

CHARACTERIZATION OF EXTRACHROMOSOMAL CIRCULAR DNA (ECCDNA) STRUCTURE, FUNCTION, AND DYNAMICS IN A CHO K1 CLONE

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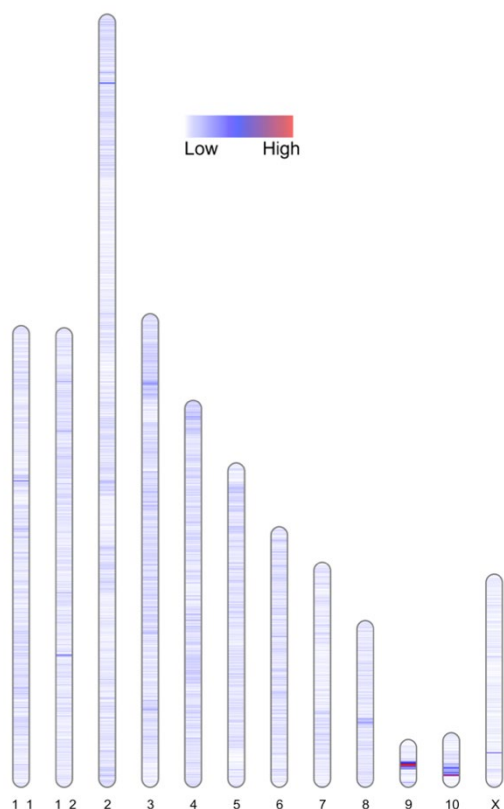


Figure 1: Map of eccDNA biogenesis locations observed in this study.

Despite the broad popularity of Chinese hamster ovary (CHO) cell lines in biomanufacturing, the intrinsic plasticity of the CHO genome causes unintended genomic rearrangements and variations that manifest as cell line instability. Undesired genomic variations can have a variety of consequences including reduced productivity, poor product quality, and/or early culture termination. Extrachromosomal circular DNA (eccDNA), focal amplifications of the genome, have been identified in the extranuclear space of many eukaryotic organisms and have been deemed a hallmark of genomic plasticity. EccDNA are believed to have multiple functions, however they are best characterized as a vehicle for gene overexpression. Diverse examples include eccDNA-mediated genetic resistance to glyphosate (Roundup) in the agricultural pest, pigweed¹ and oncogene amplification in human cancers². EccDNA were previously identified in CHO cells in 1984³, yet little is known about their structure and function within the cell. Understanding the dynamics of eccDNA in CHO cells is critical to improving predictability of cell lines and may lead to the identification of engineering targets that could enhance stability of future cell lines.

This work presents the robust characterization of sequence structure of eccDNA in a CHO K1 clone grown in control and lactate-stressed conditions in an ambr[®]250 bioreactor. Samples for eccDNA and RNA library preparation were collected from fed-batch cultures on Days 0 and 12. Sequences confirmed to be circular were annotated for repeat motifs, genes, and tRNA motifs. Repeat analysis showed that approximately 37% of eccDNA sequences contain some form of repetitive characteristic. Gene annotation identified 1,551 unique genes encoded on eccDNA and gene ontology (GO) analysis identified multiple

biological process terms related to transcription, translation, post-translational modification, and secretion. Approximately 9% of observed eccDNAs carry tRNA motifs. Sequences were mapped to the reference genome to identify biogenesis sites. While eccDNA biogenesis was found to be ubiquitous, some hot spots were identified on chromosomes 9 and 10 (Figure 1). Finally, transcriptome data was intersected with eccDNA data to identify eccDNA genes that may be functional.

1 Molin, W. T., Yaguchi, A., Blenner, M. & Saski, C. A. The EccDNA Replicon: A Heritable, Extranuclear Vehicle That Enables Gene Amplification and Glyphosate Resistance in *Amaranthus palmeri*. *Plant Cell* 32, 2132-2140, doi:10.1105/tpc.20.00099 (2020).

2 Kim, H. et al. Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. *Nat Genet*, doi:10.1038/s41588-020-0678-2 (2020).

3 Stanfield, S. W. & Helinski, D. R. Cloning and characterization of small circular DNA from Chinese hamster ovary cells. *Mol Cell Biol* 4, 173-180, doi:10.1128/mcb.4.1.173 (1984).