)
C. Sperm held on dry-ice (Demonstration)
15.00–15.15 ***TEA and COFFEE***
15:15–16:00 Preparation for Thursday's IVF
Media preparation
A. Pre-incubation dish containing MBCD
B. Fertilisation drops containing GSH
C. Wash dish containing mHTF
16.00–16.30 Animal welfare aspects on cryopreservation
Marcello Raspa, CNR-IBCN, Monterotondo, Italy
16:30–17:00 Recap/Review
19:30 ***MEET IN PRAGUE FOR BAR DINNER***

Day 2—Thursday 17th March 2016

07:30–10:30 IVF Introduction
(a) Set up an IVF using freshly harvested sperm and vitrified oocytes—(Demonstration)
(b) Set up an IVF using sperm held on dry ice -(Demonstration)
(c) Set up an IVF using sperm harvested from epididymides held overnight in Lifor—(Practical)
(d) Set up an IVF using sperm frozen in LN_2 (Practical)
10:30–11:00 ***TEA AND COFFEE***
11:00–12:00 New development and ideas in sperm freezing techniques—Toru Takeo, CARD, Kumamoto University, Japan
12:00–13:00 ***LUNCH***
13:00–15:30 Wash IVF—(Demonstration followed by practical training)
15.30–16:00 INFRAFRONTIER-EMMA resource, principles to efficiently share mouse models—Susan Marschall (Helmholtz Zentrum Munich, Germany)
16.00–16:15 Recap/Review
16:15–17:00 CRISPR technology (joint lecture)
Lluis Montoliu, National Centre of Biotechnology, Madrid, Spain

Day 3—Friday 18th March 2016

08:30–10:00 Assess IVF dishes—(Demonstration followed by practical)
10:00–10:15 ***TEA AND COFFEE***
10:15–12:00 Embryo vitrification—(Demonstration followed by practical)
12:00–13:00 Thawing vitrified embryos—(Demonstration followed by practical)
13:00–14:00 ***LUNCH***
14.00–16.00 Non-surgical embryo transfer—Barbara Stone, Paratechs Corporation
16.00–16.15 ***TEA and COFFEE***
16:15–17:15 Preparing frozen samples for shipment—Soren Knudsen—Cryoport
17:15–17:30 Wrap up presentation and closure of the course

CCP programmable nucleases (CRISPR/Cas9) Transgenesis Course

Day 1—Wednesday 16th March 2016

08:30–09:00 Arrival and Registration
09.00–09:15 Welcome and Introduction to IMG and CCP
Radislav Sedlacek (IMG/CCP)
09:15–09:25 Introduction to the Course and the Teaching Material
Björn Schuster (IMG/CCP)
09:25–10:00 Lecture: Mouse transgenesis: *Pros-and-cons* of programmable nucleases
Radislav Sedlacek
10:00–10:45 Sponsored lecture: Guide design and demonstration of web based tool
Leigh Brody—Desktop Genetics
10:45–11:00 ***TEA and COFFEE***
11:00–13:00 Practical training session I.
A. CRISPR/Cas9 Zygote injection—*Ronald Naumann*
B. Electroporation of zygotes—*Haoyi Wang*
C. Bioinformatics, experimental design & targeting & genotyping—*Björn Schuster*
13:00–14:00 ***LUNCH***
14:00–14:30 Targeting design—validation of the tools—genotyping
Björn Schuster (IMG/CCP)
14:30–16:00 CRISPR validation practical (cell culture & transfection)
Björn Schuster & Jana Kopkanova (IMG/CCP)
16.00–16.15 ***TEA and COFFEE***
16:15–17:00 Lecture: oocyte—embryo transition in mammals
Petr Svoboda—Institute of Molecular Genetics, CZ
19:30 ***MEET IN PRAGUE FOR DINNER***

