Northern blot analysis for detection and quantification of RNA in pancreatic cancer cells and tissues

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Investigation of gene expression significantly contributes to our knowledge of the regulation and function of genes in many areas of biology. In this protocol, we describe how northern blot analysis is used to identify gene expression patterns at the RNA level in human cancer cells as well as in cancerous and normal tissues. RNA molecules are separated by gel electrophoresis and are subsequently transferred to a porous membrane by capillary action. Specific sequences in the RNA are detected on the membrane by molecular hybridization with radiolabeled nucleic acid probes. Despite the development of newer methods, such as real-time PCR, nuclease protection assays and microarrays, northern blot analysis is still a standard technique used in the detection and quantification of mRNA levels because it allows a direct comparison of the mRNA abundance between samples on a single membrane. This entire northern blotting protocol takes ~4 d to complete.

INTRODUCTION

Understanding the expression patterns of genes is essential for understanding and elucidating their functions. Northern blotting, also known as northern hybridization, is a technique used for detection and quantification of specific RNA levels. This technique was originally developed by James Alwine and George Stark, who named it on the basis of its analogy to Southern blotting[1]. This protocol involves several steps. First, RNA is isolated using, e.g., the acid guanidinium thiocyanate-phenol-chloroform extraction method described in 1986 by Chomczynski and Sacchi[2], followed by gel electrophoresis for separation. Formaldehyde is present both in the gel and in the migration buffer, ensuring the inhibition of ribonuclease activity. Next, the negatively charged RNA is transferred onto a nylon membrane driven by capillary forces. The procedure usually runs overnight. The membrane is exposed to intense UV light to induce RNA cross-linking. Subsequently, the RNA fixed in the membrane is hybridized with the labeled probe, forming a double-stranded RNA–DNA or RNA–RNA structure. Hybridization is usually carried out overnight and detected by autoradiography or with the use of a phosphoimager apparatus. A flowchart showing the individual steps in northern blotting and the time needed to complete them is presented in **Figure 1**.

The identification of altered mRNA levels in cell lines and tissues leading to overexpression of oncogenes or downregulation of tumor-suppressor genes reveals one possible initial event that, in addition to mutations, sets the cells along the tumorigenic pathway[3,4]. Therefore, the identification of genes differentially expressed in comparison with normal 'healthy' tissue helps to clarify gene functions. When expression analysis is studied, microarray analysis[5] is one of the most popular methods initially used to provide an overview of deregulated genes. Subsequently, the expression pattern of potentially interesting genes has to be confirmed by other methods, such as RT-PCR[6] or northern blot. RT-PCR has become increasingly popular because it is rapid, simple and sensitive. Nevertheless, northern blot has remained a standard method because it allows for the comparison of message abundance on a single membrane and can be used in determining transcript size and in detecting alternative spliced products. Northern hybridization is extremely versatile, in that radiolabeled or nonisotopically labeled DNA, RNA or oligonucleotides can be used. Furthermore, sequences with only partial homology may be used as a probe.

