



**APPENDIX A THE BLASTN RESULT BETWEEN THE 470 bp DFR
CANDIDATE SEQUENCE AND DENDROBIUM HYBRID DFR**

Dendrobium hybrid cultivar dihydroflavonol-4-reductase (dfr)
mRNA, complete cds
Sequence ID: gb|FJ426271.1|Length: 1164Number of Matches: 1
Related Information
Range 1: 381 to 813GenBankGraphicsNext MatchPrevious Match

Query	16	AAAGGC-GGC-GCGTTCAGCGAGTGATATTCACGTCTTCTGCAGGAACAGTAAATGTGGA	73
Sbjct	381	AAAGGCCGGCAGCGTTCAGCGAGTGATATTCACGTCTTCTGCAGGAACAGTAAATGTGGA	440
Query	74	GGAACACCAAGCAGCAGTGTATGACGAGAGCTGCTGGAGTGACCTTGACTTCGTCAACCG	133
Sbjct	441	GGAACACCAAGCAGCAGTGTATGACGAGAGCTGCTGGAGTGACCTTGACTTCGTCAACCG	500
Query	134	AGTCAAGATGACCGGTTGGATGTACTTCTGTCAAAAACACTTGCTGAGAAGGCTGCTTG	193
Sbjct	501	AGTCAAGATGACCGGTTGGATGTACTTCTGTCAAAAACACTTGCTGAGAAGGCTGCTTG	560
Query	194	GGAGTTTGTGAAGGACAATGACATTCATTTAATAACCATTATTCCGACTTTGGTGGTGGG	253
Sbjct	561	GGAGTTTGTGAAGGACAATGACATTCATTTAATAACCATTATTCCGACTTTGGTGGTGGG	620
Query	254	GTCCTTCATAACATCTGAAATGCCACCAAGCATGATCACTGCACTATCATTAATTACAG	313
Sbjct	621	GTCCTTCATAACATCTGAAATGCCACCAAGCATGATCACTGCACTATCATTAATTACAG	680
Query	314	AAATGATGCCCATTAATCAATTTTAAAGCAAGTTCAATTTGTTTCAATTTGGATGACGTATG	373
Sbjct	681	AAATGATGCCCATTAATCAATTTTAAAGCAAGTTCAATTTGTTTCAATTTGGATGACGTATG	740
Query	374	TGATGCTCACATTTTCTTTTGGAGCATCCCATAGGAAATGGTAGATACATTTGCTCTTC	433
Sbjct	741	TGATGCTCACATTTTCTTTTGGAGCATCCCAAGCAAATGGTAGATACATTTGCTCTTC	800
Query	434	CTATGACTCCACA	446
Sbjct	801	CTATGACTCCACA	813

Dendrobium hybrid cultivar mRNA for dihydroflavonol-4-reductase
(dfr gene), cultivar Ear sa kul

