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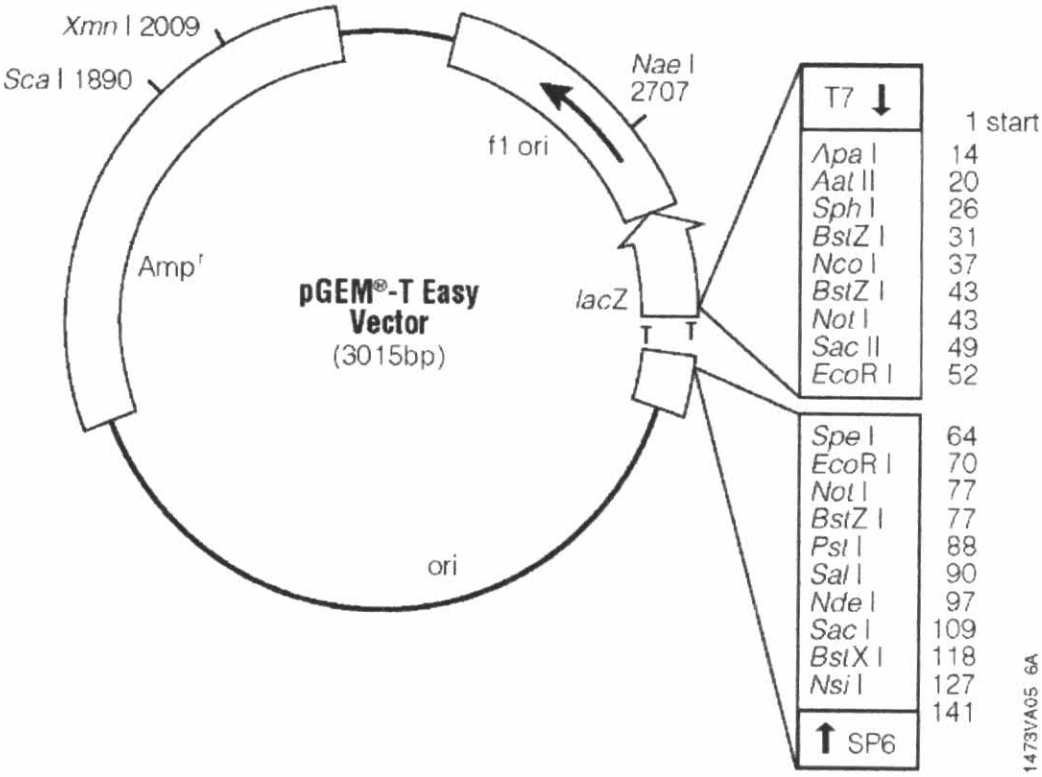
APPENDICES

APPENDIX A

Map of vector used in experiment

1. pGEM®T-essy vector



APPENDIX B

ER FR/59

GCAACAGGCTACCACTACGGGGTGTGGTCCTGTGAGGGCTGCAAGGCCTTCTTCAAGAGGAGCATCCAGGGTCACAATGACTACAT
GTGCCCGGCGACCAATCAGTGCACCATCGA

3'*ER*/5

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GACAAGCGGCGGGCCGGCGACAGAGACAAGGTTGCCAAAGATCCAGAGCACAAAGCGTCGGCGCCCCCTCAGGACGGGAGGAAGCG
CAGCAGCAGCTGTAGCAGCACCGGAGGAGGAGGAGGAGGATCTTCTGTGACTAACTTGCCTCCAGACCAGGTGCTCCTCCTGCTCC
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TTCCTCTCGCATTCAAGGGGGTAAAGGCATTATTTTTACTTTTGCATTTAAAATAGGGTAAAAATAAATCGCGATAAACCAGGTC
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3'*ER*/32

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AAAA

3'*ER*/36

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3'*ER*/49 M13F

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3'*ER*/49 M13R

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5'*ERα* 1.2/37

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ERα full/8 M13F

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5'*ER*/23

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5'*ER*/36

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ACGCGGGGAGTGAGAGACCGAGCTCGAGAGCTCGAGGGGGGAAGAGCGGAAACGTTTGATTGAGTCTCAGGAGAAGTTTGTCGGAG
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5'*ER* 900/14

