UniversitätsKlinikum Heidelberg

External Seminar Speaker

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Place: **Analysezentrum 3, 2. OG, Room 02.332**
Date: **Wednesday, May, 8th**
Time: **2.00 pm**

Mouse genome engineering using the *CRISPR/cas9* system

CRISPR/Cas9 mediated genome editing has rapidly evolved into a key technology for molecular genetics. TRAM (TRAnsgenic animal and genetic engineering Models) Core Facility of the Medical Faculty of the University of Münster (Germany) routinely applies the CRISPR/cas9 system to generate new transgenic mouse lines. Among them are: conventional and conditional gene knock-out models, knock-in at specific mouse loci, point mutations or epitope insertions in the desired gene regions, etc. The efficiency of gene modification at specific locus using the NHEJ mechanism reaches almost 90%, whereas efficiency of targeting using HDR mechanism is up to 50%. Despite the advantages of CRISPR/Cas9 based genome editing, a number of potential problems such as genome rearrangements and off target effects still impede the CRISPR/Cas9 technology for use in biomedical research and further efforts are necessary to overcome these hurdles. Our study examines problems that affect direct knock-in genome targeting. During the generation of six different conditional knock-out mouse models, we discovered that frequently (sometimes solely) homology-directed repair and/or non-homologous end-joining mechanisms caused multiple unwanted head-to-tail insertions of donor DNA templates. Disturbingly, conventionally applied PCR analysis - in most cases -failed to identify such multiple integration events, which led to a high rate of falsely claimed precisely edited alleles. We caution that comprehensive analyses of modified alleles are essential, and offer practical solutions to correctly identify precisely edited chromosomes. Our findings are important to unlock the full potential of the CRISPR/Cas9-mediated genome editing protocols for the generation of custom designed gene variants for biomedical research and gene therapy.

Host: **Prof. Dr. med. Johannes Backs**
Director of the Department Molecular Cardiology and Epigenetics
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