Transposon-based innovative method for sequencing highly repetitive heterochromatic DNA in BAC/oriV clones. M. Mendez Lago[1, 2], J. Wild[1], J.P. Abad[2], A. Martin-Gallardo[3], A. Villasante[2], W. Szybalski[1]. 1) McArdle Lab., University of Wisconsin, Madison, WI; 2) Centro Biología Molecular (CBMSO, CSIC-UAM), Madrid, Spain; 3) Servicio Interdepartamental de Investigación (SIdI) UAM, Madrid, Spain.

The contiguous finished sequence from rigorously assembled contigs is needed to understand the role of heterochromatin regions in chromosome behavior. However, when using the current aligning approaches, based on sequence overlaps, it has been hardly possible to align the newly acquired repetitive heterochromatic sequences, because they are often nearly identical. Moreover, some present methods are laborious and not open to the extensive mapping technologies currently employed by sequencing centers. Here we present a novel transposon (Tn)-based strategy for sequencing heterochromatic BACs that contain *oriV*, which permits DNA amplification in *trfA* hosts.

First, we modified Tn*5*/oriV by adding rare restriction sites, I-SceI and PI-SceI. Using this Tn, we constructed a library of BAC/oriV clones with two PI-SceI sites. One site was at a fixed position in the backbone of the BAC plasmid, while the other was in Tn, randomly inserted into the cloned heterochromatic DNA. In the next step, sequences (500-1000 nt) are to be collected, from the outward priming sites in Tn, from several hundreds of such clones. Finally, all Tns were precisely mapped using PFGE and appropriate hybridization probes from the BAC vector and transposon. Combined results of sequencing and determination of Tns precise positions and orientation will permit us to assemble the entire sequence of the heterochromatic clone, since the principle of our approach is the assembly of all the newly acquired sequences according to their physical positions instead of aligning by the conventional search for overlaps. This strategy, we hope, especially when automated by optical mapping with labeled probes, will become crucial for heterochromatin sequencing.