ACGCGGGGAAAGCGGTTGAGCCACAGAGCCTGGACAAGAAAGTCTTCCTCCTGTTCCCGATGTCGTGTGAGTCTTGAAGTCGGCCT
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CTTCAAGAGGAGCATCCAGGGTCACAATGGCTACATTTGCCCCGCCACAAATCAGTACACTATCGGCAAGAATCGGCGTAAAAGCT
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3'*ER*β 600/38

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AAAAAA

chg-L 900

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5'*chg*-L-500/12

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3'*chg*-L 500/19

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A

3'*chg*-L 500/37

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chg-L full length/19

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TGA

5'*chg*-H 1.5/10-M13F (*chg*-L)

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GTTTCCTTTTC

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CTACCCTGTGGCCAAAGTATTGAGGGATCCTGTGTATGTGGAGGTTGAGCTCCTTGAAATGACAGATCCAGCACTTGTCCTGACTC
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ACGGGCAAAAAAAAAAAAAAAAA

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3’*vtg*-1 4.3/51 3’v1 i F2

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3'*vtg*-1 4.3/51 3'v1 i R1

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AGCTGACAACATGATGCAGGGATACCAACTTGCTGCCTATTTGGACAAACCCAGTGCCAGACTCCAGATCATTCTCGCTGCCTTAG
CTGCTGACAACAACTGGAAACTCTGTGCTGACGGAGTTTTGCTTAGCAAGCATAAAGTCACAGCTAAAGTGGCCTGGGGAGTAGAA
TGCAAGGAGTACGATACCATGATCACAGCTGAAACTGGTCTTGTTGGTCCAAGCCCTGCAGCTCGTCTGAGAGTGGCCTGGAATGA
ACTACCATCTGCTCTTAAACGCTATGCAAGGATATTGTATAGCTACATTCCATATGACTTGCTGGCTGGCTTGATTCAAGGAAAGG
ATGAAAACAGTGCCAAGCAGCTCTCATTCACTGTGGTTGCAACATCTGAACGGACCATTGACTTTATTTGGAAAACACCTACACGT
ACTGTCTATAAGATGGCTTTGCACCTTCCCATCGCTCTGCCACTTGACGAAATCAAAGGTCTCACTCCTTTCGATGACATCATTGA
TAAAGTCCACTACTTGCTTGCAAAGGCTGGAGCAGCTGAATGTAGCTTAATCGGAGAAACACTGACCACATTCAACAACAGAAGAT
ACAACAACGAGATACCCTTGTCTTGCAATCAGATTCTTCACAGGATGCACAGATGAGATAAATCAGTTCAACC

3'GW v1-H-33-M13F

TGGTGCTGAACATCCACAGAGGCATCCTGAATCTCCTTCAGCTCAACATCAAGAAGACTCAGGATGTATACGAGCTGCAGGAGGTT
TGTTAAAGACCCTATTTCTGATTACTAGTCCTTGGCAGGACGTTAAATATCCCTACTAAACTTTGCTCTTTCTAATAGGCTGGAAC
TCAGGGAGTGTGCAAGACCCTTTATGCCATCAGTGAAGATGAAAGGGCTGAACGTATCCTTCTGACAAAGACCAGGGACATGAACC
AATGTCAGGAAAAGATCATCAAGGATATGGGGTTGGCGTATACAGAGAAATGTGCCAAGTGTCAGCAGGTAGTTTCAAACACATTC
TGTGCCATTCATACATCTGTGAAAGCAGAATAAGCAAGCAAATACATGGTTTGTCTTTACAGGATTCCAAAAACCTGAGAGGTGCA
ACATCATTCAATTATATCTTGAAGCCAGTTGCTAGTGGCGTCCTTATCCTGGAGGTAGCTGTGAATGAGGTGATCCAGTTCTCACC
ATTTGCCGAGTTGAAAGGAGCTGCTCAGATGGAAACCAAGTACGCCAATTAATAGTGGACATGTAAAAAATGTAGTATTTTAATTT
AATGGATCTGCTATTCAACTTCTTTTCAAATTTCAATTTACTTTCAAGGCAATCATTGGTCTTCCTTGAGATTCAGAGAGCCCCCA
TTGCACCCATTGAGGCTCAGTATATTCATCAAGGATCTCTTAAGTACGAGTTCTCCACTGAGCTTCTTCAGACACCCATTCAGCTA
ATAAAGATC

