

Appendix 1

Recipe for PCR reaction, final volume was 25 μ l

Ingredients	Final concentration
AmpliBuffer A (EURx, Poland)	1 \times
MgCl ₂ (EURx, Poland)	1.5mM
dNTP (Fermentas, Canada)	200 μ M
Forward and reverse primers (BioNEER, Korea)	0.5mM
DNA sample	100ng
<i>Taq</i> Polymerase (EURx, Poland)	1U
Distilled water	Top up until final volume

Recipe for preparation 5 \times TBE, final volume was 1000ml

Ingredients	Final concentration
C ₄ H ₁₁ NO ₃ (Vivantis, Malaysia)	445mM
H ₃ BO ₃ (Vivantis, Malaysia)	445mM
EDTA (Promega, USA)	10mM
dH ₂ O	Top up until final volume

For preparation 1 \times TBE, 200ml of 5 \times TBE was diluted with 800ml dH₂O

Recipe for preparation 10 LB/Ampicillin/IPTG/X-Gal agar plates

Ingredients	Final concentration and amount need
LB agar powder (Pronadisa, Spain)	7g
dH ₂ O	200ml
Ampicillin (Sigma, USA)	50 μ g/ml
IPTG (Fermentas, Canada)	0.5mM
X-gal (Vivantis, Malaysia)	0.08mg/ml

Recipe for DNA ligation, final volume was 10µl

Ingredients	Volume
2× Rapid Ligation Buffer, T4 DNA Ligase (Promega, USA)	5µl
50ng pGEM [®] -T Easy Vector (Promega, USA)	1µl
Gel extraction product	3µl
3U T4 DNA Ligase (Promega, USA)	1µl

Recipe for preparation 100ml of RF1

Ingredients	Final concentration
RbCl ₂ (aMReSCO, USA)	0.1M
MnCl ₂ .4H ₂ O (BIO BASIC INC, Canada)	0.05M
KoAc (BIO BASIC INC, Canada)	0.03M
CaCl ₂ .4H ₂ O (BIO BASIC INC, Canada)	0.01M
Glycerol (System, Malaysia)	15%
Distilled water	Top up until final volume
Adjust PH	5.8 PH

Recipe for preparation 100 ml of RF2

Ingredients	Final concentration
MOPS (BIO BASIC INC, Canada)	0.01M
RbCl ₂ (aMReSCO, USA)	0.01M
CaCl ₂ .4H ₂ O(BIO BASIC INC, Canada)	0.07M
Glycerol (System, Malaysia)	15%
Distilled water	Top up until final volume
Adjust PH	5.8 PH

Recipe for PCR colony, final volume was 25 μ l

Ingredients	Final concentration
AmpliBuffer B (EURx, Poland)	1 \times
dNTP (Fermentas, Canada)	200 μ M
Forward and reverse primers (BIO BASIC INC, Canada)	0.5mM
DNA sample	100ng
<i>Taq</i> Polymerase (EURx, Poland)	1U
dH ₂ O	Top up until final volume

Recipe for preparation 50ml of Solution I (stored at 4°C)

Ingredients	Final concentration
C ₄ H ₁₂ NO ₃ Cl pH 8.0 (aMReSCO, USA)	25mM
EDTA pH8.0 (Promega, USA)	10mM
Glucose (BDH, England)	50mM
dH ₂ O	Top up until final volume

Recipe for preparation 500 μ l of Solution II (freshly prepared)

Ingredients	Final concentration
NaOH (Merck, Germany)	0.2N
SDS (Promega, USA)	1%
dH ₂ O	Top up until final volume

Recipe for preparation 100ml of Solution III (stored at 4°C)

Ingredients	Final concentration
KoAc (BIO BASIC INC, Canada)	3M
Glacial Acetate acid (BDH, England)	11.5%
dH ₂ O	Top up until final volume

Recipe for restriction enzyme digestion, final volume was 20 μ l

Ingredients	Final concentration
Buffer H (Promega, USA)	1 \times
BSA (Promega, USA)	0.1mg/ml
DNA	5 μ g
<i>Eco</i> RI (Promega, USA)	5U

Appendix 2

Mega alignment

Sequence alignment of ITS regions of DNA fragment (~700bp) of *Aphelenchus* spp.

