REFFERENCES

Agrawal, N., Dasaradhi, P., Mohommed, A., Malhotra, P., Bhatnagar, R. K. and Mukherjee, S. K. (2003) RNA interference: Biology, mechanism, and applications. *MMBR*. 67: 657-685.

Alicja, Z. (2001). Plant selectable markers and reporter genes. *APP.* 23: 363-374.

Angela, F., Fabio, F. and Chris, B. (2002). Reporter genes and *in vivo* imaging. In P.M. Gilmartin and C. Bowier (eds.), β-glucuronidase (GUS), pp.265-270. New York: Oxford Universty Press.

Arenz, C. and Schepers, U. (2003). RNA interference: from an ancient mechanism to a state of the art therapeutic application. *Naturwissenschaften* 90: 345-359.

Bartel, D. P. (2004). MicroRNAs: Genomics, biogenesis, mechanism and function. *Cell* 116: 281-297.

Bernstein, E., Caudy, A., Hammond, S. and Hannon, G. (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409: 363-366.

Binns, A. N. and Thomashow, M. F. (1988). Cell biology of *Agrobacterium* infection and transformation of plants. *Annu. Rev. Microbiol.* 42: 575-606.

Birch, R. G. (1997). Plant transformation: Problem and strategies for practical application. *Annul. Rev. Plant Physiol. Plant Mol. Biol.* 48: 297-326.

Bradford, M. M. (1976). A rapid and sensitive method for the quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.

Brant, S. (2002) Antisense-RNA regulation and RNA interference. *BBA.* 1575: 15-25.

Brummell, D. A., Balint-Kurti, P. J., Harpster, M. H., Palys, J. M., Oeller, P. W. and Gutterson, N. (2003). Inverted repeat of a heterologous 3′-untranslated region for high-efficiency, high-throughput gene silencing in plants. *Plant J.* 33: 793-800.

Citovsky, V., Zupan, J., Warnick, D. and Zambryski, P. (1992). Nuclear localization of *Agrobacterium vir*E2 protein in plant cells. *Science* 256: 1802-1805.

Crazzolara, C. S., Klem, M. and Reiss, B. (1995). Method in cell biology. California: Academic Press. pp. 425-438.

Dongqin, T., Hongmei, Q., Lingxia, Z., Danfeng, H. and Kexuan, T. (2005). Transgenic tobacco plants expressing *BoRS1* gene from *Brassica oleracea* show enhanced tolerance to water stress. *J. Biosci.* 30: 647-655.

Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured Mammalian cells. *Nature* 411: 494-498.

Enriquez-Obrigon, G. A., Vazquez-padron, R. I., Pretio-sansonov, D. L., de la Riva, G. A. and Selman-Housein, G. (1998). Herbicide resistant sugarcane (Saccharum officinarum L.) plants by *Agrobacterium*-mediated transformation. *Planta* 206: 20-27.

Filichkin, S. A. and Gelvin, S. B. (1993). Formation of a putative relaxation intermediate during T-DNA processing directed by the *Agrobacterium tumefaciens vir*D1, *vir*D2 endonuclease. *Mol. Microbiol.* 8: 915-926.

Galun, E. and Breiman, A. (1997). Tansgenic plants. London, Imperial College Press. pp. 44-75.

Gregory, R. I., Chendrimada, T. P., Cooch, N. and Shiekhattar, R. 2005. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. *Cell* 123: 631-640.

Guo, H. S., Fei, J. F., Xie, Q. and Chua, N. H. (2003). A chemical-regulated inducible RNAi system in plant. *Plant J.* 34: 383-392.

Hamilton, A. J. and Baulcombe, D. C. (1999). A species of small antisense RNA in post-transcriptional gene silencing in plants. *Science* 286: 950-952.

Hammond, S., Bernstein, E., Beach, D. and Hannon, G. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. *Nature* 404: 293-296.

Hiei, Y., Komari, T. and Kubo, T. (1997). Transformation of rice mediated by *Agrobacterium tumefaciens*. *Plant Mol. Biol.* 35: 205-218.

