In situ hybridization of chick and mouse embryonic tissue

Shan Sockanathan (ssockan1@jhmi.edu)
Sockanathan Lab

Method Article

Keywords: embryo, sections, mRNA

Posted Date: May 18th, 2015

DOI: https://doi.org/10.1038/protex.2015.040

Abstract

The detection of transcripts in sectioned tissue by in situ hybridization is a useful method to localize sites of gene expression during development. This protocol is one that we have modified from Scharen-Wiemers and Gerfin-Moser Histochemistry 100:431-440.

Introduction

This protocol describes a step by step method to detect mRNA transcripts on tissue sections cut using a cryostat.

Reagents

Dig Probe In vitro Transcription H2O = 13 µl 10x transcription buffer = 2 µl dig-NTP =2 µl \(1mg) DNA =1 µl polymerase =1. 5 µl RNasin =0.5 µl 2 hr 37 C add 30 µl DEPC H20 check 2.5 µl on 1x TAE minigel \(Ethidium RNA staining stronger than vector band) spin column add hyb mix to a total volume of 200 µl typically 10-20 µl probe added to 1 ml Hyb mix **0.2M Phosphate Buffer \(P.B) \(4L)** 165.3 gr Na2HPO4 7H2O \(MW 268.07) 25.6 gr NaH2PO4 H20 \(MW137.99) water to 4000 ml Note: Made with RNASE free reagents **4% paraformaldehyde \(made fresh) \(400ml)** 200 ml water 16 gr paraformaldehyde heat to 60-70 C, add 1 drop 10 N NaOH, and stir 10 minutes to dissolve add 200 ml 0.2M P.B. \(final conc. 0.1M) add 3 gr NaCl sterile filter and store on ice **PBS \(4L)** \(0.1 M PB and 0.15 M NaCl) Made with RNASE free reagents 4L 2L 0.2 M PB 35 gr NaCl H2O to 4L **Proteinase K buffer \(400ml)** \(1mg/ml Prot K, 5 mM EDTA, 50 mM Tris) 400ml 5 ml 0.5 M EDTA pH8.0 20 ml 1M Tris pH7.5 20 ml 20mg/ml Prot K H2O to 400 ml **Hybridization Solution 1L** 50% formamide 500 ml of 100% 5xSSC 250 ml of 20x 5x Denharts 100 ml of 50x 250mg/ml bakers yeast RNA \(Sigma R6750) 0.25 gr 500 mg/ml herring sperm DNA 0.5 gr 150 ml H2O **B1** \(0.1 M Tris pH 7.5, 0.15 M NaCl) 1L 100 ml 1 M Tris 30 ml 5M NaCl 870 ml H2O **B2** \(B1 + 1% heat inactivated goat or sheep serum) **B3 \(0.45mm filtered)** 0.1 M Tris pH 9.5 0.1 M NaCl 50mM MgCl2 **B4** 4.5 µl/ml NBT \(75 mg/ml) \(NBT from BMB no. 1383213, in 70% dimethylformamide). 3.5 µl/ml BCIP \(50 mg/ml) \(BCIP from BMB no. 1383221 in 100% dimethylformamide). 0.24 mg/ml levamisole diluted in buffer B3

Equipment

standard equipment for molecular biology

Procedure

DIG-label In Situ Hybridizations **Adapted from Scharen-Wiemers and Gerfin-Moser Histochemistry 100:431-440.** **I. Tissue section:** 1. Fix embryo overnight at 4 C in 4% paraformaldehyde, 0.1 M PB. 2. Transfer embryo to 30% sucrose in 0.1M PB for 4 hr at 4 C, addition of some fixative probably reduces risk of RNase activity. 3. Mount in tissue tek \(can store at -70 C several weeks if necessary). 4. Cut

12.5µm sections \(thicker sections may increase signal). 5. Section with fisherbrand superfrost plus slides \(No 12-550-15). 6. After sectioning air dry 20 minutes \(maximum is 3 hr). **II. Tissue preparation** **NOTE Eliminase treat all glassware before starting** 1. Fix in freshly made 4 % paraformaldehyde/PBS for 10 minutes at RT. 2. Wash three times with PBS for 3 minutes each. 3. Digest in freshly diluted proteinase K \(1mg/ml in 50 mM Tris 7.5, 5 mM EDTA) 5 minutes at RT. \(Note: these conditions will have to be adjusted for various embryo stages.) 4. Fix in 4 % paraformaldehyde/PBS for 5 minutes at RT. 5. Wash three times with PBS for 3 minutes each. 6. Acetylation : 197 ml H2O, 2.4 ml triethanolamine \(Fluka 90279); mix well. Add 0.52 ml acetic anhydride, and mix by dipping slides several times. Acetylate 10 minutes room temp. Start preparing mix when slides are in last wash. 7. Permeabilize with 1% TritonX100 in PBS for 15 minutes at room temp. 8. Wash 3 times with PBS for 5 minutes each **III. Hybridization** 1. Place 500 µl hybridization buffer on slide. 2. Incubate at room temperature for 2 hr \(overnight also works well) at room temp in a 50% formamide/5xSSC humidified chamber horizontal without coverslips. 3. Replace pre-hyb \(pour off, dab off edge with paper towel to remove excess) with 75 µl hybridization solution containing probe at 200-400 ng/ml DIG RNA which was heated to 80 C for 5 min. and iced. 4. Coverslip slides, place in humidified \(5xSSC, 50% Formamide) chamber overnight at 70˚C. Note: It is best to separate slides with different probes since some contamination is possible from neighboring slides in the chamber. **IV Washes/Immunological staining** 1. Place slides in rack, submerge in 70˚C 5xSSC to remove coverslips. Carefully remove slides with forceps. 2. With forceps, transfer slides into 0.2XSSC at 70˚C for 1-3 hr. 3. Transfer to 0.2xSSC at RT for 5 min. 4. Transfer to buffer B1 5 minutes at RT. 5. Place 1-2 ml B1 with 1% heat inactivated goat \(or sheep) serum \(HINGS) on horizontal slides for 1 hr at RT. 6. Put 0.5 ml anti-DIG Ab \(1:2000 dilution in B1 + 1 % HINGS) on each slide \(=B2) 7. Place humidified chamber at 4˚C overnight \(for abundant RNA 1 hr RT okay, but overnight greatly enhances signal and reduces color reaction time). 8. Rinse with B1 once, 3x20 minutes wash \(more extensive washes may reduce background if this is a problem). 9. Equilibrate with B3. 10. Place 200 µl B4 on parafilm, and invert slide onto solution. 11. Incubate at RT 6 hr-3 days in humidified chamber in dark. 12. Stop reaction with PBS \(can be kept for up to several days at 4˚C). **V. Mounting** 1. Rinse slide with water. 2. Air dry completely 3. coverslip with Glycerol \(warm to 60 ˚C, 3 drops on slide, add coverslip)

Timing

3-4 days

References

1.Scharen-Wiemers, N. and Gerfin-Moser, A. A single protocol to detect transcripts of various types and expression levels in neural tissue and cultured cells: in situ hybridization using digoxigenin-labelled cRNA probes. Histochemistry 100:431-440 \(1993)