APPENDIX 5

SOUTHERN TRANSFER AND NICK TRANSLATION (BUFFERS USED)

Prehybridization solution

5 x SSC, 50% Formamide, 5 x Denhardt's solution, 50 mM Tris HCl (pH 7.5), autoclaved water to make the desired volume and 200 /ug/ml Salmon sperm DNA.

20 x SSC (Sodium Saline Citrate)

| 175.3 g | NaCl |
| 88.2 g  | Sodium Citrate |

Dissolved in 800 ml of distilled water. pH adjusted to 7.0 with HCl. Volume made up to 1 L.

Denhardt's solution

2 g Polyvinyl pyrrolidone
2 g BSA
2 g Ficoll

Dissolved in 200 ml distilled water. Filter sterilized.

Salmon Sperm DNA

To 350 mg Salmon sperm DNA, 35 ml distilled water was added. The DNA was dissolved by incubating the contents at 37°C overnight.