(Aph3), *Helicotylenchus dihystera* (Hd6), *Rotylenchulus reniformis* (Rr27), *Xiphinema* spp.

(Xip9 and Xip10), *Pratylenchus* spp. (Pra8) and *Meloidogyne* spp. (C4m1 and C4m2)

samples with out-group (*C.elegans*).

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#Aph3a3      TTGATTACGT CCCTGCCCTT TGTACACACC GCCCGTCCCT AGCCCCGG [48]
#Hd6c2      .....G.. G--..... [48]
#Rr27a1     .....G.. G--..... [48]
#Xip10a3    .....G.. G--..... [48]
#Xip9a1     .....G.. G--..... [48]
#Pra8c3     .....G.. G--..... [48]
#C4m1d4     .....G.. G--..... [48]
#C4m2a2     .....G.. G--..... [48]
#C4m2b3     .....G.. G--..... [48]
#C.elegans  .....G.. --T.... [48]
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#Aph3a3      GACTGAGCCA TTTCGAGAAA TTTGGGGACC GTTGATTTAA --TTTATC [96]
#Hd6c2      ..... --..... [96]
#Rr27a1     ..... --..... [96]
#Xip10a3    ..... --..... [96]
#Xip9a1     ..... --..... [96]
#Pra8c3     ..... --..... [96]
#C4m1d4     ..... --...T.. [96]
#C4m2a2     ..... --...T.. [96]
#C4m2b3     ..... --...T.. [96]
#C.elegans  .....A.TG A.....G AG.....T ..C.C..CG. GG....A. [96]
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#Aph3a3      TAAATTATTT TGATGGAAAC CAATTTAATC GCAGTGGCTT GAACCGGG [144]
#Hd6c2      ..... [144]
#Rr27a1     ..... [144]
#Xip10a3    ..... [144]
#Xip9a1     ..... [144]
#Pra8c3     ..... [144]
#C4m1d4     .....C.. [144]
#C4m2a2     .....C.. [144]
#C4m2b3     .....C.. [144]
#C.elegans  G.CT.CG..G .TGC..... ..T...T... ..T...T... [144]
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#Aph3a3      CAAAAGTCGT AACAAGGTAG CTGTAGGTGA ACCTGCTGCT GGATCATT [192]
#Hd6c2      ..... [192]
#Rr27a1     ..... [192]
#Xip10a3    ..... [192]
#Xip9a1     ..... [192]
#Pra8c3     ..... [192]
#C4m1d4     ..... [192]
#C4m2a2     ..... [192]
#C4m2b3     ..... [192]
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#C.elegans	T.....A...C	[192]
#Aph3a3	ACTTTAT---	-----GTG	ATGTTCA---	ATTTTGA-AT	-CGCAAT-	[240]
#Hd6c2---	---------	-.....-	[240]
#Rr27a1C---	---------	-.....-	[240]
#Xip10a3---	---------	-.....-	[240]
#Xip9a1---	---------	-.....-	[240]
#Pra8c3---	---------	-.....-	[240]
#C4m1d4---	-----G---	.A.....-	T.....C-	[240]
#C4m2a2---	-----G---	.A.....-	T.....C-	[240]
#C4m2b3---	-----G---	.A.....-	T.....C-	[240]
#C.elegans	G.CG.GCCTC	TGTGCCC...	..A.C..CAA	.CC..A.G..	G.T.G.CT	[240]
#Aph3a3	-----G-AA	ATA-ATTGTT	GTGTAACGGC	TGTCGCTGGT	GTCTAGGT	[288]
#Hd6c2	-----.-.-	[288]
#Rr27a1	-----.-.-	[288]
#Xip10a3	-----.-.-	[288]
#Xip9a1	-----.-.-	[288]
#Pra8c3	-----.-.-	[288]
#C4m1d4	-----AT..	...T.....	...A.....	[288]
#C4m2a2	-----AT..	.C.T.....	...A.....	[288]
#C4m2b3	-----AT..	...T.....	...A.....	[288]
#C.elegans	GGCTTCACGG	TC.GT.GAG.	..CG..AT.T	CAA..T.CCA	..TG..A.	[288]
#Aph3a3	GTTGCTGATA	C-----AG	TTGTGAACGT	CCGTGGCTGT	ATATGTGG	[336]
#Hd6c2	-----	[336]
#Rr27a1	-----	[336]
#Xip10a3	-----	[336]
#Xip9a1	-----	[336]
#Pra8c3	-----	[336]
#C4m1d4	-----	[336]
#C4m2a2	-----	[336]
#C4m2b3	-----	[336]
#C.elegans	.CCA.AACA.	.TGGCAAG..	.A...CT..	...ACC.ATG	GA.C....	[366]
#Aph3a3	TGAC-ATGTT	AGGACTATAA	TGAGTTTAAG	ACTTAATGA-	GCCTCTTA	[384]
#Hd6c2--	[384]
#Rr27a1--	[384]
#Xip10a3--	[384]
#Xip9a1--	[384]
#Pra8c3--	[384]
#C4m1d4--	[384]
#C4m2a2--	[384]
#C4m2b3-C-	[384]
#C.elegans	CAGTT..TCC	.AA....CTT	.TGTG.C.G.	G.....T	.AG.GAA.	[384]
#Aph3a3	AGTGA-----	GGACGCCAGC	ACTCTTTTTT	T-----AT	ATTTAAAA	[432]
#Hd6c2	[432]
#Rr27a1	[432]
#Xip10a3	[432]
#Xip9a1	[432]
#Pra8c3	[432]
#C4m1d4CTCTAT-..	T.....	[432]
#C4m2a2CTCTAT-..	T..A....	[432]
#C4m2b3CTCTAT-..	T.....	[432]
#C.elegansTTTGCT	.TT.TG...T	.GA.G..G..	CACGAGTCG.	C.CA.C.C	[432]
#Aph3a3	AAGAACAAAA	TTCCTAGCCT	TTCCGGTGGA	TCACTAGGCT	CGTGGATC	[480]
#Hd6c2	..A.....	[480]