3'GW v1-H-33-M13R

TGGGGCTGAACATCCACAGAGGCATCNTGAATCTCCTTTCAGTTCAACATCAAGAAGACTCAGGATGTATACGAGCTGCAGGAGGT
TTGTTAAGGACCTTATTTCTGATTACTAGTCCTTGGCAGGACGTTAAATATCCCTACTAAACTTTGCTCTTTCTAATAGGCTGGAA
CTCAGGGAGTGTGCAAGACCCTTTATGCCATCAGTGAAGATGAAAGGGCTGAACGTATCCTTCTGACAAAGACCAGGGACATGAAC
CAATGTCAGGAAAAGATCATCAAGGATATGGGGTTGGCGTATACAGAGAAATGTGCCAAGTGTCAGCAGGTAGTTTCAAACACATT
CTGTGCCATTCATACATCTGTGAAAGCAGAATAAGCAAGCAAATACATGGTTTGTCTTTACAGGATTCCAAAAACCTGAGAGGTGC
AACATCATTCAATTATATCTTGAAGCCAGTTGCTAGTGGCGTCCTTATCCTGGAGGTAGCTGTGAATGAGGTGATCCAGTTCTCAC
CATTTGCCGAGTTGAAAGGAGCTGCTCAGATGGAAACCAAGTACGCCAATTAATAGTGGACATGTAAAAAATGTAGTATTTTAATT
TAATGGATCTGCTATTCAACTTCTTTTCAAATTTCAATTTACTTTCAAGGCAATCATTGGTCTTCCTTGAGATTCAGAGAGCCCCC

ATTGCACCCATTGAGGCTCAGTATATTCATCAAGGATCTCTTAAGTACGAGTTCTCCACTGAGCTTCTTCAGACACCCATTCAGCT
AATAAAGATCAACAATGCACAGGACCTGCCCGGGCCGTCG

3'GW v1-H-45

CAGCAGGTAGTTTCAAACACATTCTGTGCCATTCATACATCTGTGAAAGCAGAATAAGCAAGCAAATACATGGTTTGTCTTTACAG
GATTCCAAAAACCTGAGAGGTGCAACATCATTCAATTATATCTTGAGGCCAGTTGCTAGTGGCGTCCCTATCCTGGAGGTAGCTGT
GAATGAGGTGATCCAGTTCTCACCATTTGCTGAGTTGAAAGGAGCTGCTCAGATGGAAACCAAGTACGCCAATTAATAGTGGACAT
GTAAAAAATGTAGTATTTTAATTTAATGGATCTGCTATTCAACTTCTTTTCAAATTTCAATTTACTTTCAAGGCAATCATTGGTCT
TCCTTGAGATTCAGAGAGCCCCCATTGCACCCATTGAGGCTCAGTATATTCATCAAGGATCTCTTAAGTACGAGTTCTCCACTGAG
CTTCTTCAGACACCCATTCAGCTAATAAAGATCAACAATGCACAGGACCTGCCCGGGCCGTCG

vtg-3 F1-alpha 500

AGGGTTGGGACTCAACACCTGAAGCTGTGTTCAATATCAAAGCATTTGCCATGAGTGGCAACCAGAAGCCCGAGGGTTATGATGCC
TCCGTGTACTACACGCCAGAAGCAAACACTCAGAATGCCCAACTGATTGTGTCCCAAGTTGGAGAAGACACCAACTGGAAGATGTG
TGTCGACACCACTGTGAATGCTGGCTCTGGGGCAAAGGCACACATCAGATGGGGAGCTGAATGTCAGTCCTATGAAATTTCAATGA
GAGCTGCTACTGCATATCTGCCTGGCTCCAAGCCAGCACTCAAAGCCAAAGTACACTGGACCAGGGTCCCAGAAGCCATGGAGGAC
ATGGGCACAAGAATTGAAAGCTACATCCCA