Hirotaka, K. and Hiroshi, K. (2003). Gene silencing by expression of hairpin RNA in *Lotus japonicus* roots and root nodules. *MPMI.* 16: 663-668.

Howard, E. A., Zupan, J., Citovsky, V. and Zambryski, P. (1992). The *vir*D2 protein of *A.tumefaciens* contains a C-terminal bipartile nuclear localization signal: Implications for nuclear uptake of DNA in plant cells. *Cell* 68: 109-118.

Hutvagner, G. and Zamore, P. D. (2002). RNAi: nature abhors a double-strand. *Curr. Opin. Genet. Dev.* 12: 225-232.

Kahl, G. and Weising, K. (1993). Biotechnology. In H. J. Rehm and G. Reed (eds.), Genetic engineering of plant cells, pp. 547-625. New York: VCH Publisher.

Karen, M., Vicki, C., Karen, C., Heidi, K. Shwan, K., Arthur, K., Craig, P., Lyudmila, S. Eric, R., Todd, S. Nathan, S. and Tuya, W. (2005). Methods in enzymology, vol. 392, pp. 1-18. California: Elesvier Academic Press.

Klee, H., Horsch, B. and Rogers, S. G. (1987). *Agrobacterium*-mediated plant transformation and its further applications to plant biology. *Annu. Rev. Plant Physiol.* 38: 467-486.

Knee, R. and Murphy, P. R. (1997). Regulation of gene expression by natural antisense RNA transcripts. *Neurochemistry International.* 31: 379-392.

Kohler, R. H., Cao, J., Zipfel, W. R., Webb, W. W. and Hanson, M. (1997). Exchange of protein molecules through connections between higher plant plastids. *Science* 276: 2039-2042.

Koncz, C., Nemeth, K., Redei, G. P. and Scell, J. (1994). Homologous recombination and gene silencing in plants. Natherlands: Kluwer, Dordrechtp.

Laurian, S. R., Pauline, A. D., Christine, L. Illimar, A., Paul, G. A. and Steven F. F. (1989). Antisense RNA inhibition of β-glucuronidase gene expression in transgenic tobacco plants. *Plant Mol. Biol.* 13: 399-409.

Lawrence, R. J., and Pikaard, C. S. (2003). Transgene-induced RNA interference: A strategy for overcoming gene redundancy in polyploids to generate loss-of-function mutations. *Plant J.* 36: 141-121.

Lipardi, C., Wei, Q. and Paterson, B. M. (2001). RNAi as random degradative PCR: siRNA primers convert mRNA into dsRNAs that are degraded to generate new siRNAs. *Cell* 107: 297-307.

Louisa, M. (2004). RNAi for plant functional genomics. *Comparative Function Genome.* 5: 240-244.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular cloning: A laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Mansoor, S., Amin, I., Hussain, M., Zafar, Y. and Briddon, R. W. (2006). Engineering of novel traits in plants through RNA interference. *Trends Plant Sci.* 11, 559-565.

Matt, W., Gregory, C., Kanagasabapathi, S. and Ahmad S. I. (2004). RNA interference and its application in crop improvement. pp.1-18.

Marathe, R., Anandalakshmi, R., Smith, T. H., Pruss, G. J., and Vance, V. B. (2000) RNA viruses as inducers, suppressors and targets of post-transcriptional gene silencing. *Plant Mol. Biol.* 43: 295-306.

Matzke, M., Aufsatz, W., Kanno, T., Daxinger, L., Papp, I., Mette, M. F., and Matzke, A. J. M. (2004) Genetic analysis of RNA-mediated transcriptional gene silencing. *BBA.* 1677: 129-141.

Muhammad, S., Nawaz, U. R., Shahid, M., Asif, A. K. Yusuf, Z. and Rob, W. B. (2007). RNAi-mediated male sterility of tobacco by silencing TA29. *Mol. Biotechnol.* 36: 159-165.