Sequence ID: emb|FM209431.1|Length: 1059Number of Matches: 1

Related Information

Range 1: 351 to 784GenBankGraphicsNext MatchPrevious Match

Query	16	AAAGGC-GGC-GCGTTCAGCGAGTGATATTCACGTCTTCTGCAGGAACAGTAAATGTGGA	73
Sbjct	351	AAAGGCCGGCAGCGTTCAGCGAGTGATATTCACGTCTTCTGCAGGAACAGTAAATGTGGA	410
Query	74	GGAACACCAAGCAGCAGTGTATGACGAGAGCTGCTGGAGTGACCTTGACTTCGTCAACCG	133
Sbjct	411	GGAACACCAAGCAGCAGTGTATGACGAGAGCTGCTGGAGTGACCTTGACTTCGTCAACCG	470
Query	134	AGTCAAGATGACCGGTTGGATGTACTTCCTGTCAAAAACACTTGCTGAGAAGGCTGCTTG	193
Sbjct	471	AGTCAAGATGACCGGTTGGATGTACTTCCTGTCAAAAACACTTGCTGAGAAGGCTGCTTG	530
Query	194	GGAGTTTGTGAAGGACAATGACATTCATTTAATAACCATTATTCGGACTTTGGTGGTGGG	253
Sbjct	531	GGAGTTTGTGAAGGACAATGACATTCATTTAATAACCATTATTCGGACTTTGGTGGTGGG	590
Query	254	GTCCTTCATAACATCTGAAATGCCACCAAGCATGATCACTGCACTATCATTAAATTACAGG	313
Sbjct	591	GTCCTTCATAACATCTGAAATGCCACCAAGCATGATCACTGCACTATCATTAAATTACAGG	650
Query	314	AAATGATGCCCATTAATCAATTTTAAAGCAAGTTCAATTTGTTCAATTTGGATGACGTATG	373
Sbjct	651	GAATGATGCCCATTAATCAATTTTAAAGCGAGTTCAATTTGTTCAATTTGGATGACGTATG	710
Query	374	TGATGCTCACATTTTCCTTTTGGAGCATCCCATAGGAAATGGTAGATACATTTGCTCTTC	433
Sbjct	711	TGATGCTCACATTTTCCTTTTGGAGCATCCCAAAGCAATGGTAGATACATTTGCTCTTC	770
Query	434	CTATGACTCCACAG	447
Sbjct	771	CTATGACTCCACAG	784

Dendrobium moniliforme dihydroflavinol reductase (DFR) mRNA, complete cds

Sequence ID: gb|HQ412559.1|Length: 1056Number of Matches: 1

Related Information

Range 1: 357 to 783GenBankGraphicsNext MatchPrevious Match

Query 21 CGGC-GCGTTCAGCGAGTGATATTCACGTCTTCTGCAGGAACAGTAAATGTGGAGGAACA 79
 |||| |||| ||||||||||||||||||||||||||||||||||||||||| 416
 Sbjct 357 CGGCAGCGTACAGCGAGTGATATTCACGTCTTCTGCAGGAACAGTGAACGTGGAGGAACA

Query 80 CCAAGCAGCAGTGTATGACGAGAGCTGCTGGAGTGACCTTGACTTCGTCAACCGAGTCAA 139
 ||||||||||| ||||||||||| ||||||||||||||||||||||||| 476
 Sbjct 417 CCAAGCAGCAGCGTATGACGAGACCTGCTGGAGTGACCTTGACTTGTGAACCGAGTCAA

Query 140 GATGACCGGTTGGATGTA CTTCTGTCAAAAACACTTGCTGAGAAGGCTGCTTGGGAGTT 199
 ||||||||||| ||||||||||| ||||||||||||||||||||||||| 536
 Sbjct 477 GATGACCGGTTGGATGTA CTTCTGTCAAAAACACTTGCTGAGAAGGCTGCTTGGGAGTT