vtg-3 F1-*Sal*I 1kb/7 M13F

AGTGTGTGGACACCACTGTGAATGCTGGTTCTGGGGCAAAGGCACACTTCAGAGGGGGAGCGGAATGTCAGTCCTAGGAAATTTCA
AGGAGAGCTGTTACGGCATTTTTGCCGGGTTCCAAGCCAGCATTCAAAGCCAAAGTCCACTGGCCCGGGGTCCCAGAACCCATGGG
GGACATGGGCCCAAGAATTGAAAGTTACTTCCCGGGCAGGGCTTTCCTTTTGGGTTTTTCCCAGCAGAATGAGAGAAACGCAGCCC
GGGGGGTTTTTCCATTGGTTGTTGCTGAATCAGCAGACAGCGTTGATGTGAAGATTAAATTCCCAAAGTTTCCAGTTTCCCCCCGG
GTTATTCCATTCCTGTTGCCCCCAGCCAATTTTCGGGGGTTTCTTCCCGGCTTCACAAATACACCAGTGGTTGCTGGGGGGGCATA
AAAACATAAATACGTCAAAGCCCGGAGGGAAGCCGGGCGCTTTTTTTCACTGGGATAATGCGGCACCCGGAAATCCCAGGGTTTTG
ATGAAAGGGGGGATCTTTAAAGAGGTTTCGGTAATCCATCGGATGTTTCCAGTTGGGAAATACCCGGTAAAGATTTTGTTTTTTTA
ATTTAAAAGTTAATAAATTTTGGACCCCCTTCCTTTAAAGGAATAAGCTGCTGCTTTGGAGGATCTTTAAGATATGGGATTTCCAG
AAAACTGTTTTTGTCCTATTTCACCCCTAAAAATGCTTTGGGCTTCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAA

vtg-3 F1-*Sal*I 1kb/7 M13R

AGGGTTGGGACTCAACACCTGAAGCTGTGTTCAATATCAAAGCATTTGCCATGAGTGGCAACCAGAAGCCCGAGGGTTATGATGCC
TCCGTGTACTACACGCCAGAAGCAAACACTCAGAATGCCCAACTGATTGTGTCCCAAGTTGGAGAAGACACCAACTGGAAGATGTG
TGTCGACACCACTGTGAATGCTGGCTCTGGGGCAAAGGCACACATCAGATGGGGAGCTGAATGTCAGTCCTATGAAATTTCAATGA
GAGCTGCTACTGCATATCTGCCAGGCTCCAAGCCAGCACTCAAAGCCAAAGTACACTGGACCAGGGTCCCAGAAGCCATGGAGGAC
ATGGGCACAAGAATTGAAAGCTACATCCCAGGCATGGCTTTCCTTTTCGGTTTCTACCAGCAGAATGAGAGAAACGCAGCGCAGGA
GGTTTCTGCATTGGTTGTTGCTGAATCAGCAGACAGCGTTGATGTGAAGATTAAATTCCCAAAGTTTACAGTTTACCACCAGGCTA
TTCCATTCCTGCTGCCGCCAGCCAATTTTCAGGAGTTTCATCCCAGCATCACAAATACAACAGTAGATGCTGGACGAGCATAAAAA
CATAAATACGTCAAAGCCCAGATGGAAGCCAGGCGCCTCTTTTCACTGCGATAATGCTGCAGCCTGAAATCACATGGCTCTGATGA
AAGTGGGGATCATTAAAGATGTATCA

5'V3-1-5-10-M13F

CGCATGGACTGGTTTAGCGATGATTTTCTGATTGGCACAGCTGCAGAAGTCTTCATGCTAAGAAGTGCGAACAACATCTTCCCCAC
TGAGTTCATAACGAAAGGAAAATTTTATTTCATTGGTAGAATTCTGCAGCTCTTGGAGCTTGGAATTCGTGCTGAAGGACTTAAGG
AGCTGTTCGGTGCCAGCATCCCTGGCTTTAAAGGAGATTTTAGTTTTAGTGACTTCCGGGCTATTTTCAATGTTCTTCAAAACTGG