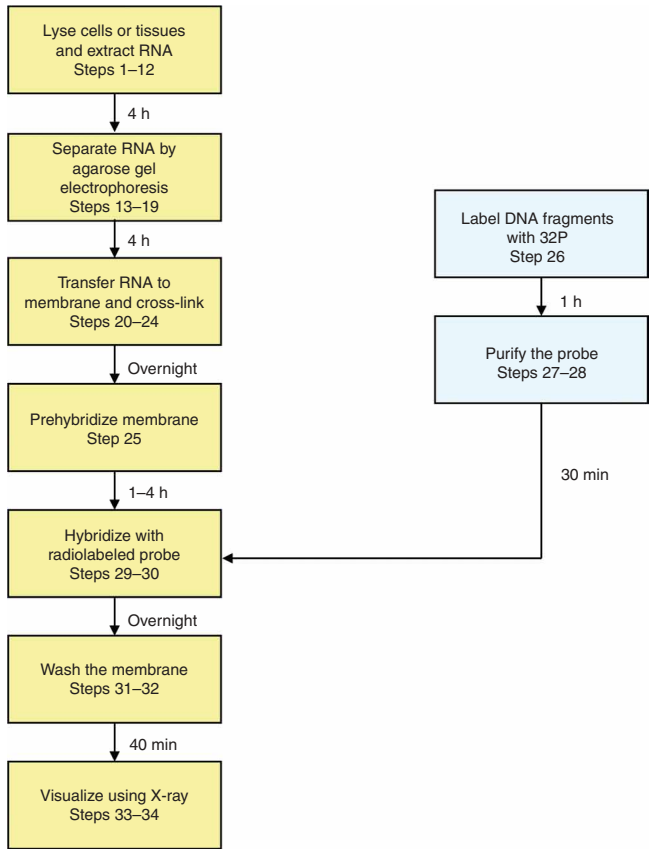

Figure 1 | Summary of steps involved in northern blotting and the approximate time needed.

TABLE 1 | Advantages and disadvantages of northern blotting.

Disadvantages
Risk of mRNA degradation during electrophoresis: quality and quantification of expression are negatively affected.
High doses of radioactivity and formaldehyde are a risk for workers and the environment.
The sensitivity of northern blotting is relatively low in comparison with that of RT-PCR.
Detection with multiple probes is difficult.
Use of ethidium bromide, DEPC and UV light needs special training and attention.
Advantages
The strength of this method is its simplicity.
Specificity is relatively high.
Sequences with even partial homology can be used as hybridization probes.
mRNA transcript size can be detected.
RNA splicing is visible because alternatively spliced transcripts can be detected.
The cost of running many gels is low once the equipment is set up.
Blots can be stored for several years and reprobed if necessary.
Quantity and quality of RNA can be easily verified after electrophoresis and before further processing is done.

The major limitations of northern blot analysis are RNA degradation and low sensitivity. If RNA samples are even slightly degraded, the quality of quantification and our ability to perform it are severely compromised. Improved sensitivity can be achieved by using highly specific antisense RNA probes or positively charged nylon membranes. Further, enrichment of mRNA by selection of oligo dT RNA will increase the sensitivity. A third limitation is the need to use multiple probes. To use more than one probe, it is usually necessary to strip the initial probe before rehybridizing with another probe. The advantages and disadvantages of northern blot analysis are summarized in **Table 1**. However, northern blot analysis is widely used in molecular biology as a gold standard for the direct study of gene expression at the level of mRNA and to detect transcript sizes. Further applications involve studies of RNA degradation or RNA splicing as well as RNA half-life. Northern blotting is also frequently used to check or confirm genetic manipulations in transgenic or knockout mice.

Microarray analysis is now commonly used as an initial step by our group and others[7–9] to identify deregulated gene expression patterns in pancreatic cancer samples and to compare these patterns with those of tissues derived from the normal pancreas and chronic pancreatitis. For a long time, the analysis of RNA levels in pancreatic tissues was hampered by the high RNAse content in these tissues. However, in the 1990s, using specific protocols for tissue handling and RNA extraction, these problems were finally solved[10–12]. Here we describe in detail the protocol for northern blotting as used by our group for mRNA profiling in pancreatic cancer[7,8,10–12].

Experimental design

Sample type and preparation. There are no limitations in the choice of cell lines. For continuous cell line propagation and subculturing conditions, one should follow instructions from the provider or from the American Type Culture Collection. Tissues can also be used. The protocol is similar, except that the tissue should be frozen in liquid nitrogen immediately upon surgical resection to avoid degradation and autolysis.

Optimization of RNA extraction. For the isolation of intact RNA strong denaturants, such as guanidinium thiocyanate, are necessary to disrupt cells, solubilize their components and denature RNAses simultaneously. In the RNA extraction method proposed by Ramadori *et al.*[13] and Chirgwin *et al.*[14], 4 M guanidinium thiocyanate is used to homogenize cells or tissues and the lysate is subsequently layered onto a dense cushion of CsCl. Owing to the density, rRNAs and mRNAs migrate to the bottom of the tube during ultracentrifugation, proteins remain in the guanidinium lysate, whereas DNA floats on the CsCl cushion. As it is difficult to process many samples simultaneously, the single-step technique of Chomczynski and Sacchi[2], in which the guanidinium thiocyanate homogenate is extracted with phenol:chloroform at reduced pH, is often preferred. Today, commercially available column-based systems are common, which use an ion-exchange RNA isolation method, on the basis of the differential binding capacity of the column. The major disadvantage of these systems is that an RNA column typically cannot absorb RNA transcripts shorter than 200 bp.

