BRC Science Highlight
February 2022

CUT&RUN Identifies Centromeric DNA Regions of *Rhodosporidium toruloides* IFO0880

Background/objective

Rhodosporidium toruloides — also known as *Rhodotorula toruloides* — has been increasingly explored as a host for bioproduction of lipids, fatty acid derivatives and terpenoids. Various genetic tools have been developed, but neither a centromere nor an autonomously replicating sequence (ARS), both necessary elements for stable episomal plasmid maintenance, has yet been reported. Here, a method for genome-wide mapping of DNA–protein interactions, **C**leavage **U**nder **T**argets and **R**elease **U**sing **N**uclease (CUT&RUN), was used to identify *R. toruloides* IFO0880 genomic regions associated with the centromeric histone H3 protein Cse4, a marker of centromeric DNA.

Approach

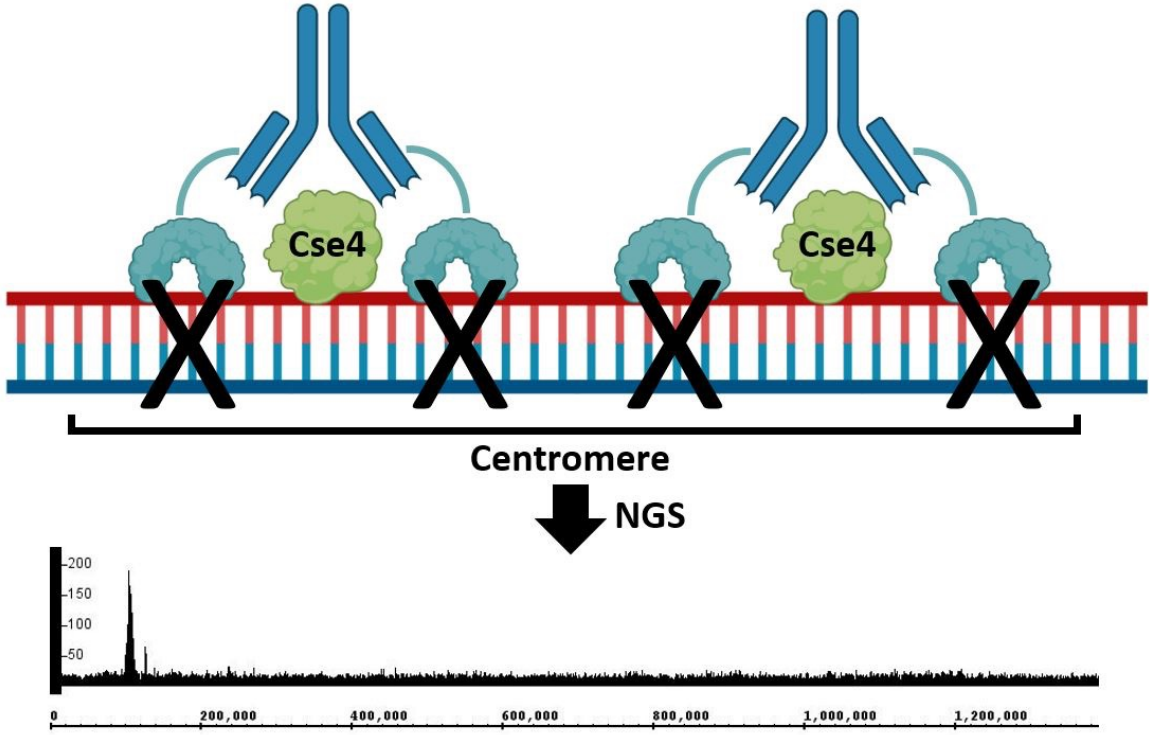
- *R. toruloides* centromeric histone Cse4 was fused with human influenza hemagglutinin (HA) epitope tag.
- CUT&RUN was used to isolate and sequence genomic DNA regions bound by Cse4.

Results

- 15 centromeres ranging from 8 to 19 kb in length were identified and characterized based on structure, sequence similarity, and transcriptional silencing.
- 4 centromeres were evaluated for, but did not show, ARS activity. These sequences displayed some characteristics of centromere sequences such as below average GC content but did not show significant sequence conservation.

Significance

- Identification of the *R. toruloides* centromeres will allow stabilization of future episomal plasmids developed for this yeast by improving the partitioning of the plasmid population between daughter cells during cell division.
- Future efforts to identify an ARS can use these centrometric DNA sequences.



Cleavage of centromeric regions creates CUT&RUN peak corresponding to centromere location on each chromosome.

Schultz, C.J., Cao, M., Mejia, A., Zhao, H. Dec. 13, 2021. "CUT&RUN Identifies Centromeric DNA Regions of *Rhodosporidium toruloides* IFO0880." *FEMS Yeast Research* 8(21). DOI: 10.1093/femsyr/foab066.

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