GAAATTCTGCCAAACAATAAGCCAGTCCTCTCTGCCTATCTTCGCGCCTCTGGACAAGAGTGCTTCTTTGGCGATATCAACAAATA
CTTGATCCAGAATATCGTCAGGGCTTTCAGTCCTTCGGCAGGGAAAGAAAGTCCTGTGTTTGCGGCAATTCAGAATTTACAGAAGG
GAATTTCGTGGCATCGGACCAAGCCATTCTTGATTTTTGAGGCTCGTTACTTTCAAGCTACCACCCTGGGCCTCCCAGTGGAGATA
AGCAAATATTACGAAACAGTCTACGGGATCACCGTTAATGCTAAAGCTGCAGTAAATCCACCACCAACTGAACATCTGGCACAGCT
GCTGAATTCTGAAATTTCACTGGAATCTGATGGTTTTGTTGGTTTCACAAAAGGATTTCTGGGTTTTCTATGGCACCAACACCGAG
CTGTTCCCAAGTGGTTTGCAAATTGAAAAGCAAAATGCCTCCTTGCCC

5'V3-1-5-10-M13R

CATCCGGGGAAGTTTGCCTCCCAAGTTCAAGTTTGAGGAAAGAAAATTGGGGAAGGTTTCGCGGGTTTTAAAAAAGGAGAGTGAGT
TTTTGGCACGGACCTAGAAGATTAATTCGGTTAACAGGTACAAAGATGTGGCGTATATTGGAAAGCGAACTCCTTTTATTCCTAGA
ATTTTTTTATACAGGGGAACCTCCCATGCCGCTTTTTTGAGGGTCTTGGATCACTAGGGGTCCAGAAGGCAATTGTCTGGCAGCCA
AGATTCGAAATGTGTGTTGGAATCAAATTTTAGAGAGTTGGTTTCTGGGTAGAGTATGAGGCGTGGAGATTAGATTGATCGCGAGG
AATACCCCCTGAATGTTTTACTGGGGTTCACTTACATAGCAGTTAAATTATTGCGTTCCAGGCAAGAAAAACTGGTGACAAAATTC
ACTTTGAAGTTTATGCCGACCCCTGCAGACATCCAATGTGTACACGCCCAAGACTTGAGATTTTGTGGACGCTTTCCAAAGAAGCA
ACCCGGTGAGCAAAGTCAGTCTTCAGATTCAGCCTCAAGTGACAGAGGATCGCATCAGAGCAAACGAGATATGATTATGGGGGGTT
GGGAATCAACACTTGAAGATGTGTTCAATATCAAAACATTTGACCAGTGAGTGGTCAACCAGAAGCCCGAGGGTTATGATGCCTCC
G

5'v3 2kb/1 M13F

CGCGGGGTTTCAGATTTGGCCTTCCAGGAGTTCAATGGGTTCCCAGGGAAAAATGGCTTTAATGCCTCCCCAAAGCTCACCAAACG
CATCGCTGCACAGCTCACCAAACCTTTCATGTTTGAGTTTGCCAGTGGACACGTTGGTGACATCCGTGCCGCTGCAGAGATCTCTG
ACACTGTTGTCAACATTGTGAGAGGGATCCTGGGTTTTTTCCAAGTCACTGTCAAGACCACCCAGAGGATCTATGAGCTAGAGGAG
GTTGGAATCCATGGCAAGTGTCAGAGTAACTATGCTATGGAGGAAAATACGGAAACACAGGACATGACTATCACTCAGGTTGTGGA
TGTCAGTAACTGCAGGGAGAAAGCAGCAATCTACAGGGGCATGGCCACCGCTGTGCTCGACAAAGTCTCCAAACAGAGAGGGGAAT
CTGTCATTTCCACAGTGAGATATGTTTACACAGTCAAGCCAACGGCAGAGGGAGGTCTCATTACCAGAGCTCATGGCCTGGAGCGA
CAGCACTTCAGTCCCTTCAATGTGAAGGGCGGCAGTTTCAAGATGCAAGCGATGAAGGAAATGGTGCTGCTTGGTGTGAGCGACAC
AGCTAGAGCCATCACGTTCGGGCCAATGGAGAGCAAGGGCAACCTTGTTTACAAGTTTGTCAGAGCAGCTAATATCCCTATTATAA
TGGAGAACCTGGACAACCCCTCCCCAAGGCGGGTGAGCTGATTAAACAACTGGCTCAGGCTAACAGATA