Napoli, C., Lemieux, C. and Jorgensen, R. (1990). Introduction of chimeric chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous gene in trans. *Plant Cell* 2: 279-289.

Nelson, P., Kiriakidou, M., Sharma, A., Maniataki, E., and Mourelatos, Z. (2003). The microRNA world: small is mighty. *TiBS.* 28: 534-540.

Preall, J. B., He, Z., Gorra, J. M. and Sontheimer, E. J. (2006). Short interfering RNA strand selection is independent of dsRNA processing polarity during RNAi in Drosophila. *Curr. Biol.* 16: 530-535.

Rovere, C. V., del Vas, M., and Hopp, H. E. (2002). RNA-mediated virus resistance. *Curr. Opin. Biotechnol.* 13: 167-172.

Sadaf, K. and Julie, M. S. (2007). *Arabidopsis thaliana GH3.9* influences primary root growth. *Planta* 226: 21-34.

Sasha, D., Alex, M., Nigel, S., Harry, V. O. and Malcolm, E. (2007). Effect of seed-specific expression of the ipt gene on *Nicotiana tabacum* L. seed composition *J. Plant Growth Regul.* 51: 217-229.

Sambrook, J., and Russell, W. D. (2001). Molecular cloning: A laboratory manual. 3rd. New York: Cold Spring Harbor Laboratory Press.

Schramke, V. and Allshire, R. (2004). Those interfering little siRNAs: Silencing and eliminating chromatin. *Curr. Opin. Genet. Dev.* 14: 174-180.

Sen, G. L., Wehrman, T. S. and Blau, H. M. (2005). mRNA translation is not a prerequisite for small interfering RNA-mediated mRNA cleavage. *Differentiation*. 73: 287-293.

Smith, N. A., Singh, P., Wang, M. B., Stoutjesdijk, P. A., Green, A. G. and Waterhouse, P.M. (2000). Total silencing by intron spliced hairpin RNAs. *Nature* 407: 319-320.

Stachel, S. E. and Nester, E. W. (1986). The genetic and ranscriptional organization of the *vir* region of the A6 Ti plasmid of *Agrobacterium tumefaciens*. *EMBO J.* 5: 1445-1454.

Stoutjesdijk, P. A., Singh, S. P., Liu, Q., Hurlstone, C. J., Waterhouse, P. A. and Green, A.G. (2002) hpRNA-mediated targeting of Arabidopsis FAD2 gene gives highly efficient and stable silencing. *Plant Physiol.* 129: 1723-1731.

Tinland, B. (1996). The integration of T-DNA into plant genomes. *Trends Plant Sci.* 1: 178-184.

Walden, R. (1993). Cell culture, transformation and gene technology. In P. J. Lea and R. C. Leegood (eds.), Plant biochemistry and molecular biology, pp. 275-295. England: John Wiley & Sons.

Waterhouse, P., Graham, M. and Wang, M. B. (1998). Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc. Natl. Acad. Sci.* USA. 95: 13959-13964.

Wesley, S. V., Helliwell, C. A., Smith, N. A., Wang, M. B., Rouse, D. T., Gooding, P. S. Liu, Q., Singh, S. P., Abbott, D., Stoutjesdijk, P. A., Robinson, S. P., Gleave, P., Green, A. G. and Waterhouse, P. M. (2001). Construct design for efficient, effective and high-throughput gene silencing in plants. *Plant J.* 27: 581-590.

Winans, S. C. (1992). Two-way chemical signaling in *Agrobacterium*-plant interaction. *Microbiol Rev.* 34: 913-922.

Zambryski, P., (1988). Basic processes underlying *Agrobacterium*-mediated DNA transfer to plant cells. *Annul. Rev. Genet.* 22: 1-30.