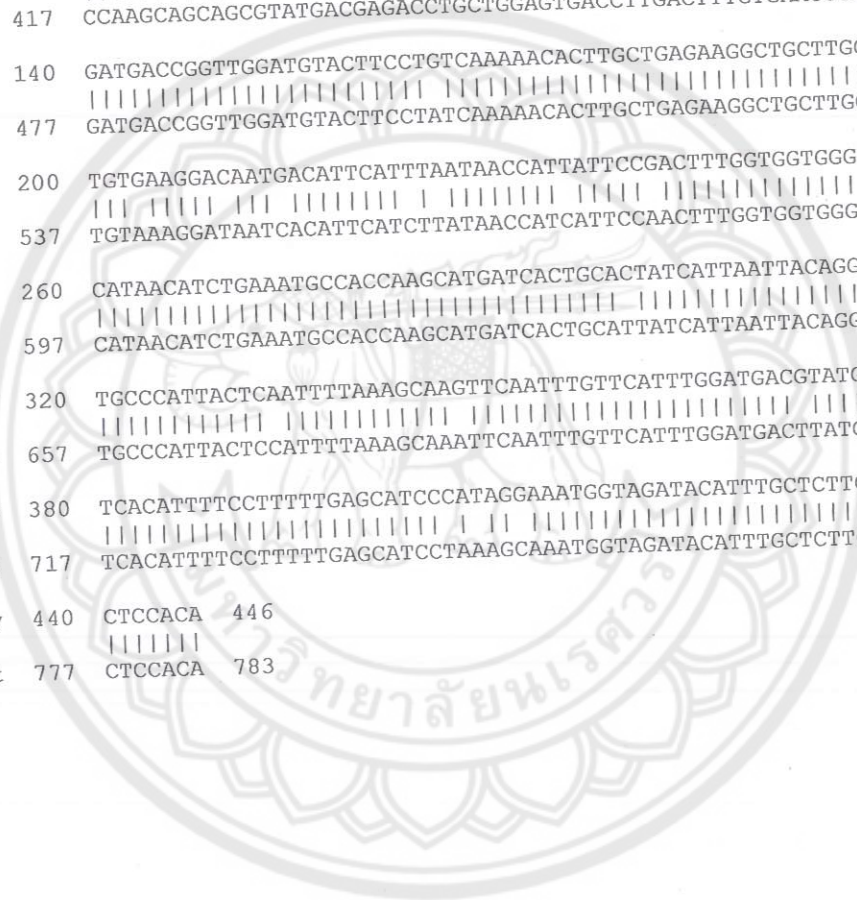
Query 200 TGTGAAGGACAATGACATTCATTTAATAACCATTATCCGACTTTGGTGGTGGGGTCCTT 259
 ||| ||||| ||| ||||||| | ||||||| ||||| ||||||||||| 596
 Sbjct 537 TGTAAAGGATAATCACATTCATCTTATAACCATCATTCCAACCTTTGGTGGTGGGGTCCTT

Query 260 CATAACATCTGAAATGCCACCAAGCATGATCACTGCATTATCATTAAATTACAGGAAATGA 319
 ||||||||||| ||||||||||| ||||||||||||||||||||||||| 656
 Sbjct 597 CATAACATCTGAAATGCCACCAAGCATGATCACTGCATTATCATTAAATTACAGGAAATGA

Query 320 TGCCCATTA CTAATTTAAAGCAAGTTCAATTTGTTCAATTTGGATGACGTATGTGATGC 379
 ||||||||||| ||||||||||| ||||||||||||||||||||||||| 716
 Sbjct 657 TGCCCATTA CTAATTTAAAGCAATTTCAATTTGTTCAATTTGGATGACTTATGTGATGC

Query 380 TCACATTTTCCTTTTTGAGCATCCCATAGGAAATGGTAGATACATTTGCTCTTCCTATGA 439
 ||||||||||| ||||||||||| ||||||||||||||||||||||||| 776
 Sbjct 717 TCACATTTTCCTTTTTGAGCATCCTAAAGCAAATGGTAGATACATTTGCTCTTCCTATGA

Query 440 CTCCACA 446
 |||||
 Sbjct 777 CTCCACA 783



Oncidium sp. 'Sharry Baby' dihydroflavonol 4-reductase (DFR)
mRNA, partial cds

Sequence ID: gb|JQ928173.1|Length: 554Number of Matches: 1

Related Information

Range 1: 211 to 554GenBankGraphicsNext MatchPrevious Match

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Query 36  GTGATATTCACGCTTCTGCAGGAACAGTAAATGTGGAGGAACACCAAGCAGCAGTGTAT 95
          |||
Sbjct 211  GTGATATTCACGCTTCCGGCAGGAACAGTGAATGTGGAAGAAAAGCAAGCAGAGGTCTAC 270

Query 96  GACGAGAGCTGCTGGAGTGACCTTGACTTCGTC AACCGAGTCAAGATGACCCGGTTGGATG 155
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Sbjct 271  GACGAGAGCTCCTGGAGCGACCTTGACTTCGTC AACCGAGTCAAGATGACCCGGCTGGATG 330