Membrane choice. To increase the sensitivity of northern blotting, use of a positively charged nylon membrane is recommended.

Probe design and labeling. Northern blots can be probed with RNA, DNA or oligonucleotide probes. *In vitro*-transcribed RNA probes have the advantage that they can be hybridized and washed under more stringent conditions, which results in lower background. Probes can be labeled radioactively or nonisotopically either with fluorochrome or enzyme or with a hapten, such as biotin or digoxigenin; however, radioactive labeling provides the most sensitive method for detection. Probes can be prepared either by random primed labeling or by nick translation. The random primed labeling is inherently simpler than nick translation because the two nuclease activities of DNase I and 5′–3′ exonuclease are no longer required. The reactions are therefore more homogeneous and the average size of the probe DNA is an inverse function of the concentration of the primer, with an optimum length of 400–600 bp (see ref. 15). The optimal length for labeling of double-stranded DNA templates is between 200 and 1,000 bp. Shorter templates generate probes of low specific activity, whereas the use of longer templates may result in enhanced background levels[16,17].

Hybridization and optimization. Factors that influence hybridization efficiency and specificity in northern blot analyses include temperature, ionic strength, destabilizing agents, mismatched base pairs, duplex length and base composition. High salt content favors hybridization reactions (i.e., less specificity and higher background), whereas decreased salt and/or increased detergent or temperature increases hybridization specificity and reduces background. There are two main problems that can occur: either the signal is too weak or the background is too high. Usually 25 ng of probe is sufficient, but if the target gene has a very low expression level, either the amount of DNA or radioactivity can be increased. This, however, could also lead to higher background. When the background is high, more stringent washing conditions can be used or the washing time and/or number of washes can be increased.

Controls. For northern blotting, use samples with and without the target gene expression as determined by microarray or RT-PCR.

MATERIALS

REAGENTS

- Diethylpyrocarbonate (DEPC; Sigma, cat. no. D 5758k)
- Sodium chloride (NaCl; Merck, cat. no. 567441)
- Tris-HCl (Serva, cat. no. 37192)
- Magnesium chloride ($MgCl_2$; Sigma, cat. no. 248614)
- Sodium dodecyl sulfate (SDS; Merck, cat. no. 428023)
- Phenol:chloroform:isoamyl alcohol (25:24:1) (Merck, cat. no. 516726)
  ! CAUTION Hazardous; Phenol is a toxic and corrosive solution.
- Agarose (Invitrogen, cat. no. 5510-019)
- Formaldehyde (Merck, cat. no. 344198)
- Ethidium bromide (Roth, cat. no. 2218.1) ! CAUTION Mutagenic properties.
- Formamide (deionized, nuclease-free; Ambion, cat. no. AM9342) ! CAUTION Causes eye and skin irritation.
- Sodium citrate (Merck, cat. no. 567446)
- Ficoll (Sigma, cat. no. F-4375)
- Salmon sperm (sheared; Ambion, cat. no. AM9680)
- Sodium phosphate (Sigma, cat. no. 5915) ! CAUTION Irritant; wear suitable gloves.
- Dextran sulfate (Merck, cat. no. 265152)
- Sodium hydrogen phosphate (Na_2HPO_4; Merck, cat. no. 1.06586)
- EDTA (Sigma, cat. no. ED4SS) ! CAUTION Irritant.
- RediPrime II kit (GE Healthcare, Amersham Biosciences, cat. no. RPN1633)
- Qiagen nucleotide removal kit (Qiagen, cat. no 28304)
- QiaShredder (Qiagen, cat. no. 79654)
- Sodium acetate (NaOAC; Merck, cat. no. 567418)
- Ethanol (Merck, cat. no. 1.00983.1000)
- Sodium hydroxide (NaOH; Merck, cat. no. B545695948) ! CAUTION Caustic.
- Guanidinium thiocyanate (Promega, cat. no. V2791) ! CAUTION Avoid contact with eyes, skin and clothing.
- β-Mercaptoethanol (Merck, cat. no. 444203) ! CAUTION Hazardous.
- *N*-lauroylsarcosine (Sarcosyl; Sigma, cat. no. 61743)
- α^{32}P-labeled dCTP (10 μCi μl^{-1}; Amersham Biosciences) ! CAUTION When dealing with radioactive material, appropriate safety precautions must be followed.
- 3-(*N*-morpholino)propanesulfonic acid (MOPS; Merck, cat. no. 475922)
- Isopropanol (Merck, cat. no. 1010401000)
- RNA ladder (Fermentas, cat. no. SM1831)