5'v3 2kb/1 M13R

GTGGGGAAGATGTTGTTCGCACTTCTTAGCATGAAGACTTCTGCAGCTGTGCCAATCAGAAAATCACCGCTAAACCAGTCCATGCG
TCTTGCTTGGCTGTAGTAATAGCTGGTACGACCAAATTTAGGTGCGAGGATCTTCACAGCCACATTGCAGGCTGTTGAGAGGAAGT
GATTGTCTGGAGTCGTGGATCTTGCAACACTTTGTAAATAAGAGTAAACAAAGCTAACCACATGGAAGTCTTTTTCTTCCAGTAGA
TGTGTTGTCACCGTAGACACAAGAGCCATTGATGGATTTGTGTCAAACAGGATCGTGATAGCCATCATGCGAATTTCAGCAGGAAG
GTGTTTTTGCAGGAACAGACTCAGGGTGATGCCTTGGACACTGTGACGGTCTCTGGCAGCTGTGAGTCTCATAGACTGCACAGCAG
CACTCAGGACACGAGGTGGCAGATCCACAGGGTTGGCAGCAACTCCAGGGAGGAAGCGCATGATGGTTTTAATGCTGCCTGGATGA
CCTGTGTTCCCCAGGGCTTTCAGAGCGAGGACCATGTCTTCCTCATTGTTATTCCTCAGACTCTCCGTGGCCATGTCCAGCAGCGG
CTGAACAGCAGATACTGGACATGGTGTATAATATGCGCAGTGCTTGTACACCAGAGAGCCATAGGCAAGCACCACAGTGTGCCACA
GAAACGTGTTAGATTTACTGAATGGCATTGTCAGGAAAACTTTA

5'v3 1.2kb/15 M13F

ACGCGGGTCTCCAAACAGAGAGGGGAATCTGTCATTTCCACAGTGAGATATGTTTACACAGTCAAGCCAACGGCAGAGGGAGGTCT
CATTACCAGAGCTCATGGCCTGGAGCGACAGCACTTCAGTCCCCTCAATGTGAAGGGCGGCAGTTTCAAGATGCAAGCGATGAAGG
AAATGGTGCTGCTTGGTGTGAGCGACACAGCTAGAGCCATCACGTTCGGGCCAATGGAGAGCAAGGGCAACCTTGTTTACAAGTTT
GTCAGAGCAGCTAATATCCCTATTATAATGGAGAACCTGGACAACCCCATCCCAAAGGCGGTTGAGCTGATTAAACAACTGGCTCA
GGCTAACAGATACCAGGTTGACAGTGCAACAACTGAGGACACTATAAAGCTGTATCAGCTCCTGAGAGTGATGCCATATGAAGGAT

TGGATGTTATGTGGGCGCAATTTGCAGGAAATGAAGAACACAGACGTTGGTTTCTGGACATGATTGTTGAGATTGGTGATGCCCGA
ATCCTAAAGTTCCTGGAAACAAGGTTTAAGGCAGGTGACGTGCCTGCAACCGAAGCTCTGGAAACTCTTCTGTTGTCCATTAACCA
CCTGCAGGCTATCCCTGAGCTAGTTGAGATGGCTAAAGTTTTCCTGACAATGCCATTCAGTAAATCTAACACGTTTCTGTGGCACA
CTGTGGTGCTTGCCTATGGCTCTCTGGTGTACAAGCACTGCGCATATTATACACCCTGTCCAGTATCTGCTG

5'v3 1.2kb/15 M13R

TCCTGACAATGCCATTCAGTAAATCTAACACGTTTCTGTGGCACACTGTGGTGCTTGCCTATGGCTCTCTGGTGTACAAGCACTGC
GCATATTATACACCATGTCCAGTATCTGCTGTTCAGCCGCTGCTGGACATGGCCACGGAGAGTCTGAGGAATAACAATGAGGAAGA
CATGGTCCTCGCTCTGAAAGCCCTGGGGAACGCAGGTCATCCAGGCAGCATTAAAACCATCATGCGCTTCCTCCCTGGAGTTGCTG
CCAACCCTGTGGATCTGCCACCTCGTGTCCTGAGTGCTGCTGTGCAGTCTATGAGACTCACAGCTGCCAGAGACCGTCACAGTGTC
CAAGGCATCACCCTGAGTCTGTTCCTGCAAAAACACCTTCCTGCTGAAATTCGCATGATGGCTATCACGATCCTGGTTGACACAAA
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ACAAAGTGTTGCAAGATCCACGACTCCAGATAATCACTTCCTCTCAACAGCCTGCAATGTGGCTGTGAAGATCCTCGCACCTAAAT
TTGGTCGTACCAGCTATTACTACAGCCAAGCAAGACGCATGGACTGGTTTAGCGATGATTTTCTGATTGGCACAGCTGCAGAAGTC
TTCATGCTAAGAAGTGCGAACAACATCTTCCCCAC