Day 2—Thursday 17th March 2016

09:00–09:15 Recap/Review
09:15–10:00 Lecture: Genotyping strategies and tools
Jana Kopkanova & Björn Schuster (IMG/CCP)
10:00–10:45 Sponsored Lecture: CRISPR genome editing workflow
Caroline Becket—Merck Millipore Sigma
10:45–11:00 ***TEA AND COFFEE***
11:00–13:00 Practical training session II.
A. CRISPR/Cas9 Zygote injection—*Ronald Naumann*
B. Electroporation of zygotes—*Haoyi Wang*
C. Bioinformatics, experimental design & targeting & genotyping—*Björn Schuster*
13:00–14:00 ***LUNCH***
14:00–14:20 Targeting design—validation of the tools—genotyping
Björn Schuster (IMG/CCP)
14:20–16:00 CRISPR validation practical (DNA isolation and PCR based genotyping I)
Jana Kopkanova & Björn Schuster (IMG/CCP)
16.00–16:15 ***TEA & COFFEE***
16:15–17:00 Lecture—CRISPR technology
Lluis Montoliu—CNB, Spain
This lecture will be a joint lecture with the Cryopreservation workshop
19:30 ***DINNER***

Day 3—Friday 18th March 2016

09:00–09:45 Development CRISPR-Cas9 technology & Cpf1
Bernd Zetsche—Broad Institute, USA
09:45–10:00 ***TEA AND COFFEE***
10:00–12:00 Practical training session III.
A. CRISPR/Cas9 Zygote injection—*Ronald Naumann*
B. Electroporation of zygotes—*Haoyi Wang*
C. Bioinformatics, experimental design & targeting & genotyping—*Björn Schuster*
12:00–13:00 ***LUNCH***
13:00–15:00 CRISPR validation practical (PCR based genotyping)
Jana Kopkanova & Björn Schuster (IMG/CCP)
15:00–15:15 ***TEA and COFFEE***
15:15–16:00 Collection of results and preparation of short presentations
16:00–17:15 Short group presentations and discussion
17:15–17:30 Summary & Conclusions
Radislav Sedlacek
17:30 End of course

Zebrafish Genome Editing

Day 1—Wednesday 23rd March 2016

18:00–18:45 Registration (Clarion Congress Hotel, Prague)
18:45–19:00 Welcome and Introduction (P. Bartunek and Z. Kozmik)
19:00–19:45 Talk-Zebrafish transgenesis and genome editing: state-of-the-art (C. Mosimann)
20:00–22:00 Get together/roundtable discussion/course expectations and ***LIGHT DINNER***

Day 2—Thursday 24th March 2016

08:30–09:00 Arrival at Institute of Molecular Genetics, Prague—***TEA/COFFEE***
09:00–12:30 Collection of embryos, microinjections—morpholino oligonucleotides, CRISPR-Cas9 (mRNA and protein)
12:30–13:30 ***LUNCH***
13:30–14:00 Talk-sgRNA design, genotyping and analysis of results (C. Mosimann)
14:00–15:30 *in vitro* transcription and purification of sgRNA, discussion of alternative methods
16:00–16:30 ***TEA and COFFEE***
16:30–18:00 Embryo manipulations, mounting, imaging, analysis (stereo, macroscope)
18:00–18:30 Setup of adult zebrafish to generate injection-ready embryos
19:00 ***DINNER***

Day 3—Friday 25th March 2016

09:00–12:00	Collection of embryos, microinjections (Tol2 transposase, fluorescent constructs)
12:00–13:00	***LUNCH***
13:15–14:00	Special lecture (G. Lieschke)
14:00–15:00	Molecular analysis of mutagenesis efficiency (T7 endo assay, gel electrophoresis)
15:00–15:30	***TEA and COFFEE***
15:30–17:30	Embryo manipulations, mounting, imaging, analysis (time-lapse, confocal)
17:30–18:00	Discussion and closing remarks
18:00	End of workshop

Oral Presentations

Utilising transposon-delivered transgenes for understanding reprogramming

Andras Nagy

Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada; Department of Obstetrics & Gynaecology and Institute of Medical Science, University of Toronto, Toronto, Canada

The discovery of a defined set of transcription factors that can induce reprogramming of somatic cells to pluripotent stem cells (iPSCs) has had an unprecedented impact on our view on future cell transplantation-based tissue repair and restoration of faulty physiological functions. Somatic cell reprogramming is a several weeks long process through which cells reach pluripotency, the developmental state similar to embryonic stem cells. This cascade of events and the driving forces behind the phenomenon are very poorly understood. It is, however, crucial to uncover the fine details of this process in order to comprehend the true properties of iPSCs and so better tailor their future therapeutic use.

