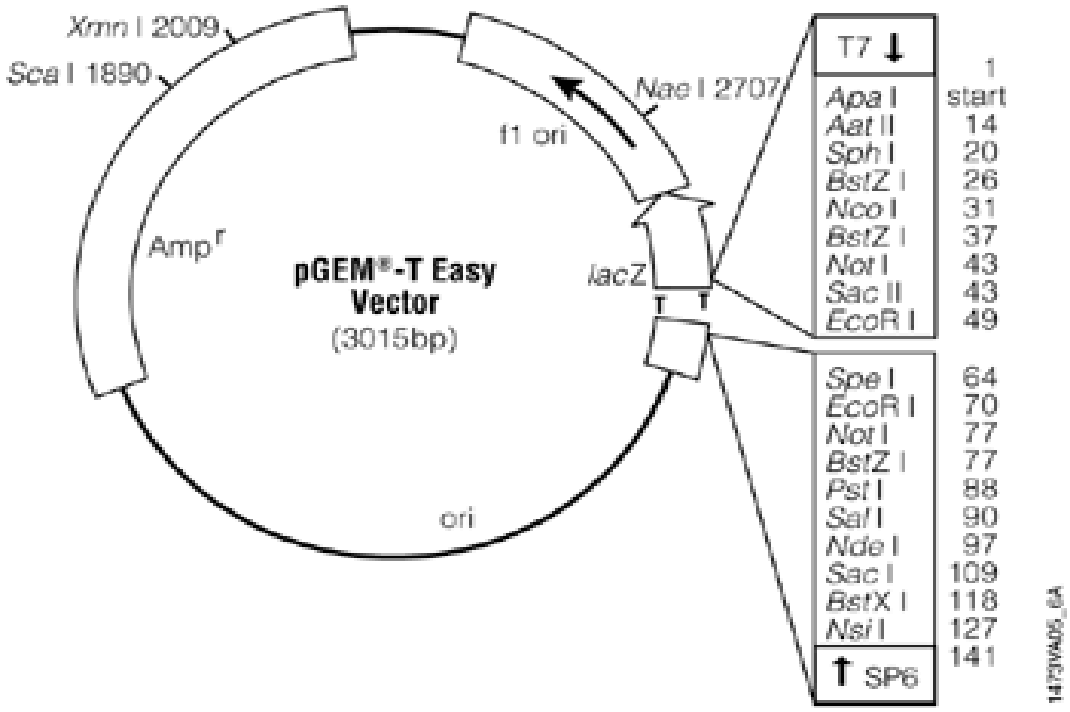
APPENDIX 1: pGEM-T-Easy vector Map



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APPENDIX 2: REAGENTS AND SOLUTIONS

Solution	Composition
10X TBE	0.9 M Tris (pH 8.3) 0.89 M Boric acid 25 mM EDTA
DNA loading buffer	30% Glycerol (v/w), 15mM EDTA (pH 8), 0.05% Bromophenol Blue (w/v)
Luria broth	1% Bacto-tryptone 0.5% Yeast Extract 1% NaCl
Ampicillin	0.1g Ampicillin in 1 ml sdH_2O
Formaldehyde gel loading buffer	720 µl formamide, 160 µl 10X MOPS buffer, 260 µl 37% formaldehyde, 100µl sdH_2O, 100 µl EtBr (10 mg/ml, 80µl BPB in DEPC H_2O.
GTE	1% glucose, 50mM Tris HCl, 10 mM EDTA
0.1 M PBS, pH 7.4 (w/v)	5 PBS tablets in 1000ml H_2O
20X SSC	175.3 g Trisodium citrate in 800 ml DEPC H_20, pH 7 with HCL and add up to a litre.
0.1 % v/v DEPC treated H_20	1 ml DEPC to 1 L dH_2O, shake overnight at 37^oC and autoclave.