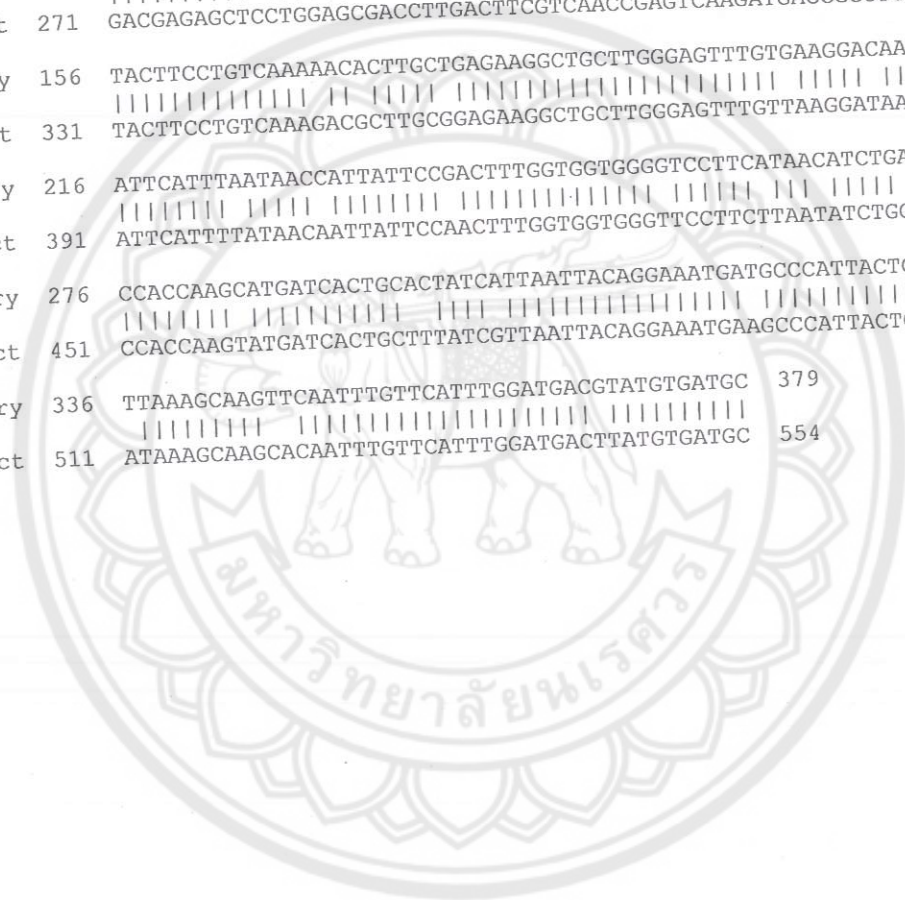
Query 156  TACTTCCTGTCAAAAACACTTGCTGAGAAGGCTGCTTGGGAGTTTGTGAAGGACAATGAC 215
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Sbjct 331  TACTTCCTGTCAAAGACGCTTGGCGAGAAGGCTGCTTGGGAGTTTGTGAAGGATAATGAT 390