#Rr27a1 ..A..... [480]
 #Xip10a3 ..A.....T [480]
 #Xip9a1 ..A..... [480]
 #Pra8c3 ..A..... [480]
 #C4m1d4 ..A...T... A.T..... .AT..... .C... [480]
 #C4m2a2 ..A...T... A.T..... .AT..... .C...G [480]
 #C4m2b3 ..A...TG... A.T..... .AT..... .C... [480]
 #C.elegans ..CCG.T.TG .GT...CCTGG .GG.TA.ATG CGT..... TC.TCT.. [480]

#Aph3a3 GATGAAGA-A CGCAGCAAAC TG-CGATAAG TTTTGCGAAC -TGCAGCA [528]
 #Hd6c2-..... [528]
 #Rr27a1-..... [528]
 #Xip10a3-..... [528]
 #Xip9a1-..... [528]
 #Pra8c3-..... [528]
 #C4m1d4-..... C.-.....T ..G..... -.....A. [528]
 #C4m2a2-..... -.....T ..G..... -.....A. [528]
 #C4m2b3-..... -.....T ..G..... -.....A. [528]
 #C.elegans TT..CG..G. G.A..TT.GT ..A.....GT C..GAT..T. G..GTT.G [528]

#Aph3a3 GCCTTGA--- -----GCA TAAAAGTTTT GCACGCATAT TGCCGCAT [576]
 #Hd6c2---..... [576]
 #Rr27a1---..... [576]
 #Xip10a3---..... [576]
 #Xip9a1---..... [576]
 #Pra8c3---..... [576]
 #C4m1d4 A.....---..... C..... [576]
 #C4m2a2 A.....---..... C..... [576]
 #C4m2b3 A.....---..... C..... [576]
 #C.elegans .TT.C.GCCG AGCCAAG..G CC...AACAC AT.A...CC. .TT.AT.. [576]

