

2110 AF=1+AF

2120 RETURN

2200 AH=AG+AH

2210 AI=1+AI

2220 RETURN

2300 AK=AK+AJ

2310 AL=1+AL

2320 RETURN

2400 AN=AM+AN

2410 AO=1+AO

2420 RETURN

2500 AQ=AP+AQ

2510 AR=1+AR

2520 RETURN

3000 END

APPENDIX 4

ELECTROPHORESIS BUFFERS

- TRIS-EDTA -

TRIS-Cl....(pH 7,6).....10 mM

EDTA.....(pH 8).....1 mM

Adjust to pH 7,6

- TRIS-BORATE-EDTA -

TRIS BASE.....54 g/l

BORIC ACID.....27,5 g/l

EDTA (0,5 M, pH 8).....20 ml

- FYCOLL -

BROMOPHENOL BLUE.....0,25%

XYLENE CYANOL.....0,25%

FYCOLL TYPE 400 (in water).....25%

Store at room temperature.

APPENDIX 5

INCUBATION BUFFERS FOR THE RESTRICTION ENDONUCLEASES

- *ECO RI* -

TRIS-HCl.....100 mM
NaCl.....50 mM
MgCl₂.....10 mM
Adjusted to pH 7,5

- *HIND III* -

TRIS-HCl.....10 mM
NaCl.....50 mM
MgCl₂.....10 mM
DITHIOERYTHRITOL.....14 mM
Adjusted to pH 7,6

- *BGL II* -

GLYCINE-NaOH.....20 mM
MgCl₂.....10 mM
MERCAPTOETHANOL.....7 mM
Adjusted to pH 9,5

APPENDIX 6

HYBRIDIZATION BUFFERS

- SODIUM PHOSPHATE BUFFER -

Na_2HPO_4(ph 8,9).....1 M

NaH_2PO_4(pH 3,9).....1 M

Adjusted to pH 6,5

- DEIONIZING FORMAMIDE -

Mix 50 ml of formamide and 5 g of mixed-bed, ion-exchange resin (i.g. Bio-Rad AG 501-X8, 20-50 mesh). Stir for 30 minutes at room temperature. Filter twice through a Whatman no 1 filter paper (Whatman, USA). Dispense into 1 ml aliquots and store at -20°C .

DENHARDTS SOLUTION (50 x) (grams)

FICOLL.....10

POLYVINYLPYRROLIDONE.....10

BSA (Pentax fraction V).....10

H_2Oup to 11

Filter through a disposable Nalgene filter. Dispense into 25 ml aliquots and store at -20°C .

- BOVINE SERUM ALBUMIN (BSA) -

BSA (Pentax fraction V).....1g
H₂O.....10 ml

Dispense into aliquots and store at -20°C.

PREHYBRIDIZATION SOLUTION

DEIONIZED FORMAMIDE.....50 ml (50%)
NaCl.....4,37 g
SODIUM CITRATE.....2,2 g
DENHARDT (20 x)......25 ml
SODIUM PHOSPHATE BUFFER..(pH 6,5).....5 ml
HERRING SPERM DNA..(5 mg/ml).....1,5 ml
H₂C.....up to 100 ml

- HYBRIDIZATION SOLUTION -

DEIONIZED FORMAMIDE.....50 ml
NaCl.....4,37 g
SODIUM CITRATE.....2,2 g
DENHARDT (20 x)......25 ml
SODIUM PHOSPHATE BUFFER..(pH 6,5).....5 ml
HERRING SPERM DNA..(5 mg/ml).....1,5 ml
DEXTRAN SULPHATE (50% sol.).....20 ml
H₂O.....up to 100 ml

- POSTHYBRIDIZATION -

DEIONIZED FORMAMIDE.....5 ml
NaCl.....4,37 g
SODIUM CITRATE.....2,2 g
DENHARDT (20 X).....25 ml
SODIUM PHSPHATE BUFFER..(pH 6,5).....5 ml
H₂O.....up to 100 ml

APPENDIX 7

NICK TRANSLATION REACTION MIXTURE

DNA.....	0,5 µg
DNA ENZYMES.....	5 µl
NUCLEOTIDE SOLUTION.....	10 µl
H ₂ O.....	15 µl
RADIOACTIVE NUCLEOTIDE.....	2 µg

APPENDIX 8

SCINTILLATION SOLUTION

POPOP.....0,1 g/l

PPO.....5 g/l

POPOP - 1'4-bis-2-(5-Phenyloxazolyl-benzene)