EQUIPMENT

- Gel cast apparatus (Bio-Rad)
- Gel tank for electrophoresis (Bio-Rad)
- Power supply (Bio-Rad)
- Nylon membrane (Fermentas)
- Whatman paper (GE Healthcare Life Sciences)
- Stratalinker (Stratagene)
- Kodak XAR-5 film (Sigma)
- Kodak intensifying screens (Sigma)
- Scintillation counter (Beckman Coulter)
- Geiger counter (Berthold)
- Spectrophotometer (GE Healthcare Life Sciences)
- Film developer (Röntgen-Bender)
- Densitometry software (ImageJ, National Institutes of Health)
- UV light, camera (Syngene)
- Oven (Thermo Scientific)
- Mortar and pestle (Roth)
- Vortex (Ika)
- Microwave (Siemens)
- Water bath (Lauda)

REAGENT SETUP

▲ CRITICAL Prepare solutions in RNase-free glassware using autoclaved DEPC water to inhibit RNase activity. Wear gloves.

DEPC-treated water Prepare a 0.1% (wt/vol) solution and autoclave for 20 min; stable at room temperature (RT; 25 °C).

SSC Mix 0.75 M NaCl and 0.75 M sodium citrate; stable at RT.

Lysis buffer Mix 4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% (wt/vol) sarcosyl, 0.1 M β-mercaptoethanol and 10 mM EDTA containing β-mercaptoethanol; stable in the dark up to 3 months at RT.

Denhardt's solution Mix 0.5% (vol/vol) Ficoll, 0.5% (wt/vol) polyvinylpyrrolidone and 0.5% (wt/vol) bovine serum albumin, and store at 4 °C.

Agarose gel Mix 1.2% (wt/vol) agarose dissolved in electrophoresis buffer and 1% (vol/vol) formaldehyde; prepare fresh.

Electrophoresis buffer 10× MOPS (10% (wt/vol)); store at RT.

Prehybridization buffer Mix 50% (vol/vol) formamide, 0.2% (wt/vol) sodium dodecyl sulfate, 5× SSC, 5× Denhardt's solution, 100 μg ml^{-1} salmon sperm and 50 mM sodium phosphate (pH 6.5); prepare fresh.

SSPE Mix 150 mM NaCl, 10 mM NaH_2PO_4 and 1 mM EDTA.

2 M sodium acetate Dissolved in DEPC water (pH 4.0; adjust pH by adding acetic acid).

2× RNA loading dye Mix 50% (vol/vol) formamide, 20% (vol/vol) glycerine, 0.2% (wt/vol) bromphenol blue, 0.2% (vol/vol) xylene cyanole, 2 mM EDTA, 2.2 M formaldehyde and 3 M NaOAC.

10× MOPS Mix 0.2 M MOPS, pH 7.0, 50 mM sodium acetate and 1 mM EDTA.

PROCEDURE

Total RNA extraction ● TIMING 4 h

1| Cells or tissues can be lysed using options A or B, respectively.

(A) Lysis of cells

(i) Harvest cells when 80–90% confluency is reached; remove the culture medium and add cell lysis buffer according to the size of the culture dish (e.g., for one 150-mm-diameter dish, 4 ml of cell lysis buffer is recommended).

(ii) Dislodge cells with a cell lifter.

(B) Lysis of tissue

(i) Place frozen tissue in a mortar containing liquid nitrogen and pulverize with a pestle. Immediately add lysis buffer according to the weight of the tissue sample (e.g., for 100 mg of frozen tissue, 3 ml are recommended).

▲ CRITICAL STEP Immediately freeze tissue in liquid nitrogen upon surgical removal, and maintain at −80 °C until use.