Query 216  ATTCATTTAATAACCATTATTCCGACTTTGGTGGTGGGGTCCTTCATAACATCTGAAATG 275
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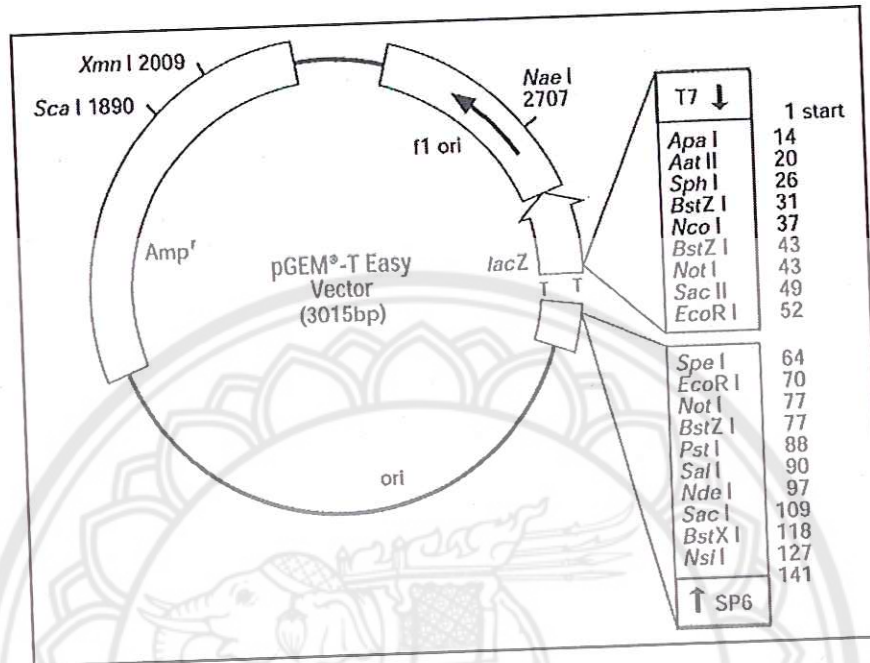
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Sbjct 451  CCACCAAGTATGATCACTGCTTATCGTTAATTACAGGAAATGAAGCCCATTAACAATA 510

Query 336  TTAAAGCAAGTCAATTTGTTTCATTTGGATGACGTATGTGATGC 379
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Sbjct 511  ATAAAGCAAGCACAATTTGTTTCATTTGGATGACTTATGTGATGC 554

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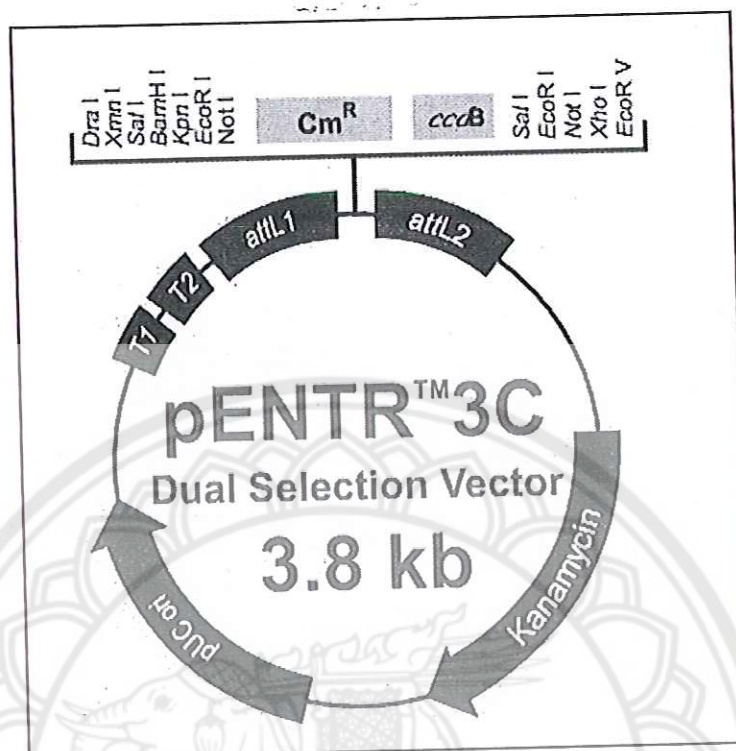
APPENDIX B PHYSICAL MAPS OF pGEM®-T EASY, pENTR™ 3C,
pSTARGATE AND pWATERGATE VECTORS



pGEM®-T Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10-128
SP6 RNA polymerase promoter (-17 to +3)	139-158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176-197
<i>lacZ</i> start codon	180
<i>lac</i> operator	200-216
β -lactamase coding region	1337-2197
phage f1 region	2380-2835
<i>lac</i> operon sequences	2836-2996, 166-395
pUC/M13 Forward Sequencing Primer binding site	2949-2972
T7 RNA polymerase promoter (-17 to +3)	2999-3

Source: Technical Manual, Promega, USA



Comments for pENTR™ 3C
3756 nucleotides

rrnB T1 transcription termination sequence: bases 106-149

rrnB T2 transcription termination sequence: bases 281-308

attL1: bases 358-457 (complementary strand)

