Expansion microscopy on *Saccharomyces cerevisiae*

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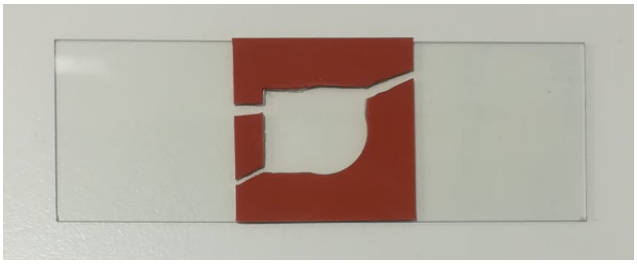
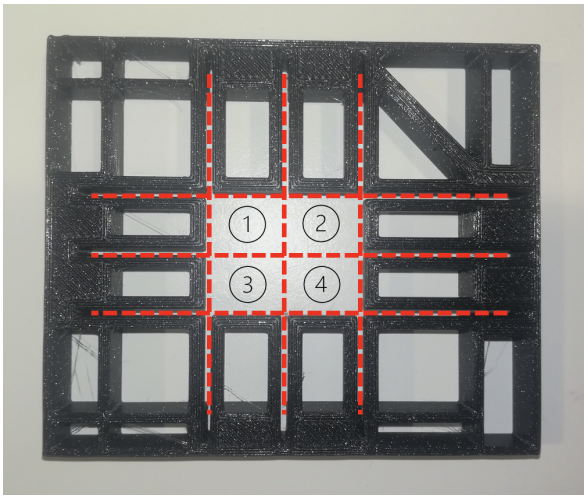
Supplementary Files



Supplementary Figure 1. Photograph of a gelation chamber slide. The orange silicon surface (CoverWell™ Modular Hybridization System, Depth 0.6mm, E70334-A, Science services) was custom cut and sticked on a regular microscope glass slide. The gelation solution is added within the area delimited by the silicon surface, and the sample on the coverslip is put on top. After gelation is completed, the gel is removed. The gel will have the shape of the delimited area - a rectangle with one rounded corner, making it is possible to quickly identify the side of the gel that contains the sample.



Supplementary Figure 2. Photograph of a custom cutter shape. The shape is designed to easily obtain samples with a consistent size that fit an Attofluor™ Cell Chamber. Its dimensions correspond to a sample that was gelated on the gelation chamber slide (Supplementary Figure 1) and expanded four times. It is put on top of the gel, and cuts in the gel are made by passing a blade along the red dashed lines to get the four central pieces (numbered). The shape was 3D printed in polylactic acid (PLA). The .stl file is available at https://github.com/HenriquesLab/YeastExM.

Supplementary Table 1. Troubleshooting table.

Problem	Possible cause	Possible solution
The gelation solution was polymerised or partially gelled before the coverslip was placed on top of it	The polymerisation reaction proceeded too fast	The polymerisation is quickly initialised after TEMED addition, so immediate contact with the sample is crucial. It is important to ensure that the reagents and the gelation chamber are placed on ice and remain sufficiently cold.
	The monomer solution is too old	Make sure that the monomer solution is not older than 3 months. If so, prepare a fresh solution.
The NHS-Ester and the DAPI solutions are not homogenous	NHS-Ester and DAPI solutions are not completely thawed	The NHS-Ester and DAPI staining are crucial to achieving efficient staining. Make sure that the solutions remain at RT for a long time until completely thaw before use.
Distorted cell structure	The distorted cell structures can result from insufficient fixation step of the cells on the coverslip	Use a fresh batch of the fixation solution.
Cell structures appear to be discontinuous	This can be attributed to a problem in the denaturation step	Confirm that the denaturation buffer is completely homogenised before use. Make sure that the solution looks transparent and not white. If needed, warm the solution and then vortex until it's completely transparent.
The fluorescence signal is weak	This problem may be due to poor labelling of the proteins of interest during staining	Labelling overnight at 4 °C or increase the concentration of fluorescent marker.