#Aph3a3 AGGGGTCAA- --ACCCTTTG CTAT--GTCT -----GGTTC AGGGTCAT [624]
 #Hd6c2-..... [624]
 #Rr27a1-..... [624]
 #Xip10a3-..... [624]
 #Xip9a1-..... [624]
 #Pra8c3-..... [624]
 #C4m1d4 T.....-.....T..... ..C--..... [624]
 #C4m2a2 T.....-.....T..... ..C--..... -----A..... [624]
 #C4m2b3 T..A.....-.....T..... ..C--..... [624]
 #C.elegans TT.AA...GT AC..TGA... .C.AAA.... TCACG.ACAT GC...G.. [624]

#Aph3a3 TATCTTTCAA AGCGAAAGCT T---TATTGT ATAATTGCTT TTGTGGCT [672]
 #Hd6c2 [672]
 #Rr27a1 [672]
 #Xip10a3 [672]
 #Xip9a1T.. [672]
 #Pra8c3 [672]
 #C4m1d4 .T.....T.. [672]
 #C4m2a2 .T.....T.. [672]
 #C4m2b3 .T.....T.. [672]
 #C.elegans .TGT.G.TG. GATTTGCATC .CGA..A.AC G..C.A.... CA.C.ATG [672]

#Aph3a3 TCTTTAT-GT TTAAATATG ATTGTTAGAT ATGTTTAATT GTTCGCAT [720]
 #Hd6c2-..... [720]
 #Rr27a1-..... [720]
 #Xip10a3-..... [720]
 #Xip9a1-..... [720]
 #Pra8c3-..... [720]

#C4m1d4	--.....-A.GA-G.GTT.	..-CC..G..	..AT.A..	[720]
#C4m2a2	--.....-A.GA-G.GTT.	..-CC..G..	..AT.A..	[720]
#C4m2b3	--.....-A.GA-G.GTT.	..-C...G..	..AT.A..	[720]
# <i>C.elegans</i>	GA.CGG.T.C	A.CG.G...C	GA..AAGA.C	GCAGC.TGC.	.CGT-T.C	[720]
#Aph3a3	GTATCATCAA	CA-TATTGAT	TTTTTGACCT	GAA--CTCAG	TCCAAGAG	[768]
#Hd6c2A.....--.....	..-G....	[768]
#Rr27a1A.....--.....	..-G....	[768]
#Xip10a3A.....--.....	..-G....	[768]
#Xip9a1A.....--.....	..-G....	[768]
#Pra8c3A.....--.....	..-G....	[768]
#C4m1d4	A..A.TA...	G.ACCAAACA	A.....	...--.....	..-G....	[768]
#C4m2a2	A..A.TA...	G.ACCAAACA	A.....	...--.....	..-G....	[768]
#C4m2b3	A..A.TA...	G.ACCAAACA	A.....	...--.....	..-.....	[768]
# <i>C.elegans</i>	T..C..CG..	TTGC.GACGC	..AGA.TGG.	...ATT..GA	A.GC.T..	[768]
#Aph3a3	CACCCGCTGA	ACTTA-AGCA	TAT-----	-----	-----	[816]
#Hd6c2-	...-----	-----	-----	[816]
#Rr27a1-	...-----	-----	-----	[816]
#Xip10a3-	...-----	-----	-----	[816]
#Xip9a1-	...-----	-----	-----	[816]
#Pra8c3-	...-----	-----	-----	[816]
#C4m1d4-	...-----	-----	-----	[816]
#C4m2a2-	...-----	-----	-----	[816]
#C4m2b3-	...-----	-----	-----	[816]
# <i>C.elegans</i>AA...G	G.C.CC..TT	GG.ACGTCTG	G TTCAGGGTT	GTTTAACT	[816]


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#C.elegans ..G..... ..T..... ..C..... ..... [768]

#Xip9a2 GTGGTGAAAT TTTGAACGCA TAGCGCCGTT GGGTTTTTCCC TTCGGCAC [816]
#Xip10a2 ..... [816]
#Rr27a1 .....A.... [816]
#Aph3a1 ..... [816]
#Hd6d3 ..... [816]
#C.elegans .....C..... ..A..AAC T..GCC...A G.T..T.. [816]