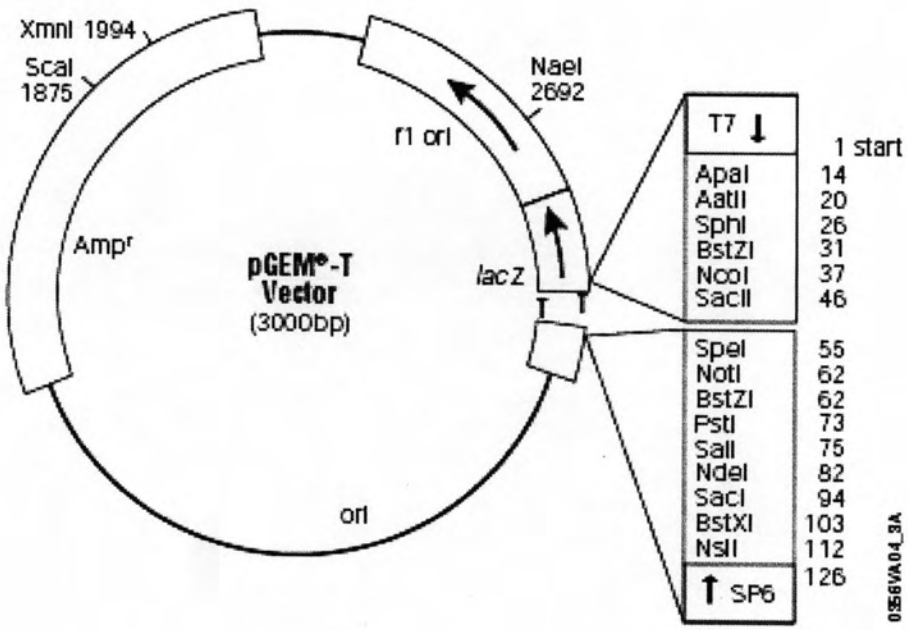
Zhen, W., Changbin, C., Yunyuan, X., Rongxi, J., Zhihong, X. and Kang, C. (2004). A practical vector for efficient knockdown of gene expression in rice (*Oryza sativa* L.). *Plant Mol. Biol. Reptr.* 22: 409-417.

Zupan, J. R. and Zambryski, P. (1995). Transfer to DNA from *Agrobacterium* to the plant cell. *Plant Physiol.* 107: 1041-1047.