5'v3 900/44 M13F

ACCGGGAAATGAAGAACACAGACGTTGGTTTCTGGACATGATTGTTGAGATTGGTGATGCCCGAATCCTAAAGTTCCTGGAAACGA
GGTTTAAGGCAGGTGACGTGTCTGCATCCGAAGCTCTGGAAACTCTTCTGTTGTCCATTAACCACCTGCAGGCTATCCCTGAGCTA
GTTGAGATGGCTAAAGTTTTCCTGACAATGCCATTCAGTAAATCTAACACGTTTCTGTGGCACACTGTGGTGCTTGCCTATGGCTC
TCTGGTGTACAAGCACTGCGCGTATTATACACCATGTCCAGTATCTGCTGTTCAGCCGCTGCTGGACGTGACCACGGAGAGTCTGA
GGAATAACAATGAGGAAGACATGGTCCTCGCTCTGAAAGCCCTGTGGAACGCAGGTCATCCATGCACCATTAACACCATAATGCCC
TTCCTCCCTGGAGTTCCTGCCAACCCTGTGAATCTGCCACCTCTTGTCCTGAATGCTGCTGTCCTACCTTCAGCACCCACAGATGA
CCCTACACATTCACAGTGCCTCAAGGCATAGCCTGAATCTGCCCCCGTAAAAAACACGAACCACTGCTTTTCCCCTTTATAGCTAT
CAATATATCCTGTCTCTTACTCAAACAACTCTTGATTACCCGAATTCCTAGGCTGACCTTTACACCTCTCCCACAAACATCACCCA
TTCTATTATACAACGCCTCTCTATCTATCACCACACCTTGAAC

5'v3 900/44 M13R

CATGCCATTCAGTAAATCTAACACGTTTCTGTGGCACACTGTGGTGCTTGCCTATGGCTCTCTGGTGTACAAGCACTGCGCGTATT
ATACACCATGTCCAGTATCTGCTGTTCAGCCGCTGCTGGACGTGGCCACGGAGAGTCTGAGGAATAACAATGAGGAAGACATGGTC
CTCGCTCTGAAAGCCCTGGGGAACGCAGGTCATCCAGGCAGCATTAAAACCATCATGCGCTTCCTCCCTGGAGTTGCTGCCAACCC
TGTGGATCTGCCACCTCGTGTCCTGAGTGCTGCTGTGCAGTCTATGAGACTCACAGCTGCCAGAGACCGTCACAGTGTCCAAGGCA
TCACCCTGAGTCTGTTCCTGCAAAAACACCTTCCTGCTGAAATTCGCATGATGGCTATCACGATCCTGTTTGACACAAATCCATCA
ATGGCTCTTGTGTCCACGGTGACAATACATCTACTGGAAGAAAAAGACTTCCATGTGGTTAGCTTTGTTTACTCTTATTTACAAAG
TGTTGCAAGATCCACGACTCCAGATAATCACTTCCTCTCAACAGCCTGCAATGTAGCTGTGAAGATCCTCGCACCTAAATTTGGTC
GTACCAGCTATTACTACAGCCAAGCAAGACGCATGGACTGGTTTAGCGATGATTTTCTGATTGGCACAGCTGCAGAAGTCTTCATG
CTAAGAAGTGCGAACAACATCTTCCCCAC