PPO - 2-5-Diphenyloxazole

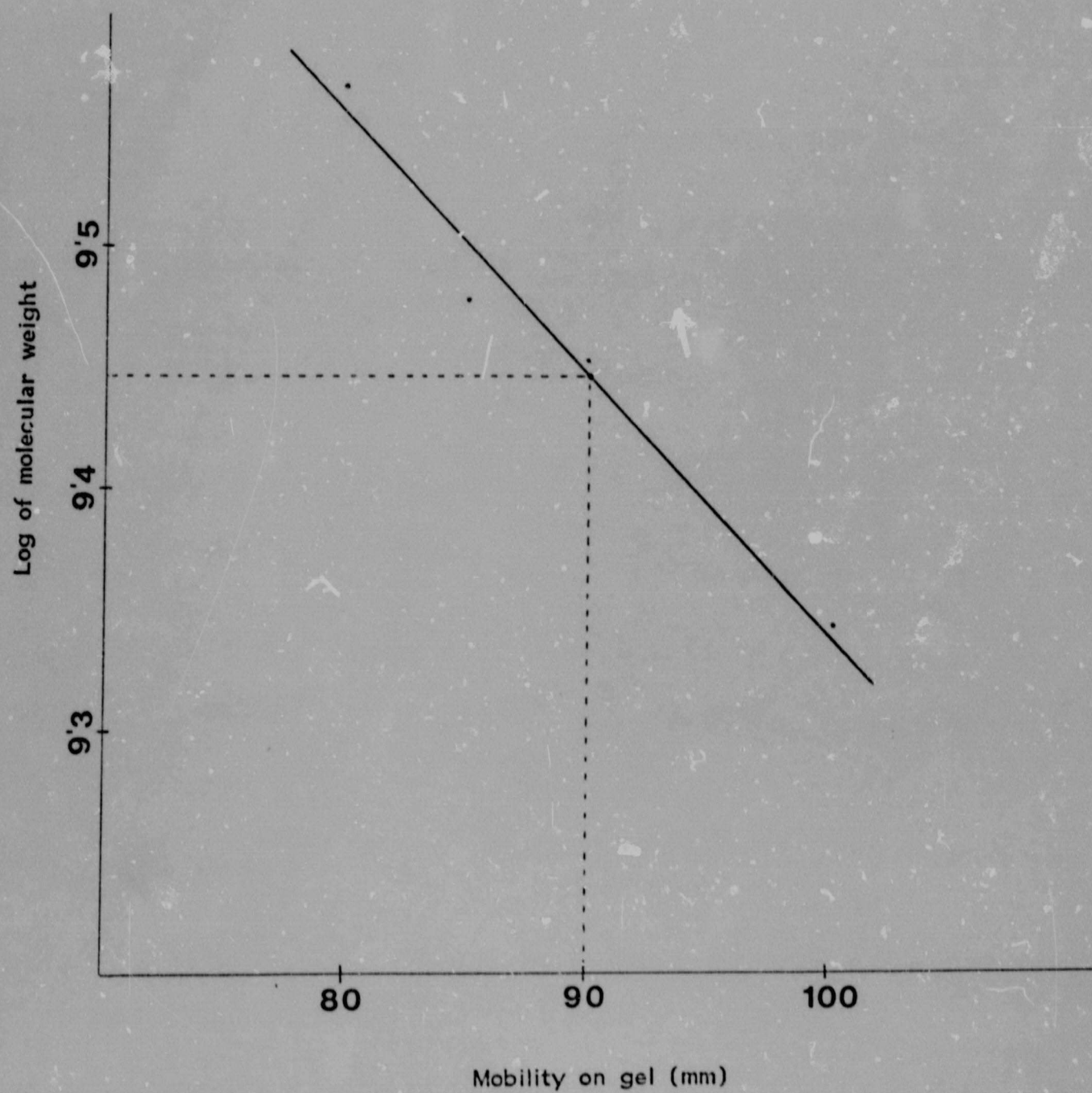
APPENDIX 9

STANDARD MOLECULAR WEIGHT DNA MARKERS

MICROORGANISM	MOLECULAR WEIGHT
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<i>Agrobacterium tumefaciens</i>	3,65 x 10 ⁹ daltons
<i>Bacillus subtilis</i>	3,0 x 10 ⁹ daltons
Calf thymus.....	5 x 10 ⁶ daltons
<i>Erwinia herbicola</i>	2,83 x 10 ⁹ daltons
<i>Escherichia coli</i>	2,2 x 10 ⁹ daltons

STANDARD CURVE FOR THE MOLECULAR WEIGHT ESTIMATION OF DNA
USING BACTERIAL DNA MARKERS



Author Hortelano Gonzalo

Name of thesis Genetic Study Of A Bacterium Isolated From "greening" Infected Citrus. 1986

PUBLISHER:

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