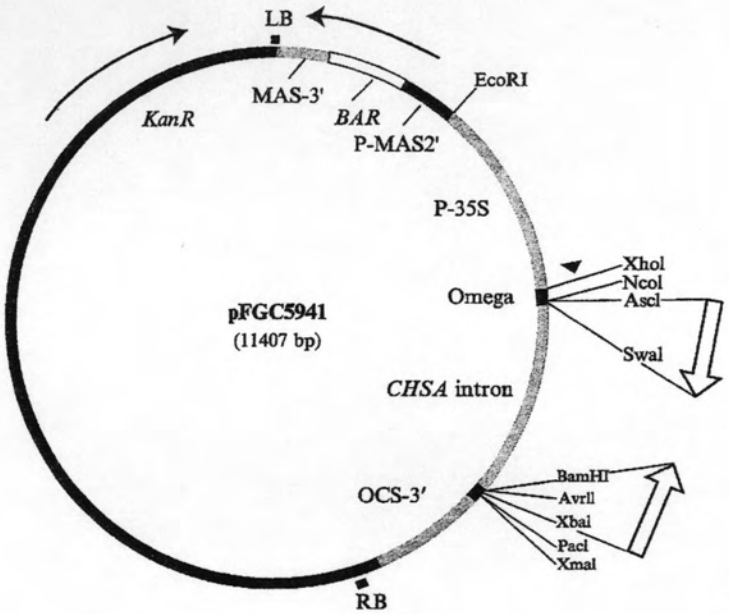
APPENDICES

APPENDIX A

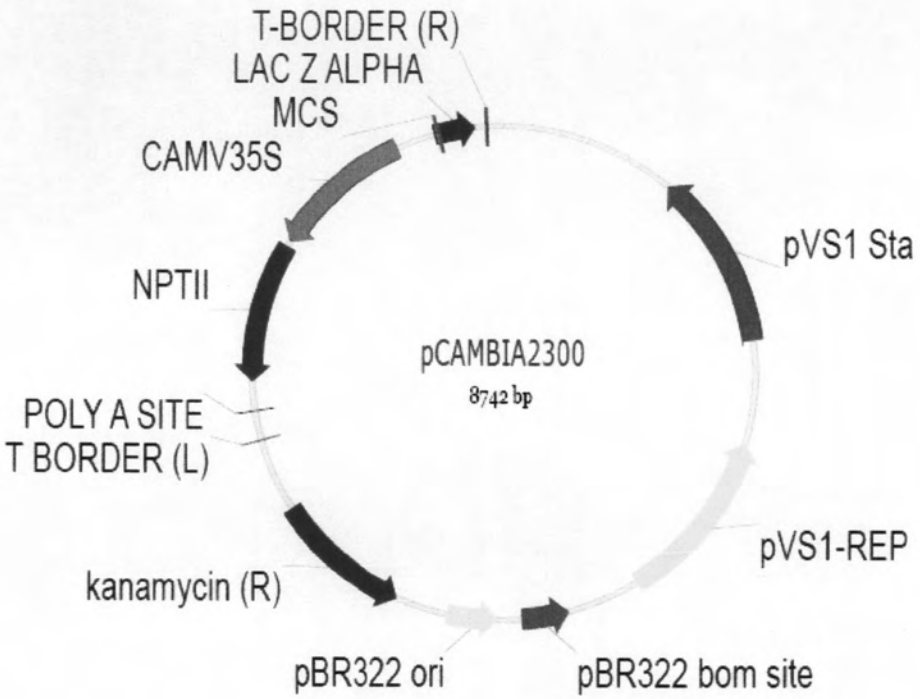
Circle map of pGEM®-T Vector



Circle map of pFGC5941



Circle map of pCAMBIA2300



APPENDIX B

The composition of tobacco tissue culture

Table 1 The composition of MS medium (Murashige and Skoog, 1962)

Solution	Chemicals	Concentration (mg/l)
Macronutrients	NH_4NO_3	1,650
	KNO_3	1,900
	$CaCl_2.2H_2O$	440
	KH_2PO_4	170
	$MgSO_4.7H_2O$	370
Micronutrients	KI	0.83
	H_3BO_3	6.2
	$CoCl_2.6H_2O$	0.025
	$MnSO_4.7H_2O$	16.9
	$ZnSO_4.7H_2O$	8.6
	$Na_2MoO_4.7H_2O$	0.25
	$CuSO_4.5H_2O$	0.025
FeEDTA	$FeSO_4.7H_2O$	27.8
	$Na_2EDTA.2H_2O$	37.8
B5 vitamins	Myo-inosital	100
	Nicotinic acid	0.5
	Pyridoxine HCl	0.5
	Thiamine HCl	0.1
	Glycine	2
	Sucrose	30,000
	Agar	8,000

pH 5.8

Table 2 The composition of CM medium (selection medium)

MS medium supplemented with

Chemicals	**concentration (mg/l)**
BAP	1 mg/l
NAA	1 mg/l

pH 5.8

Table 3 The composition of MS-selection medium

CM medium supplemented with

Chemicals	**Concentration (mg/l)**
Cefotaxime	250
Hygromycin	50

pH 5.8

Table 4 The composition of MS-shoot induction medium

MS medium supplemented with

Chemicals	**Concentration (mg/l)**
BAP	1
Cefotaxime	250

pH 5.8

APPENDIX C

Chemical solution

1. β-Glucoronidase (GUS) assays

Staining Solution

100 mM NaH_2PO_4/Na_2HPO_4 pH7.5	10 ml of 0.2 M
0.1% Triton X-100	200 µl of 10% (v/v)
10 mM EDTA	400 µl of 0.5 M
1 mM X-Gluc	1 ml of 20 mM in DMF (0.104 g/10 ml)
0.5 mM K Ferricyanide	1 ml of 10 mM (33 mg/10 ml H_2O)
0.5 mM K Ferrocyanide	1 ml of 10 mM (44 mg/10 ml H_2O)
H_2O	6.4 ml

A time-saving alternative

Mix phosphate buffer, EDTA, Triton X-100, and H_2O in the propotions given above and store at room temperature. Just before using, prepare the K-Ferricyanide and K-Ferrocyanide stocks. Mix the components in the following proportions.

