Targeted DNA sequencing of biochemically enriched regions of a yeast genome

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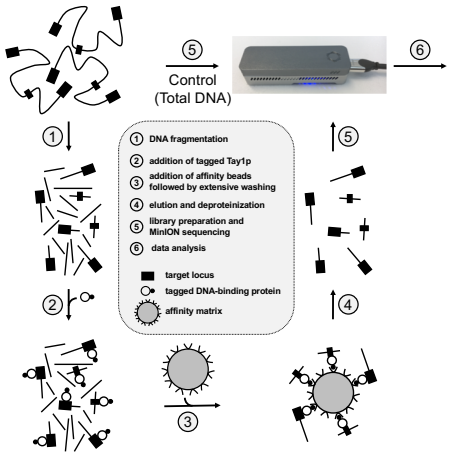
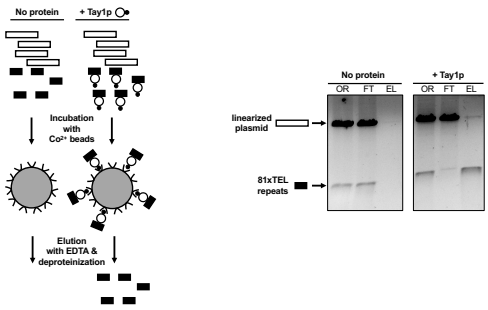
DNA-binding proteins are key factors involved in the maintenance of genome integrity and gene expression. To identify loci that are recognized by a Myb domain containing DNA-binding protein Tay1 in the genomic DNA of the yeast *Yarrowia lipolytica* [1, 2], we combined a pull-down assay and DNA sequencing on a MinION device. The sequencing library has been prepared using 1D ligation chemistry (SQK-LSK108) and sequenced in a FLO-MIN106 (R9.4.1) flow cell. Resulting sequence data were mapped to the reference genome. The comparison with the data obtained by sequencing of a control DNA sample revealed a number of genomic DNA fragments including the telomeric sequences that were biochemically enriched in the pull-down experiment. This approach can be useful in genome-wide identification of loci potentially recognized by DNA-binding proteins of interest as well characterization of sequences representing their putative binding sites.

Experimental design:



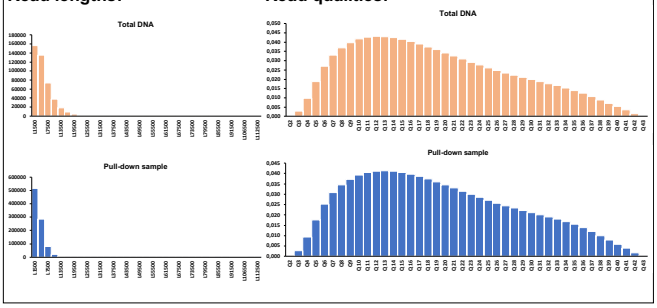
Pull-down of DNA containing telomeric tract of *Y. lipolytica* using Tay1p tagged with 6HN epitope:



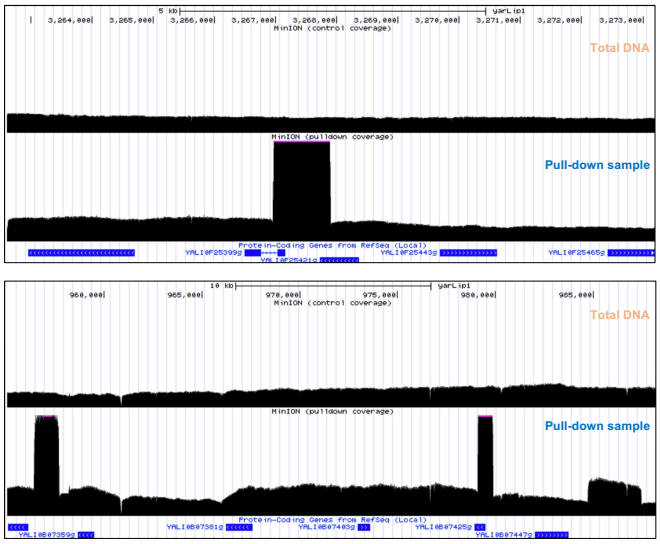
Left panel: Scheme of the experiment. Plasmid pUC19 carrying 81 telomeric repeats of *Y. lipolytica* was digested to produce equimolar amounts of linearized plasmid backbone and fragments with telomeric tracts. 160 nM of Tay1-6HN was mixed with 2 nM DNA resulting in a ratio of 1 molecule of Tay1p per 5-7 telomeric binding sites. After allowing binding of Tay1p to its target DNA, Co^{2+}-beads were added to a sample, incubated at room temperature for 30 min and the bound DNA was eluted by a combined action of EDTA and proteinase K. As a control, no protein was added to DNA.
Right panel: Representative experimental result. In control sample (no protein added) there is the same amount of plasmid DNA and telomeric fragments in original (OR) and flow-through fractions and there is no DNA in the elution (EL). In the sample containing Tay1p, the majority of telomeric fragments is bound to the beads and then successfully eluted with a small amount of contaminating plasmid DNA.

	Raw data [Mbp]	Read lengths	Mean	Median
Total DNA	**2925**	**642717**	**4551.5**	**3331**
Pull-down sample	**3790**	**1326128**	**2858.6**	**2135**

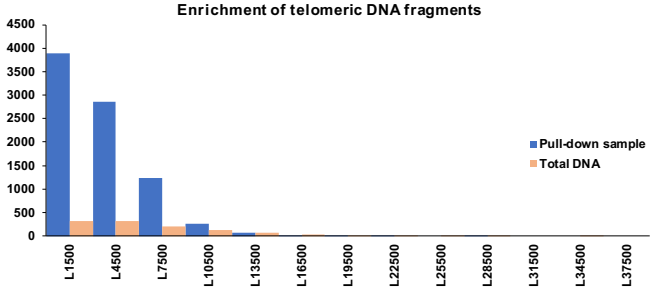
Read lengths: **Read qualities:**



Examples of biochemically enriched genomic regions:



Enrichment of telomeric DNA fragments



References:

1. Kramara, J., Willcox, S., Gunisova, S., Kinsky, S., Nosek, J., Griffith, J.D., Tomaska, L. (2010) Tay1 protein: A novel telomere-binding factor from *Yarrowia lipolytica*. *J. Biol. Chem.* 285: 38078-38092.
2. Visacka, K., Hofr, C., Willcox, S., Necasova, I., Pavlouskova, J., Sepsiova, R., Wimmerova, M., Simonicova, L., Nosek, J., Fajkus, J., Griffith, J.D., Tomaska, L. (2012) Synergism of the two Myb domains of Tay1 protein results in high-affinity binding to telomeres. *J. Biol. Chem.* 287: 32206-32215.

Acknowledgements This work was supported by the Slovak Research and Development Agency Grants APVV 15-0022 (to L.T.) and 14-0253 (to J.N.), the Slovak Grant Agency Grants VEGA 1/0052/16 (to L.T.), 1/0333/15 (to J.N.), 1/0684/16 (to B.B.) and 1/0458/18 (to T.V.), and the Comenius University Grants UK/268/2018 (V.H.) and UK/282/2018 (to H.L.).