Summary of the
2nd international Workshop on
Minicircle-DNA
07. – 09. May 2008, Bielefeld (Germany)
by Martin Schlef (PlasmidFactory, martin.schleef@plasmidfactory.com)

Introduction & Background

Minicircles (MC) are circular non-viral DNA elements that are generated e.g. by an intramolecular (cis-) recombination from a parental plasmid (PP). The difference between MC and standard plasmid vectors for gene therapy or nucleic acid vaccination is that the MC does neither contain the bacterial origin of replication (needed only in bacteria fort he amplification of plasmids in cell division) nor the antibiotic resistance markers (AB^R) or other selection systems to keep the plasmid in high amounts within the producer cell.

Since it is a regulatory requirement to avoid the AB^R and un-necessary (or CpG-containing) sequence elements from pharmaceutically used plasmids the removal of such is a major goal in non-viral vector development and also supporting the plasmid-based production of viral vectors (e.g. AAV, LV).

After on the 1st workshop on MC the knowledge on this topic was summarized, all participants coming again were interested in any progress made after 15 months.

The workshop was – as before - organized by the local biotech network "Bio-Tech OWL e.V." (www.bio-owl.de) in close collaboration with PlasmidFactory, Bielefeld, Germany (www.plasmidfactory.com). The biotech network links and bundles the local activities in the field of biotechnology and supports the transfer of research for industrial applications.

Day 1 – Technology and application

Dr. Peter Mayrofer (Mayrhofer & Jechlinger OEG, Wien, AU) and Dr. Marco Schmeer (PlasmidFactory, Bielefeld, DE) presented the progress on the PlasmidFactory minicircle system over the last 15 months overcoming the bottleneck of inducing the recombination of the parental plasmid into minicircle and miniplasmid at a defined time point and making sure that this does not happen earlier. In addition both presented the progress made in scaling the system from micro- to mg scales and presenting first transfection data with luc- and GFP- reporter genes in collaboration with various partners, one of them also participating in the workshop and later presenting some of these data (Dr. Wolfgang Walther, Charité, Berlin, DE). He observed a significant increase in the expression level of reporter genes in transfected cells in comparison with the corresponding plasmid-based vector.

The participants expressed their mourning about the surprising death of Dr. Wolfgang Jechlinger **(Picture 1)**, who passed away in December 2007 after preparing a publication on the system which will be published soon.

Dr. Eberhard Neumann (University of Bielefeld, DE) summarized the fundamentals of electro gene transfer and its influence on the membrane structure focussing on size effects of the transferred molecules.

Dr. Jürgen Bode (HZI, Braunschweig, DE) presented data on the application of S/MAR-elements within minicircle vectors and focussed on a short mutant of S/MAR (<800 bp) as well as a specific application for the production of

recombinant protein with the minicircle system – namely the production of antibodies.

Dr. Daniel Scherman (University of Paris, FR) presented the historic development of a triple-helix system for the specific purification of plasmid DNA which in between is in clinical application.

Dr. Christiane Juhls presented a linear minimalistic gene vector (MIDGE) and applications in clinical trials. The linear gene fragment is produced from a standard plasmid by restriction digestion and ligation of caps at both ends to prevent exonuclese based degradation.

A mini-plasmid was presented by Jürgen Mairhofer (University of Wien, AU), being depleted of all non-required sequences and based on the selection for an origin of replication (RNAI/RNAII titration).

Dr. Frank Jacobs (University of Leuven) presented data on a minicircle system based on Kay et al. containing an expression cassette with a special promoter-transgene-combination for hydrodynamic liver transfection in mice. Here, persistent levels of gene expression were observed. The data was controversially discussed between him and Dr. Bode.

The influence of two close promoters to each other was discussed by Dr. Manfred Gossen (MDC, Berlin, DE).

Day 2 – IP and patent situation

Dr. Martin Grund and Dr. Stacey Farmer (both GRUND IPG, München, DE) presented - on the example of patents for minicircle systems and related IP - aspects important in patent application and evaluation. A round table with all participants (60 participants, some of them on **Picture 2** in front of the town hall of Bielefeld) summarized together the workshop results.

Summary

The progress in the field of non-viral DNA vector development was clearly visible between the first and the second workshop. Both the minicircle technology and the plasmid technology were improved to obtain tools further optimizing non-viral gene transfer. Dr. Charles Coutelle lined out that the scope of this workshop may shift towards non-viral systems in general to cover all aspects of the field. Dr. Martin Schleef (scientific organizer of both workshops) requested to keep in mind that both – non-viral as viral systems – initially are based on work in plasmids and that e.g. for lentiviral or AAV vector production based on transient transfection the use of improved systems such as minicircle/miniplasmid may have a huge potential in safety.

All participants appreciated the venue and content and it was mutually agreed to have a third conference in the second half of 2009 – hopefully with more in vivo-data, aspects of DNA vaccination and an overview on other possible applications for this type of vector DNA.

Acknowledgement

The organizers thank all participants for their interest and contribution, those supporting the workshop with all different kinds of support as sponsors – mainly the Chamber of Commerce (Bielefeld) for inviting us free of charge to their conference center and to the European Community via FP6 CLINIGENE Network of Excellence for travel grants for students and members of the NoE to participate.

Picture 1: Dr. Wolfgang Jechlinger (1971 to 2007)

Picture 2: Some of the participants meeting in front of the Bielefeld town hall.