Component	For 5 ml
Buffer Stock	4.25
K-Ferro Stock	0.25
K-Ferri Stock	0.25
X-Gluc Stock	0.25

2. Determination of protein concentration

Bradford solution:

Coomassie Brilliant Blue (G250)	100 mg
Absolute ethanol	50 ml

Stir in a container protected from light for 2 hours.

Add 100 ml of 85% phosphoric acid.

Mix, and adjust volume to 1 L with dH_2O. Filter through Whatman No.1 pape and store the solution at room temperature in a brown glass bottle (usable for several weeks).

3. Northern blot hybridization

1 M Tris/HCl pH8:

Tris base (Trizma)	60.5 g
H_2O	400 ml

Stir the solution to dissolve Tris completely, adjust pH to 8.0 with 6 N HCl and adjust the volume to 500 ml with distilled water. Filter through a sterile, 0.22 μm nitrocellulose filter and autoclave for 20 minutes at 121 °C

100X Denhardt' Solution:

Prepare and store in a sterilized container

Bovine serum albumin (BSA)	2 g
(Sigma# A 6003-fatty acid free)	
Polyvinyl pyrrolidone (PVP)	2 g
(Sigma# PVP40, avg.mol.wt. 40,000)	
Ficoll(type 400, Sigma# F4375)	

Sterile ddH_2O	80 ml

Dissolve with rapid stirring and adjust the volume to 100 ml with sterile distilled water. Filter through a sterile, 0.22 μm nitrocellulose filter and store at 4 °C

Denatured DNA stock (5mg/ml):

DNA (*calf thymus- Sigma# D1501)	500 mg
TE buffer pH 8	75 ml

Dissolve by vortexing, shaking, and then by sonicating. Sonicate 5 to 10 times in 30 second bursts at a power setting that gibes cavitations, but no forming. Chill the DNA solution in an ice H_2O bath between each sonication. Shake the DNA at moderate speed on a shaker at room temperature until the DNA is completely in solution. Dialyze 4 times for 4 hours each at 4 °C against 20 volumes of TE pH 8. Take 10 μl and dilute to 1 ml for A_{260} (A_{260}=50 μg/ml). Dilute to 5 mg/ml with TE pH 8. Store at 4 °C.

20% (w/v) SDS:

Sodium dodecylsulfate	20 g
Sterile ddH_2O	100 ml

Stir rapidly to dissolve completely and filter through a sterile, 0.22 μm nitrocellulose filter.

Prehybridization Solution:

Formamide	50 ml (50% v/v)
20X SSPE	25 ml (0.75M Na^+)
100X Denhardt's	5 ml (5X)

Denatured DNA stock	2 ml (100 μg/ml)
H_2O	17 ml
20% (w/v) SDS	1 ml (0.2%w/v)

Add components, in the order given, and mix well. Filter through a 5 μm Teflon filter.

Hybridization Solution:

Formamide	50 ml (50% v/v)
20X SSPE	25 ml (0.75M Na^+)
100X Denhardt's	1 ml (5X)
H_2O	23 ml
20% (w/v) SDS	1 ml (0.2%w/v)

4. Oligolabeling DNA fragment

10 X buffer

500 mM Tris-HCl pH 6.9	0.5 ml of 1 M stock
1000 mM $MgSO_4$	100 μl of 1M stock
1 mM Dithiothreitol	1μl of 1M stock
600 μM each of dGTP, dATP and dTTP	6 μl of 100 mM stock

Adjust the volume to 1 ml with sterile distilled water.