To address this need we utilized the unique property of the transposon-mediated transgene delivery system combined with doxycycline inducible expression of the reprogramming factors. The transposon ITR-flanked transgenes were seemingly much less subjected to silencing than the virus-delivered ones. Therefore, we were able to generate highly efficient secondary reprogramming systems, allowing us a comprehensive molecular description of the reprogramming cascade toward two distinct pluripotent states. We explored alternative outcomes of somatic reprogramming by fully characterizing reprogrammed cells independent of preconceived definitions of reprogrammed iPSC states. We demonstrate that manipulating the expression level of the reprogramming factor influences the arrival of cells to a non-ES cell-like or ES cell like pluripotent state. This bifurcated process has been characterized with multiple “omic” platforms, consisting of the transcriptome (microRNA, lncRNA and mRNA), CpG methylation, ChIP-sequencing (for chromatin marks: H3K4me3, H3K27me3 and H3K36me3), in addition to quantitative mass spectrometry profiling of the global and cell surface proteome. This dataset enables cross-referencing between “omic” platforms, which facilitates deeper understanding of the cascade of molecular events and the dynamics of the epigenome driving the generation of pluripotent cells.

Keywords: stem cells, pluripotency, somatic cell reprogramming, epigenetics of stem cells

Genome and epigenome editing with CRISPR/Cas9 for gene therapy and disease modeling

Charles A. Gersbach

Department of Biomedical Engineering, Duke University, Durham, USA

The advent of genome engineering technologies, including the RNA-guided CRISPR/Cas9 system, has enabled the precise editing and regulation of endogenous human genes and epigenetic states. We have applied these tools to the correction of mutations that cause genetic disease and also adapted them to manipulate the epigenome and control cell fate decisions. For example, we engineered CRISPR/Cas9-based nucleases to correct the human dystrophin gene that is mutated in Duchenne muscular dystrophy patients. When we delivered these nucleases to cells from patients with this disease, the correct gene reading frame and expression of the functional dystrophin protein were restored *in vitro* and following cell transplantation into mouse models *in vivo*. When delivered directly to a mouse model of this disease, gene editing by the CRISPR/Cas9 system led to gene restoration and improvement of biochemical and mechanical muscle function. In other studies, we have engineered CRISPR/Cas9-based tools to regulate the expression of endogenous genes and applied these tools to control diverse genes relevant to disease, development, and differentiation. Genome-wide analysis of the DNA-binding, gene regulation, and chromatin remodeling by these targeted epigenome modifiers has demonstrated their exceptional specificity. We have recently applied these technologies to control the decisions of stem cells to become specific cell fates and reprogram cell types into other lineages that could be used for drug screening and disease modeling. Incorporating methods to dynamically control the activity of these proteins, such as optogenetic control of the proteins with light, has allowed us to pattern gene expression both temporally and spatially. Ongoing efforts include designing strategies to manipulate specific epigenetic marks that would enable deciphering the influence of epigenetics on gene regulation and disease states. Collectively, these studies demonstrate the potential of modern genome engineering technologies to capitalize on the products of the Genomic Revolution and transform medicine, science, and biotechnology.

Keywords: genome editing, epigenome editing, CRISPR, gene therapy

Direct production of mouse mutants using engineered nucleases in one-cell embryos

Ralf Kühn

Max-Delbrück Center for Molecular Medicine, Berlin, Germany

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The CRISPR/Cas9 system has emerged as a popular genome editing method because of its technical simplicity and robustness. It can be widely applied to generate genome editing animals including mice. In this talk I will present our recently developed *add-on* tools for CRISPR/Cas9 system: GONAD and Easy (*Isi*)-CRISPR.