2| Homogenize either by pushing six times through a syringe with a yellow needle (20 G) or use the QiaShredder columns by Qiagen. Transfer into a centrifuge tube.

3| Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and one-tenth volume of 2 M sodium acetate.

4| Vortex vigorously to mix the phases.

5| Centrifuge at 17,900*g* in a conventional tabletop microcentrifuge for 10 min at RT to separate the phases.

6| Remove the upper aqueous phase to a new tube.

7| Add the same volume of isopropanol to the new tube from Step 6 to precipitate the RNA and vortex.

8| Incubate at −20 °C for at least 1 h.

■ **PAUSE POINT** The extract can be stored at −20 °C for several weeks.

9| Precipitate by centrifugation at 12,000*g* for 30 min at 4 °C. Discard the supernatant.

10| Wash the pellet by adding 1 ml of 80% (vol/vol) ethanol and vortex.

11| Centrifuge at RT for 20 min at 12,000*g*. Carefully aspirate the supernatant from the tube and dry the pellet. Resuspend the RNA pellet in 20 µl of DEPC-treated water.

■ **PAUSE POINT** RNA can be stored at −80 °C.

12| Calculate the RNA concentration by measuring the OD with a spectrophotometer at 260 nm. Dilute RNA 1:100 in distilled water. RNA concentration (µg µl^{-1}) = OD (1:1,000) × 40. Also measure the ratio of A260/A280.

▲ **CRITICAL STEP** If the preparation is clean, the value should be between 1.9 and 2.

? TROUBLESHOOTING

Formaldehyde gel electrophoresis ● TIMING 4 h

13| Wash the gel apparatus including the gel plates, spacers and combs with ethanol and then rinse thoroughly using DEPC water. Set up the apparatus following the manufacturer's instructions.

! **CAUTION** Glass plates and combs must be thoroughly cleaned.

14| Mix 1.8 g of agarose in 130.5 ml of DEPC water and boil in a microwave. Let it cool down to ~60 °C, and then add 15 ml of 10× MOPS and 4.5 ml of formaldehyde solution (37% (vol/vol)) and fill the apparatus. Insert the comb and allow the gel to solidify for 40 min.

15| Remove the comb and fill the tank with electrophoresis running buffer.

16| Mix 20 µg of RNA with RNA loading dye (1:1) and incubate for 10 min at 75 °C to denature.

17| Load the samples and RNA ladder into the wells.

18| Run the gel at 120 V for 2 h.

19| Prepare a 0.05% (wt/vol) ethidium bromide solution with electrophoresis buffer, incubate gel in this solution for 15 min and destain for 30 min in electrophoresis buffer or water. After ethidium bromide staining, take a photograph under UV light to verify that equal amounts of total RNA have been loaded (see **Fig. 2a**; see **Fig. 2b** for an example of degraded RNA).

▲ **CRITICAL STEP** For gels of total RNA, the 28S and 18S ribosomal subunits are visible and act as convenient markers (~5 and 2 kb, respectively). The quality of RNA is detected by the appearance of sharp ribosomal RNA bands without smear and the 28S band should be twice as bright as the 18S band (the theoretical ratio of 28S to 18S is ~2.7:1).

Transfer of RNA from gel to membrane ● TIMING 16 h

20| Soak the gel in 20× SSC for 15 min.

21| Prepare the membranes by cutting it to the size of the gel and soak the membranes in 2× SSC.

22| Set up capillary transfer in the order described in **Box 1**. Allow the RNA to transfer overnight. The blot transfer is illustrated in **Figure 3**.

▲ **CRITICAL STEP** Plastic wrap the exposed wick. Check for air bubbles at each step. Do not move the membrane once it has been set down. Level the weights so that the pressure is distributed evenly.

? TROUBLESHOOTING

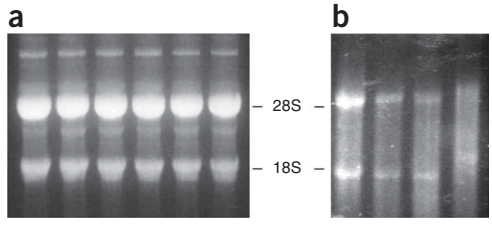

Figure 2 | Examples of RNA gels. (**a**) RNA gel electrophoresis. Six samples, 20 µg per lane of different pancreatic cancer cell lines were run on an agarose gel. The clear appearance of the 28S and 18S rRNA with no smearing indicates high quality of RNA. (**b**) An example of degraded RNA.