Scintillation Fluid (500 ml):

PPO	2 g
POPOP	0.025 g
Toluene	333.5 ml

Triton	166.5 ml

5. Formadehyde gels for fractionating RNA and northern blotting

Gel Buffer 40X:

Triethanolamine	23.6 g (1.6 M)
$Na_2EDTA.2H_2O$	29.8 g (80 mM)
ddH_2O	500 ml

Adjust pH to 7.5 with 85% H_3PO_4, bring the volume to 1 liter with distilled water and autoclave.

Sample Buffer 1.25X:

Gel buffer 40X	25 µl
Formadehyde	165 µl
Formamide	500 µl
50% (v/v) glycerol	110 µl
Bromophenol blue	a few crystals to give
Xylene cyanal	a strong blue color

Adjust the volume to 1 ml with sterile distilled water.

Diethylpyrocarbonate Solution:

In a 250 ml flask with a stir bar, in the hood.

ddH_2O	100 ml
20% (w/v) SDS	5 ml
Diethyl pyrocarbonate	100 µl

Stir rapidly until diethyl pyrocarbonate has dissolved. Use within 10 minutes to clean gel box, casting stand, glass plates, slot-forming combs.

Reservior Buffer:

Gel Buffer 40X	12.5 ml
Formadehyde	42 µl

Bring the volume to 500 ml distilled water.

0.2 M Phosphate Buffer Stock:

A. $Na_2 HPO_4.7H_2O$	26.8 g

Dissolve in a final volume of 500 ml ddH_2O

B. $NaHPO_4.H_2O$	13.8 g

Dissolve in a final volume of 500 ml ddH_2O

Place solution A in a 1000 ml beaker with a stir bar on a magnetic stirrer. Add solution B with stirring until the pH reaches 7.0.

Ethidium Bromide Stock:

Ethidium Bromide	1 g
H_2O	100 ml

Stir several hours with a magnetic stirrer and store in a dark bottle at 4 °C.

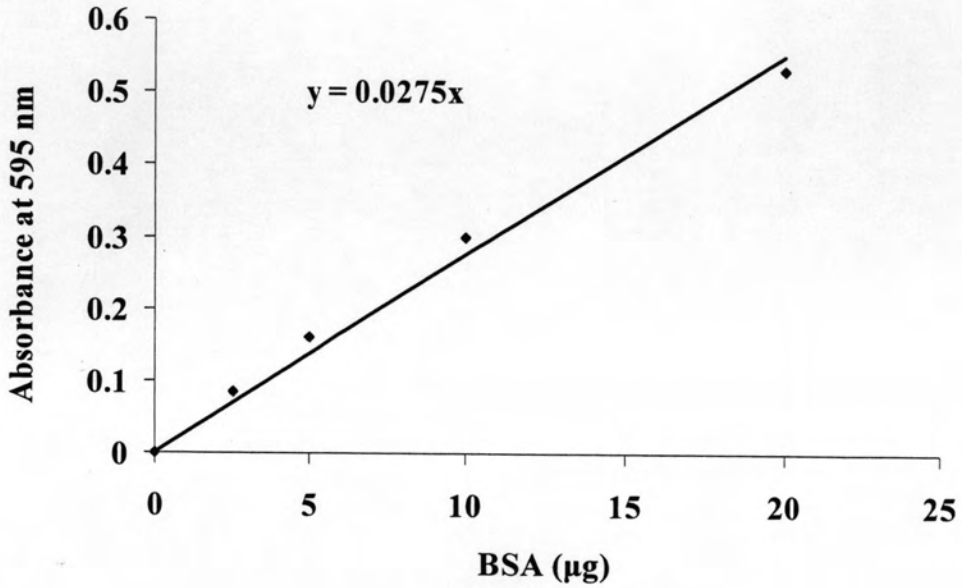
DEPC-treated H_2O:

H_2O	100 ml
DEPC	0.5 ml

Shake vigorously and leave open under a hood overnight and autoclave.