GONAD: Animal transgenesis involves three major technical steps; isolation of fertilized eggs, microinjection and subsequent transfer of eggs into recipient females. Of these, the microinjection is the most tedious step that requires sophisticated equipment as well as highly skilled personnel to perform the task. Using CRISPR system, some groups recently demonstrated that *microinjection* can be replaced with electroporation of eggs. Such ex vivo electroporation helped handling of many eggs simultaneously but it did not exclude other two steps of animal transgenesis. We developed a new system called Genome-editing via Oviductal Nucleic Acids Delivery (GONAD) method that could bypass all three critical steps of traditional genome engineering methods. The GONAD strategy allows electroporation to deliver nucleic acids (NAs) to the zygotes within the intact mouse oviduct in situ. With this method (instillation of NA solution into the oviductal lumen of pregnant females and following electroporation to the oviducts), we successfully delivered NAs into the day 0.7 and day1.5 zygotes and obtained gene-disrupted embryos/pups when delivered CRISPR/Cas9 genome editing components. The GONAD method can also be potentially applied to generate genetically engineered models in other species.

Easy-CRISPR: One of the major challenges of CRISPR/Cas9 system is very poor efficiency of insertion of longer DNA cassettes at the Cas9 cut sites. Because smaller single-stranded OligoDeoxyNucleotides (ssODNs) get inserted at relatively higher efficiency than the longer double-stranded DNA cassettes, we hypothesized that longer single-stranded DNAs (ssDNAs) could also get inserted efficiently. Because longer ssDNAs (>200-bases long) cannot be readily synthesized commercially, such strategies require the use of dsDNAs (plasmid-based constructs) even though the dsDNA repair templates generally require long homology arms and are inserted with lower efficiency compared to ssDNA. We synthesized ssDNA donors of 0.2 to 0.5-kb or longer, using a standard molecular biology technique; in vitro Transcription and Reverse Transcription (*iv*TRT) that were then used in CRISPR/Cas9-mediated targeted insertion experiments (pronuclear and cytoplasmic injection of ssDNA together with CRISPR/Cas9 genome editing components). As a result, we routinely obtained knock-in mice with an insertion efficiency of up to 83 %. We named this new method as (*Isi*)-CRISPR: ***i***vTRT-***s***sDNA ***i***nsertion CRISPR (pronounced *Easy*-CRISPR).

Keywords: CRISPR/Cas9, *in vivo* electroporation, GONAD, ssDNA, knock-in, Easy-CRISPR

CRISPR-Cas9 application in mouse model creation and transcription regulation

Haoyi Wang[1,2]

[1]Institute of Zoology, Chinese Academy of Sciences, Bejing, China; [2]The Jackson Laboratory, Bar Harbor, Maine, USA

CRISPR-Cas9 system has become the tool of choice for genome engineering. Previously we established the method of microinjecting CRISPR-Cas9 system into zygotes to generate mouse models. To overcome the technically demanding and inherently low throughput method of microinjection, we devised the Zygote Electroporation of Nuclease (ZEN) technology, which employs electroporation to deliver CRISPR-Cas9 reagents to the zygotes and generated live mice carrying targeted NHEJ and HDR mutations. The general principles discovered and described in this study have implications for high efficiency, high throughput genome engineering in animals.

To extend the utility of the CRISPR-Cas9 system, we have taken advantage of the ability of Pumilio PUF domains to bind specific 8-mer RNA sequences. By combining these two systems, we established the Casilio system, which allows for specific and independent delivery of effector proteins to specific genomic loci. We demonstrated that the Casilio system enables independent up- and down-regulation of multiple genes, as well as live-cell imaging of multiple genomic loci simultaneously. Importantly, multiple copy of PUF binding sites can be incorporated on sgRNA backbone, therefore allowing for local multimerization of effectors. In addition, the PUF domain can be engineered to recognize any 8-mer RNA sequence, therefore enabling the generation and simultaneous operation of many Casilio modules.

Keywords: Genome editing, CRISPR-Cas9, mouse model, transcription regulation

Gene cassette knock-in in mice with cloning-free CRISPR/Cas system

Tomomi Aida[1], Tetsushi Sakuma[2], Shota Nakade[2], Takashi Yamamoto[2], Kohichi Tanaka[1]

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Knock-in mice carrying functional gene cassettes have provided invaluable opportunities for *in vivo* functional analysis of genes, cells, and circuits in mammalian organisms. Although the CRISPR/Cas system has enabled one-step generation of knockout mice with high efficiency, relatively low success rates of gene cassette knock-in limit its application range. We