23| At the end of the transfer, remove the weights and paper towels. Leave the upper Whatman on and turn over the gel with membrane. Poke holes in the gel boxes to mark the lanes. Pull the gel off and dispose of it. Cut off the upper left corner of the membrane so that the lanes can be identified later.

24| With the RNA side facing upward, cross-link RNA to the nylon membrane using a UV cross-linker at a setting of 120 mJ cm^{-2}. Bake in a vacuum oven for 30 min and store the membrane when it is dry.
! CAUTION Label the membrane properly.
▲ CRITICAL STEP Quality of transfer is quickly checked under UV light (254 nm) when two major bands of ribosomal subunits, the 18S and the 28S RNA fraction, are visible.
■ PAUSE POINT The membrane can be stored at RT until use.
? TROUBLESHOOTING

BOX 1 | NORTHERN BLOT TRANSFER

1. Prepare a reservoir containing 20× SSC.
2. Place a tray (minimum the size of the gel) in the middle of the reservoir.
3. Soak a long piece of Whatman paper in 20× SSC for use as a wick.
4. Lay the wick on top of the tray with the ends dipping into the reservoirs.
5. Soak three pieces of Whatman paper in 20× SSC.
6. Lay the gel with the top side facing down.
7. Place the nylon membrane on top of the gel. Remove bubbles between the gel and the membrane.
8. Soak three pieces of Whatman paper in 2× SSC and lay them on top of the membrane.
9. Stack at least three inches of cut-to-size paper towels on top of the Whatman paper.
10. Place a plate on top of the wick and weigh down the whole setup.

Prehybridization and probe preparation ● TIMING 1–4 h

25| Put the membrane in a plastic (lunch) box with 10–20 ml of prehybridizing solution. Incubate the glass tube at 42 °C for 1–4 h in a slowly shaking water bath.
▲ CRITICAL STEP Good prehybridization or blocking is necessary to minimize background problems.

26| While the membrane incubates, prepare the probe using 25 ng of the DNA fragment and label the DNA with (α-^{32}P)dCTP (50 µCi, 3,000 Ci $mmol^{-1}$) using the RediPrime II kit according to the manufacturer's instructions.
! CAUTION Use screw-cap tubes.
▲ CRITICAL STEP Other labeling methods are also suitable.
? TROUBLESHOOTING

27| Purify the radiolabeled probe from unincorporated nucleotides using the Qiagen nucleotide removal kit according to the manufacturer's instructions. Elute in 100 µl.

28| Use 1 µl of probe to determine the counts with a scintillation counter. A total of 1 µl should be 100,000 c.p.m.
? TROUBLESHOOTING

Hybridization ● TIMING 18 h

29| Denature the probe by placing it in a boiling water bath for 5 min followed by snap cooling on dry ice for 2 min. Briefly spin down the probe.

30| Add the denatured probe directly to the prehybridization solution. Incubate blots overnight at 42 °C.
? TROUBLESHOOTING

31| Pour the probe with the prehybridization solution into a tube and store at −20 °C for later use (up to 3 months).

32| Wash the blot at 65 °C in 100–200 ml of 1× SSPE and 0.5% (wt/vol) SDS, and twice at 68 °C in 100–200 ml of 0.1× SSPE and 0.5% (wt/vol) SDS, each for 10 min.
! CAUTION Low-stringency washes remove the hybridization solution and unhybridized probe, whereas high-stringency washes remove partially hybridized molecules.

33| Dry the membrane on blotting paper and expose the blots at −80 °C to Kodak XAE-5 film with Kodak intensifying screens for 1–6 d.
? TROUBLESHOOTING

34| Following autoradiography, use laser densitometry to quantify the radiographic bands.
▲ CRITICAL STEP To quantify equal loading, probe the membrane with an 18S or 7S rRNA or any other housekeeping gene probe. Strip the blot if necessary by adding boiling water, then cool.