#Xip9a2 GTCTGGTTCA GGGTTGTT-- ATCCGA---C CTA----- CTACAGTG [864]
#Xip10a2 .....-- .....---. ....-..... [864]
#Rr27a1 .....-- .....---. ....-..... [864]
#Aph3a1 .....-- .....---. ....-..... [864]
#Hd6d3 .....-- .....---. ....-..... [864]
#C.elegans .....TA .CT.A.TGC. T..GGCTTCT ..T.G.A. [864]

#Xip9a2 -----TGGC TTGTCACAGA ACACTGTTTG TC---GAATG GTACTTAC [912]
#Xip10a2 -----..... [912]
#Rr27a1 -----..... [912]
#Aph3a1 -----..... [912]
#Hd6d3 -----..... [912]
#C.elegans AGTCTTC... ..GGGC. ...T.AG.GA G.TGA..T.C .CGTC.CG [912]

#Xip9a2 TCACGTAATT CCCATTCTAG TCTAGAAGCA TGAAAG--CA ACATGATA [960]
#Xip10a2 ..... [960]
#Rr27a1 ..... [960]
#Aph3a1 ..... [960]
#Hd6d3 ..... [960]
#C.elegans G..TACTGA. .TTG.C.... ..C..... .C.C.AGT.. .G.CAGAG [960]

#Xip9a2 TTT-----T CTTATAGAAA ATATCCCTGA ATGAT-TCGT ATTGAAAC [1008]
#Xip10a2 ...-----..... [1008]
#Rr27a1 ...-----..... [1008]
#Aph3a1 ...-----.....A. [1008]
#Hd6d3 ...-----..... [1008]
#C.elegans C..CACCGT. ..GTCTTGT. ..G.GA.GCG C.AC.C.TA. ..C.G.GG [1008]

#Xip9a2 GCGTAT---A TGGTGTTCGA TC--ACCCA- -CGATCAT-- ----- [1056]
#Xip10a2 .....-..... [1056]
#Rr27a1 .....-..... [1056]
#Aph3a1 .....-..... [1056]
#Hd6d3 .....-..... [1056]
#C.elegans AGC.GCCAA. ...C..C... CTTG..T.GG CT.....CA AGACGTGT [1056]

#Xip9a2 --AACTA-GT ACTTGATGAA T--AAGTAAG GAAAATTATG TT----- [1104]
#Xip10a2 --.....-..... [1104]
#Rr27a1 --.....-..... [1104]
#Aph3a1 --.....-..... [1104]
#Hd6d3 --.....-..... [1104]
#C.elegans TT....GC.. GTC.T....T GTG...CT.C TGC.TC.... .CAGAATG [1104]

#Xip9a2 CGTGGTCCGC CTTGTTG--T -ATTGAACAC ATGCACCGCT ATGTGCAC [1152]
#Xip10a2 .....-.....G..... [1152]
#Rr27a1 .....-.....G..... [1152]
#Aph3a1 .....-.....G..... [1152]
#Hd6d3 .....-.....G..... [1152]
#C.elegans T..C...GCT .C.T.C.AA. AC.G.G.TT. G.CT.GTCTC G....TGT [1152]

#Xip9a2 ATATACGCCG ATTCGAC--- --AACGATTC ATTGCTAA-- ----- [1200]

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#Xip10a2      .....--- --..... -- ..... [1200]
#Rr27a1      .....--- --..... -- ..... [1200]
#Aph3a1      .....--- --..... -- ..... [1200]
#Hd6d3       .....--- --..... -- ..... [1200]
#C.elegans   G.TG.TAT.. .A.TA.TTTT CG.TT...G. GGC...G.GA AGAGAGAC [1200]

#Xip9a2      -----ATTG CAACCTGAAC TCTGGCGTGA TTACCCGC [1248]
#Xip10a2     -----..... [1248]
#Rr27a1      -----..... [1248]
#Aph3a1      -----..... [1248]
#Hd6d3       -----..... [1248]
#C.elegans   GGTGCGTGTC TTGCTA..CT ..... ..A.T..... [1248]

#Xip9a2      TGAACTTAAG CATAT [1263]
#Xip10a2     ..... [1263]
#Rr27a1      ..... [1263]
#Aph3a1      ..... [1263]
#Hd6d3       ..... [1263]
#C.elegans   ..... [1263]

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