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In situ hybridisation on yeast with Locked Nucleic Acid *in situ* detection probes

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The protocol originates from the Singer laboratory (Long et al. (1997) Science 277, 383-387) and has been modified for yeast, *Saccharomyces cerevisiae*, cell cultures subjected to a heat-shock treatment. However, the outlined routines for fixation etc. should be applicable for yeast cell cultures in general.
Ref.: The protocol is modified from Thomsen et al. (2005) RNA, 11:1745-1748.

Fixation of yeast cells for RNA-FISH

- Cell cultures (10 mL) were grown at 25ºC to an OD600 of 0.1-0.3 and subjected to an instantaneous temperature-shift by addition of pre-heated media
- After incubation at the desired temperature and time, e.g. 31ºC for 90 min, cells were fixed for 15 min at the experimental temperature by adding formaldehyde to a final concentration of 4%. This was followed by 30 min incubation at 20ºC.
- Fixed cells were pelleted by centrifugation and washed three times in 1 ml wash buffer (1.2M sorbitol, 0.1M KH_2PO_4/K_2HPO4, pH 6.5), and subsequently resuspended in 200-500 µL wash buffer.

Mounting on glass slides

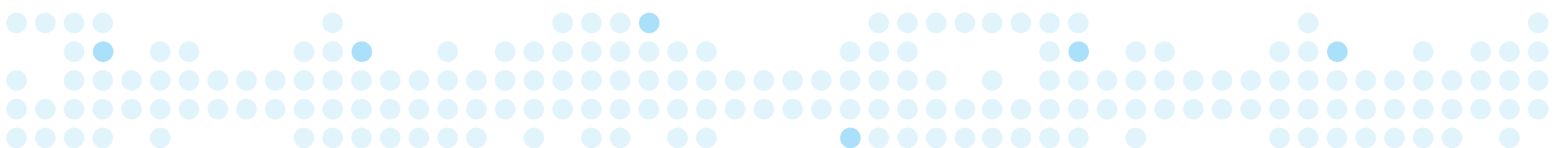
- 10µl of the individual cell suspensions were plated on 14-well, 0.1% poly-L-lysine coated glass slides (Immuno-Cell Int.), washed in wash buffer containing 1% ß-merchaptoethanol before spheroplasting for 10-15 min at 30ºC in 10 µL oxalyticase solution (20mM vanadyl-ribonucleoside, 0.2% ß-merchaptoethanol, 0.1 U/µL RNasin, 1.2M sorbitol, 0.1M KH_2PO_4/K_2HPO_4, pH 6.5, 0.1 mg/mL oxalyticase (Enzogenetics)).
- Subsequently, cells were washed for 5 min at 20ºC; twice in wash buffer, once in (0.1M KH_2PO_4/K_2HPO_4, pH 6.5, 0.1% NP40) and once in (0.1M KH_2PO_4/K_2HPO_4 pH 6.5), and finally incubated with cold 70% ethanol for 15-30 min at -20ºC.

Probe preparation

- Probe preparation was done by direct labelling of 10-20µg of 5´-end amino modified Locked Nucleic Acid in situ hybridization probe with 300µg of monofunctional Cy3 NHS-Ester (Amersham Pharmacia) in 0.1M $NaHCO_3$/Na_2CO_3, pH 9.0 overnight at 25ºC in the dark. The probe was purified on a G-50 spin column and the concentration determined by OD measurement.
- For each well, 100ng of purified probe was mixed with 10µg salmon sperm DNA and 10µg yeast tRNA, lyophilized and resuspended in 5µL solution I (80% formamide, 10mM $NaHPO_4$ pH 7.0), denatured for 5 min at 95ºC and finally mixed with 5µL of solution II (4xSSC, 20mM Vanadyl-ribonucleoside, 4µg/µL BSA, 0.1U/µl RNasin).

RNA-FISH

- Using a pipette, cells were drained for ethanol and washed in 10-20 µL (depending on well size) at 20ºC, twice for 5 min in 2xSSC and once for 10 min in 40% formamide/2xSSC, 0.1% Triton X-100, before overnight incubation at 37ºC with 10 µL of probe mix.
- Removal of probe solution was followed by washing steps in 10-20 µL volume: (i) twice in 40% formamide/2xSSC for 10 min at 37ºC, (ii) once in 2xSSC/0.1% Triton X-100 for 10 min at 20ºC, (iii) twice in 1xSSC for 10 min at 20ºC, and (iv) twice in 1xPBS for 5 min at 20ºC.
- Finally, 2.5µl of mounting solution (1xPBS pH 8.0, 80% glycerol, 0.1µg/mL DAPI) was applied to air-dried wells, which were subsequently covered with a cover slip and analysed by fluorescent microscopy.



NB: Optimisation of the standard conditions by increasing hybridization stringency conditions can be achieved by increasing the formamide concentration (normally 40%) in hybridization- and all appropriate wash-buffers to 50%, 60% and 70%, respectively.

Imaging

Images were acquired on an Olympus BX51 microscope equipped with cooled Olympus DP50 CCD camera and analysis software.

Buffers

*Please note: For optimal fixation it may be critical to use fresh formaldehyde solutions. Fresh 4% solutions can be made from 16%, methanol free, formaldehyde or from solid paraformaldehyde (4% w/v).

For preparation of buffers please refer to :
Molecular cloning : a laboratory manual / Sambrook, Joseph; Russell, David W. --
3rd ed. -- New York: Cold Spring Harbor Laboratory, 2001.

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