TBS	1 M Tris (pH 7.5),5 M NaCl in DEPC H_2O
0.1 M Sodium Citrate, pH 6	29.4 g Tri-sodium citrate in 800 ml dH_2O, pH 6.0 add up to 1litre
10X TE	100 mM Tris-HCl (pH 7.5); 10 mM EDTA
4 m Lithium chloride	8.478 g LiCl in 50 ml DEPC H_20,store at room temperature
0.5 M EDTA, pH 8	18.612 g EDTA in 50 ml DEPC H_2O with continuous stirring and constant addition of NaOH drops until pH 8.0, add up to 100 ml with DEPC H20.
10 mg/ml Proteinase K	10 mg Proteinase K in 1000 µl DEPC H_20
10% SDS	10 g SDS in 100 ml DEPC H_2O
10X MOPS	200mM MOPS, 10mM EDTA, 50mM Sodium Acetate
0.1 M HCL	10 ml of 9M (32%) HCl in 990 ml DEPC H_20
0.5% Acetic Anhydride	0.25 ml Acetic Anhydride 49.75 ml Tris (pH 8)
Hybridization Buffer	40 g Dextran sulphate sodium salt in 70 ml DEPC H_20 and heat at 68^oC for 3-4 hours and add up to 100ml, store at 4^oC
0.2 M Phosphate buffer	17.02 g Na_2HPO_4 and 12.48 g NaH_2PO_4 in

	DEPC H20
0.85% Nacl	8.5 g NaCl in 1000 ml dH_2O,autoclave store at room temperature
4% PFA	4g PFA in 100 ml 0.1 M Phosphate buffer by slow heating to 60^oC, until solution becomes clear.
1 M Tris pH 8.0	Dissolve 121 g Trizma Base in 800 ml DEPC H_20, pH 8.0 add up to 1litre.
1 M Tris pH 7.5	121 g Trizma Base in 800 ml DEPC H_2O, pH 7.5 add up to 1 litre.
5 m NaCl	292.2 g NaCl in 1000 ml DEPC H_20
Biotinylated Nucleotide Mix	250 mM Biotinylated Nucleotide Mix 10 mM Tris-HCL (pH 7.6) 1 mM EDTA
Cell Resuspension Solution	500 mM Tris-HCL (pH 7.5) 10 mM EDTA 100 µg/ml RNase A
Cell Lysis Solution	0.2 M NaOH and 1% SDS
Neutralization Solution	4.09 M Guanidine Hydrochloride 0.75 M Potassium Acetate 2.12 M Glacial Acetic Acid (pH 4.2)
Column Wash Solution	60% Ethanol 60 mM Potassium Acetate

	8.3 mM Tris-HCL (pH 7.5) EDTA
Membrane Wash Solution	10 mM Potassium Acetate (pH 5) 80% Ethanol 16.7 mM EDTA (pH 8)
Membrane Binding Solution	4.5 M Guanidine Isothiocyanate 0.5 M Potassium Acetate (pH 5)
10X Detection Buffer	1 M Tris-HCL (pH9.5) 1 M NaCl
Equilibration Buffer	200 mM Potassium Cacodylate 25 mM Tris-HCL (pH 6.6) 0.2 mM DTT 2.5 mM Cobalt Chloride 0.25 mg/ml BSA
Lysis and Binding Buffer	4.5 M Guanidine Hydrochloride 50 mM Tris-HCl 30% Triton X-100 (w/v), pH 6.6
DNase I	10 kU lyophilized DNase I
DNase incubation buffer	1M NaCl 20 mm Tris-HCl 10 mm $MnCl_2$, pH 7.0
Wash Buffer I	5 M Guanidine hydrochloride 20 mM Tris-HCl, pH 6.6

Wash Buffer II	20 mM NaCl 2 mm Tris-HCl, pH 7.5
Elution Buffer	Nuclease-free H_2O

APPENDIX 3: Structure of DWNN gene with the arrows indicating primer sites. A- 5' 1.1 kb, B- 3' 1.1 kb, C- 3'6.1 kb and D- exon 16.