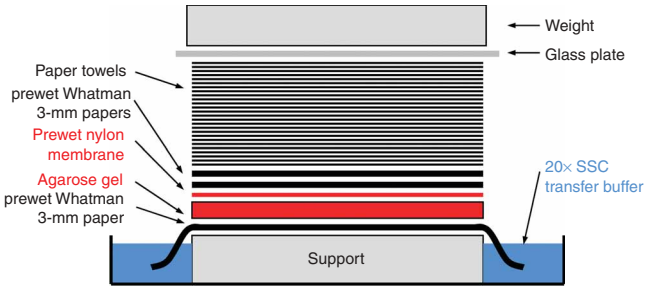

Figure 3 | Upward capillary transfer. Capillary transfer of nucleic acids from an agarose gel to solid supports is achieved by drawing the transfer buffer from the reservoir upward through the gel into a stack of paper towels.

● TIMING

Steps 1–12, RNA extraction: ~4 h
Steps 13–19, gel electrophoresis: ~4 h
Steps 20–24, transfer of RNA to membrane: ~16 h
Steps 25–28, prehybridization and probe preparation: 1–4 h
Steps 29–32, hybridization: ~18 h
Steps 33 and 34, autoradiography: 1–6 d

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 2**.

TABLE 2 | Troubleshooting table.

Problem	Possible reason	Solution
No bands are observed	Poor quality of RNA (Steps 1–12)	Check A_{260}/A_{280} ratio
	Low RNA concentration (Steps 1–12)	Increase the number of cells or amount of tissue. Poly (A)-selected RNA can also be used instead of total RNA to increase the amount of mRNA target
	Inefficient labeling (Steps 26–28)	Check labeling and prepare a new probe; use fresh ^{32}P within the first half-life
	Probe concentration is too low (Steps 26–28)	Increase the amount in hybridization
	The probe is too short (Step 26)	Longer probes will contain more labeled molecules
	Aberrations generated during subcloning (Step 26)	Check by sequencing or restriction analysis
	RNA is not transferred to the membrane (Steps 20–24)	Check northern blotting setup. Extend the transfer if necessary
	Insufficient film exposure (Step 33)	Increase exposure time
High background	Contamination of membrane (Steps 25–33)	Membrane was not completely dry. Handle the membrane carefully
	Bubbles during blotting (Steps 20–22)	Remove all bubbles during blotting
	Blot dried out during hybridization (Step 30)	Cover membrane completely
Too many bands are observed	Nonspecific probe (Step 26)	Redesign probe

ANTICIPATED RESULTS

Northern blotting is a technique that can provide information about deregulated gene expression in malignancies such as cancer. In one of the first studies that successfully isolated RNA from human pancreatic tissues, Korc and coworkers showed that in comparison with the normal pancreas, pancreatic cancers exhibited 3-fold, 15-fold and 10-fold increases in the mRNA moieties encoding the epidermal growth factor receptor (EGFR), EGF and transforming growth factor-α (TGF-α), respectively[12] (**Fig. 4**). These findings suggested for the first time that overexpression of the EGFR and its ligands EGF and TGF-α was involved in the pathobiology and carcinogenesis of pancreatic cancer.

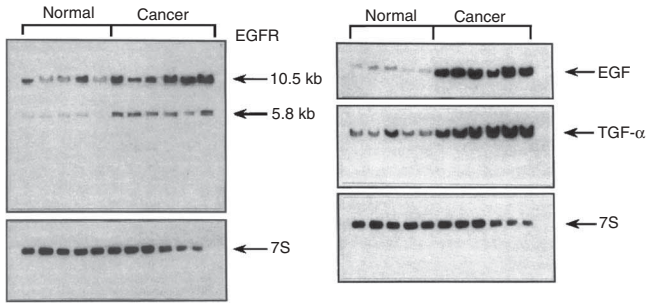

Figure 4 | Northern blot analysis of EGFR, EGF and TGF-α in the normal pancreas and pancreatic cancer. Pancreatic RNA was extracted using the guanidine isothiocyanate method. The membrane was probed with the ^{32}P-labeled EGFR, EGF or TGF-α cDNA and, as a loading control, the 7S cDNA. Figure and legend reprinted from ref. 12 with permission from the American Society for Clinical Investigation (conferred through the 'Copyright Clearance Center').

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