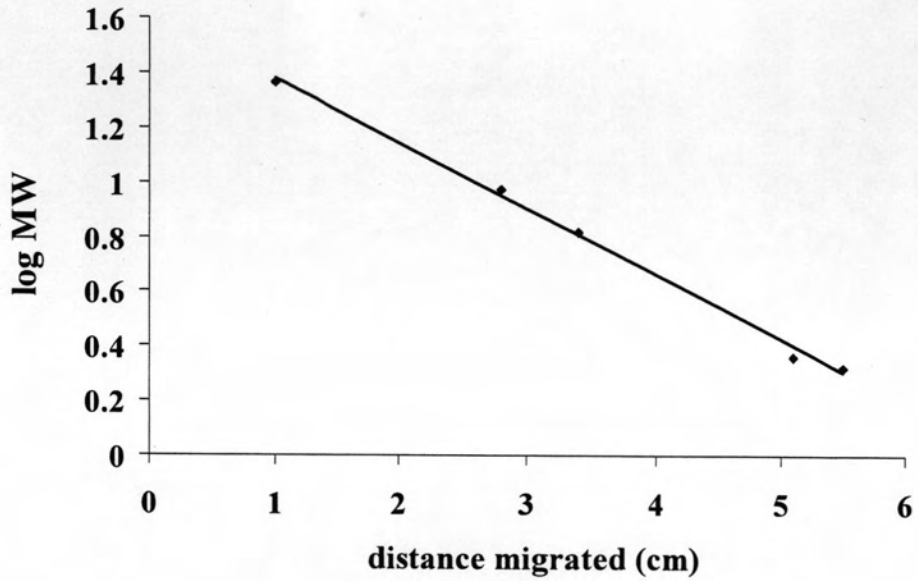
APPENDIX D

Standard curve for protein determination by Bradford's method



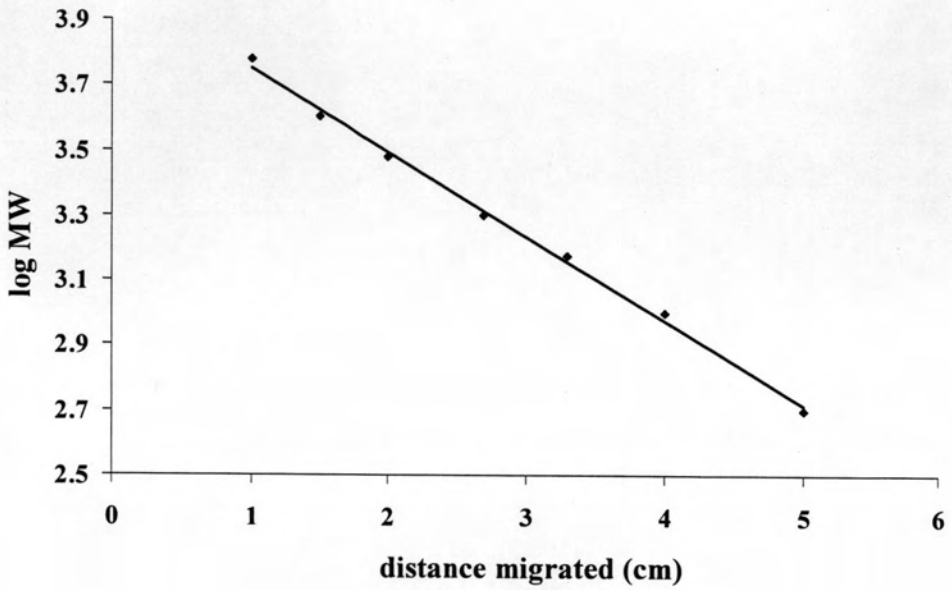
APPENDIX E

Standard curve for molecular weight determination in southern blot analysis



APPENDIX F

Standard curve for molecular weight determination in northern blot analysis



BIOGRAPHY

Mr. Veerakorn Hotimavorakul was born on February 4th, 1982 in Bangkok. After graduating with degree of Bachelor of Science from the department of Biology at Mahidol University in 2003, he keeps on studying for the Master of Science at the Biochemistry Program, Faculty of Science at Chulalongkorn University in that year.