APPENDIX
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Stock solutions:

**Phosphate Buffered Saline (PBS) pH 7.2/pH 7.6**

- Sodium phosphate (anhydrous) 1.52 gm
- Potassium dihydrogen phosphate 0.58 gm
- NaCl 8.50 gm
- Sterile ddH$_2$O 1000 ml

The pH was adjusted with 3M NaOH according to the experiment and the solution, filter-sterilized.

**Proteinase K 20 mg/ml**

- Proteinase K 20 mg
- Sterile ddH$_2$O 1 ml

Solution was filter-sterilized and stored at -20°C.

**20X SSC pH 7.0**

- NaCl 3.0 M
- Sodium citrate 0.3 M

The pH was adjusted with 10N NaOH.
Solutions for DNA extraction:

**Paraffin-embedded tissue:**

**Proteinase K mix**
- Tris-HCl pH 7.5: 10.0 mM
- MgCl$_2$: 1.5 mM
- Tween 20: 0.45 %
- Proteinase K: 60 μg/ml

**Throat washes:**

**RPMI 1640**
- RPMI 1640: 1 sachet
- Sodium bicarbonate: 2.0 gm
- HEPES: 1.0 gm
- Sterile ddH$_2$O: 1000 ml

The solution was filter-sterilized and stored at 4°C.

**Digestion buffer**
- Tris-HCl pH 8.0: 0.05 M
- EDTA pH 8.0: 0.005 M
- SDS: 1 %
- Proteinase K (added fresh): 200 μg/ml

**RNase 10 mg/ml**
- Ribonuclease: 100.0 mg
- Sterile ddH$_2$O: 10.0 mg
Solutions for plasmid isolation:

Luria-Bertani broth (LB)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Broth was autoclaved and cooled before using. LB agar was prepared by adding 1.0 gm of Agar to the broth prior to autoclaving.

Ampicillin 20 mg/ml

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>20 mg</td>
</tr>
<tr>
<td>Sterile ddH₂O</td>
<td>1 ml</td>
</tr>
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</table>

Solution was filter-sterilized and stored at -20°C.

Solution I

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>50 mM</td>
</tr>
<tr>
<td>Tris-HCl pH 8.0</td>
<td>25 mM</td>
</tr>
<tr>
<td>EDTA pH 8.0</td>
<td>10 mM</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Solution was stored at 4°C.

Solution II

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>NaOH</td>
<td>0.2N</td>
</tr>
<tr>
<td>SDS</td>
<td>1 %</td>
</tr>
</tbody>
</table>

Solution was freshly prepared as necessary.
Solution III

5 M Potassium acetate 60.0 ml
Glacial acetic acid 11.5 ml
Sterile ddH₂O 28.5 ml

Solution was stored at 4°C.

Solutions for agarose gel electrophoresis:

5X TBE

Trizma base 54.0 gm
Boric acid 27.5 gm
EDTA 0.05 M 20.0 ml

Solution was made up to 1 litre with distilled water.

6X Gel loading dye

Bromophenol blue 0.25 %
Glycerol 40 % (w/v)
ddH₂O 100 ml

Solution was stored at 4°C.

Ethidium bromide 10 mg/ml

Ethidium bromide 100 mg
ddH₂O 10 ml
Solutions for nonradioactive Southern blot hybridization:

**Denaturation buffer**
- NaCl 1.5 M
- NaOH 0.5 M

**Neutralization buffer**
- Tris-HCl 1.0 M
- NaCl 1.5 M

**Hybridization solution**
- 5X SSC 22.5 ml
- N-lauroylsarcosine, Na-salt 0.1 % (w/v)
- SDS 0.02% (w/v)
- Blocking reagent 1.0 % (w/v)

**Wash Solution 1**
- 2X SSC 100.0 ml
- SDS 0.1 % (w/v)

**Wash Solution 2**
- 0.1X SSC 100.0 ml
- SDS 0.1 % (w/v)
Solutions for detection of hybridized products

Buffer 1 pH 7.5

Tris-HCl 100 mM
NaCl 150 mM

Buffer 2

0.5 % (w/v) Blocking reagent in buffer 1.

Buffer 3

Tris-HCl 100 mM
NaCl 100 mM
MgCl₂ 50 mM

Buffer 4

Tris-HCl 10 mM
EDTA 1 mM

Colour solution

4-Nitro blue tetrazolium chloride 45 μl
5-Bromo-4-chloro-3-indoyl phosphate 35 μl
Buffer 3 10 ml
Solutions for treatment of slides:

50X Denhardt’s solution

Ficoll 5 gm
Polyvinylpyrrolidone 5 gm
Bovine serum albumin 5 gm
ddH₂O 500 ml

Solution was filter-sterilized and stored in aliquots at -20°C.

Organosilane solution pH 3.45

1 % (v/v) 3-amino-propyl-trithoxysilane in double distilled water.

pH was adjusted with 1M HCl.

Glutaraldehyde

10 % (v/v) Glutaraldehyde in PBS.

Sodium-m-periodate

0.1 M Sodium-m-periodate in double distilled water.

Solutions for in situ hybridization:

0.2 % Glycine

Glycine 0.2 gm
PBS 100 ml
4 % Paraformaldehyde pH 7.2

Paraformaldehyde 4 gm
PBS 100 ml

Solution was freshly prepared as necessary by dissolving at 60-70°C and adding a few drops of 2N NaOH to give a clear liquid.

100 % deionized Formamide

Formamide 100 ml
Resin 10 gm

Solution was stirred vigorously for 30 minutes in a fume chamber and filtered twice through Whatman #1 paper. Aliquots were stored at -20°C.