Chloramphenicol resistance gene (CmR): bases 610-1268

ccdB gene: bases 1610-1915

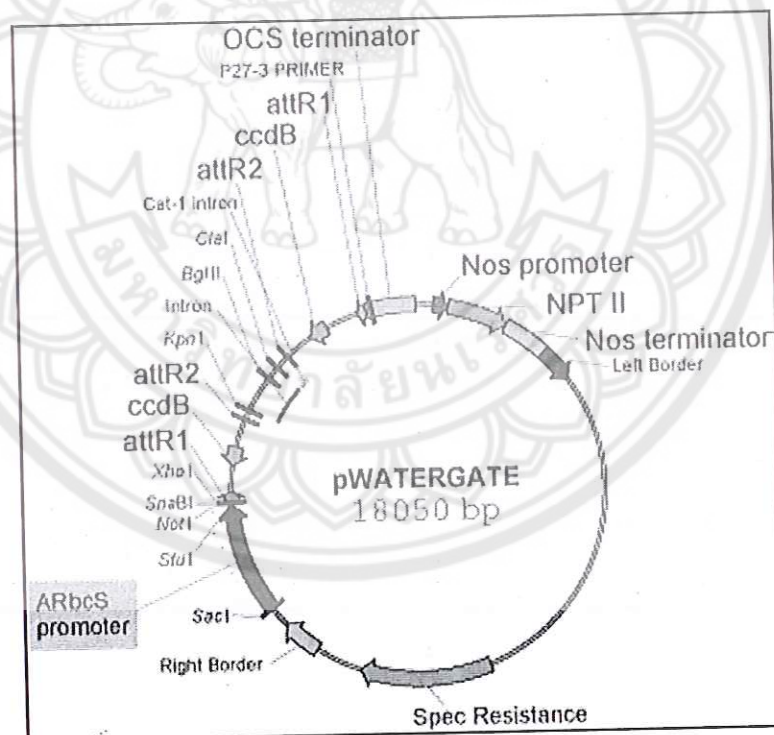
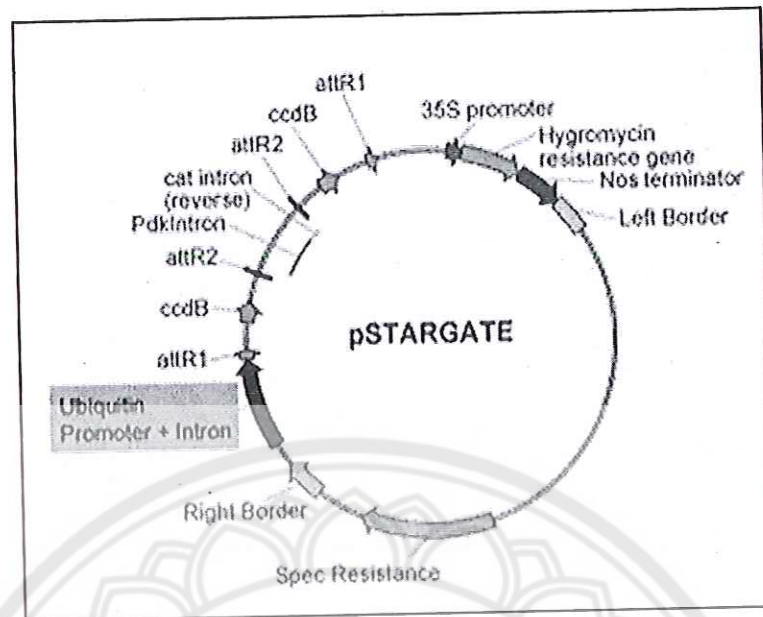
attL2: bases 1985-2084

Kanamycin resistance gene: bases 2207-3016

pUC origin: bases 3080-3753

EcoR I site: 497 824 1957

Source: Vector Information, Invitrogrn, USA



Source : hairpinRNAi vectors for plants, CSIRO, Australia