IDENTIFICATION OF TRANSGENE INTEGRATION SITES, THEIR STRUCTURE AND EPIGENETIC STATUS WITH CAS9-TARGETED NANOPORE SEQUENCING IN CHO CELLS

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The genetic plasticity of CHO cells allows efficient generation of recombinant cell lines by insertion of transgenes into the host genome. Next to random integration approaches, targeted integration of transgenes into pre-selected genomic locations is a compelling technology to generate stable producer cell lines. However, the potential of off-target transgene integration cannot be fully controlled and generated cell lines have to be screened for correct integration sites. In order to characterize transgene integrations, we have adopted a fast and accurate method to detect the entirety of transgene integration and their topology in CHO cells using Cas9 targeted nanopore sequencing (nCATS). nCATS allows targeted, directional sequencing of long DNA fragments (>>20 kbp) and simultaneously provides information about the DNA methylation status of the sequenced genomic region.

For nCATS applications, dephosphorylated high molecular weight genomic DNA is subjected to CRISPR/Cas9 mediated cleavage with gRNAs targeting genomic loci of interest. The generation of newly phosphorylated DNA ends at the induced double strand breaks allows site-specific, directional ligation of nanopore sequencing adapters and thus enriched sequencing of targeted sites. For proof of concept, we performed nCATS by targeting the Fut8 promoter in a CHO-K1 cell line after epigenetic modulation. Next, identification of transgene integration sites and their structure was analyzed in two recombinant CHO-K1 cell lines that were previously analyzed by TLA-sequencing.

Enriched sequencing of the endogenous Fut8 promoter resulted in high on-target coverage and correct identification of the targeted region. Additionally, the DNA methylation status could be accurately assessed in cell lines that were previously subjected to targeted DNA methylation. For the identification of transgene integration sites, reads spanning over exogenous and endogenous regions were used to precisely determine transgene integration sites. Interestingly, in addition to the integration sites reported by TLA sequencing, a third integration site was detected by nCATS. Furthermore, the conformation of the integrated sequence was disclosed identifying a plasmid concatemer of more than 21 kb in length, which could not be resolved previously. Importantly, nCATS is a fast (up to 48 hours until final results), versatile and affordable tool to determine integration sites in recombinant cell lines. Additionally, nanopore sequencing offers the advantage to simultaneously study epigenetic modifications (i.e. DNA methylation) of the targeted genomic sequence, which adds an additional layer of information.