**Boo**t**s**ing expression in a **t**ai**l**-made way by **n**ew promoters and terminators for *S. cerevisiae*

**Julia Pitzer**<sup>a,b</sup>, Raphaëla Wimmer<sup>a</sup>, Anton Glieder<sup>a</sup>

<sup>a</sup>Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14, 8010 Graz, Austria
<sup>b</sup>Austrian Centre of Industrial Biotechnology (ACIB GmbH), Petersgasse 14, 8010 Graz, Austria

---

**Introduction**

Efficient and well-regulated promoters and terminators are essential for the successful production of recombinant proteins and for the implementation of enzymatic pathways. In this study, new regulatory DNA elements were designed for *Saccharomyces cerevisiae* to expand the toolbox of available parts for fine-tuned transcriptional regulation.

For the construction of a random-mutation walking library a 10 bp random DNA sequence was inserted at consecutive positions in the upstream activating sequence of the bidirectional HHX1 histone promoter. The effects were quantified by fluorescence measurements (Figure 1).

---

**Create inducibility**

From this library, knowledge about neutral regions, without expression regulating effects, was obtained and utilized for the introduction of a GAL4 binding site at different positions to equip the originally constitutive histone promoter with an galactose inducible element. In this way, promoter variants with similar activity on glucose and strongly increased activity on galactose were created (Figure 2).

---

**Random-mutation walking library**

Figure 1. Screening results of the bidirectional HHX1 histone promoter random-mutation walking library in *S. cerevisiae* after 15 h growth on YPD by measuring the fluorescence of the reporter proteins eGFP (green) and Tomato (red). The fluorescence was normalized to the OD of the cell culture at 600 nm. Mean values and standard deviations of biological triplicates of three representative clones are shown.

---

**Terminators**

Heterologous terminators from three different yeasts were tested in *S. cerevisiae*, where they showed expression enhancing effects compared to standard native terminator sequences.

---

**Conclusion**

- Sequence diversification of a bidirectional histone promoter allowed the generation of a promoter library which covers a wide activity range.
- Introduction of a GAL4 binding site transformed the originally constitutive histone promoter into an galactose inducible promoter variant with similar activity on glucose and strongly increased activity on galactose.
- Heterologous terminators with expression enhancing effects were discovered.

---

**Acknowledgements**

The research leading to these results has received funding from the Innovative Medicines Initiative Joint Undertaking under grant agreement no 115360, resources of which are composed of financial contribution from the European Union’s Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contribution. [www.imi.europa.eu](http://www.imi.europa.eu)

E-mail: julia.pitzer@acib.at

This work has been supported by the Austrian BMWFW, BMVIT, SFG, Standortagentur Tirol and ZIT through the Austrian FFG-COMET Funding Program.