Fluorescence *in situ* hybridization for detection of poly(A) RNA

Material:

- 1x PBS
- 4% Paraformaldehyde (PFA) in 1x PBS
- 0.5% TritonX-100 in 1x PBS
- -20°C MeOH
- 70% Ethanol
- 2x SSC
- NaCl
- Na_3 citrate (dihydrate)
- HCl
- yeast RNA
- Formamide
- BSA
- dextrane sulfate
- Alexa555-oligo-dT50 (100 pmol/µl; Invitrogen)
- Hoechst dye (#33422, 10mg/ml)
- polyvinyl alcohol (vinol)

Solutions:

- Hybridization buffer:
  2.2 mg yeast RNA (final conc. 0.1mg/ml)
  4.4 ml 2x SSC
  4.4 ml Formamide (final conc. 20%)
  44 mg BSA
  2.2 g dextrane sulfate
  -> fill up with water to 22 ml
  heat at 65°C until BSA is dissolved, cool down and filter sterilize
  store at -20°C

- 20x SSC:
  175.3 g NaCl
  88.2 g Na_3 citrate (dihydrate)
  add H_2O, dissolve by stirring, adjust pH to 7.0 with a few drops of concentrated HCl.
  adjust the volume to 1000 ml, autoclave

- Vinol mounting medium:
  5 g polyvinyl alcohol (Sigma P8136)
  30 ml 0.1 M Tris pH 8.0

Georg Stoecklin
Sarah Hofmann A200 g.stoecklin@dkfz.de

10 ml Glycerol
1 ml 10% sodium azide
rotate for several hours at 37°C, then centrifuge at 4.000 rpm for 30 minutes to remove undissolved matierial.
aliquot and freeze at -20°C.

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Sarah Hofmann A200 g.stoecklin@dkfz.de

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Protocol:

Fixing the cells:

- Cells are grown on glass coverslips in 24-well plates
- remove the old medium, rinse in 1x PBS
- fix with 500 µl 4% PFA/1x PBS for 10 min
- add 500 µl 0.5% TritonX-100/1x PBS for 10 min or -20°C MeOH (for mammalian cells)
- to store the cells: add approx. 700µl of 70% EtOH and keep at 4°C
- otherwise: continue with Immunofluorescence or in situ hybridization

In situ hybridization:

- remove EtOH or 0.5% TritonX-100/PBS
- wash 3x with 2x SSC for 10 min at RT
- pre-hybridize coverslips with hybridization buffer for approx. 1h
- hybridize with Alexa555-oligo-dT50 (1:2.000 until up to 1:5.000 in hybridization buffer; protect from light with aluminium foil), 250 µl per coverslips, shaking 1h at RT
- wash 2x with 2x SSC for 5 min at RT on shaker
- for counterstaining of nuclei, incubate with Hoechst dye (1:10 000 in hybridization buffer) for 30 min at RT
- wash 3x with 2x SSC for 5 min at RT
- mount the coverslips onto microscope slides with Vinol mounting medium

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