chg-L D12

CGACGGCCTGGGCAGGTGTTGCATCTGTTGCTGTTGAGTGCAGAGAGAATGATGCTCATGTGGAAGTCAGGAGGGATATGTTTGGG
ACTGGCCAGTTGGTCAATCCGAATGACCTCACCCTGGGGAACTGTCCTGCTGTCGCAGAGGATCCTGCGGCTCAAGTGTTGATTTT
TGAAGCTGAACTGCATGACTGTTTGAGCTCATTGGTGGTAAGTAAAATACATAATGCTGAAACTAAACAATATCTTATAATGAATC
GTATATGAATATATCATCAAGGTTTTTGGTCATCTTTGCTAGTTGACAGAAGATTCCCTGACCTACATCTTCACTCTGAACTACGA
TCCCCGACCTCTGGGTTCCTCCCCCGTAGTAAGGACCGGCAGTGCAGCTGTTATTGTGGAATGCCACTACCCAAGGTGTGTTGACT
GAAGATATGTGGCAATAAACAATCAAAACACAAGTAAAATAATCATCTTAATATGTAACGTAATGTGTAGCCTTTAGCAGAAATGC

ACCGTGAAATCTAATCTGCACATGCCAGTGGTAGACGCATGTCACGCTGAGGATTTCTTTAGTCTTCTGTTTTAAGCGGGTATGTC
TGTCTTGTCTCTTAGAAAGCACAATGTGAGCAGCCTTCCTC

chg-L S32

CGACGGCCGGGCAGGTATATCACCAAGGTTTTTGGTCATCTTTGCTAGTTGACAGAAGATTCCCTGATCTACATCTTCACTCTGAA
CTACGATCCCCGACCTCTGGGTTCCTCCCCCGTAGTAAGGACCGGCAGTGCAGCTGTTATTGTGGAATGCCACTACCCAAGGTGTG
TTGACTGAAGATATGTGGCAATAAACAATCAAAACACAAGTAAAATAATCATCTTAATATGTAATGTAATGTGTAGCCTTTAGCAG
AAATGCATCGTGAAATCTAATCTGCACATGCCAGTGGTAGACGCATGTCACGCTGAGGATTTCTTTAGTCTTCTGTTCTAAGCGGG
TATGTCTGTCTTGTCTCTTAGAAAGCACAATGTGAGCAGCCTTCCTC

chg-L D12-S4 M13F

CCCAAACATATCCCTCCTGACTTCCACATGAGCATCATTCTCTCTGCACTCAACAGCAACAGATGCAACAGGTTGAGGACGTAGCG
GTTCAAATGGTATTTCAGGCGCAGGTTCAGGCGGGGGAGGTTCAGGGTATGTCCATTGAAGTGGTGTTTCAAATGTCTGCTTATGC
TGTTGAGTGTCTTGAGGCACTTGTTTGGGCTCCTGCTTCACAGGAGGTGCAGGTTTCTGATATTTTGAAGGCGTGTACTCTCCCCA
CTGAGCACCACAGACGCTGGCAAATAGAGCCAGTGCCACAAGGCAAGCAGCAGTCCACTTCATCACCATGGCTCCACAGTGACAAG
CGATCTGAGAGATTCTCAAGGTGCTGAAAGAGTGGTGGGAACTTGCTTGTACTTTGATAACAATGTTAACCTGTATATTTTCTTTA
CTGCCCCGCGCCTTAAGTTGGGCATCCATCGGTATACTCGTAAGGCATAGTGGTCTCCTGTAATATGAGGCTGAATACTGAGAGCT
TGCATTATGATTTTTGATCTTGAGCCTATTAGTCAAAGTTTCTATCCCTTTCAGCAGCAGAAATGGAGTAAATCAAACGTATACTC
GTAAATATGGTTGTCAATTCAGATGAAAACAAAAATTTCTGACTGGATATAAAAATTGATGTCCAGCTCAGGAGTTGAGACAAGGC
TTTACTATTCCTAAAACCATGATGATTATACTCCATCTAGCCTCTTCAACAGTATTGAAAAAAGACCCGAAACAACCAGTAGTTTC
CGCCCCTATGATTCGTCTTTCAAGGCATAAAGAACCAAAAGCCTCCTATTTAACATAAGTAATATCCCTCTACTCACGGGTAATTT
TCCTAATTATTTGTATTATG

chg-L D12-S4 M13R

CGACGGCCCGGGCAGGTATTATCACAATAATAAAACAAGATCTTCAGTCCAGTGATTTTTCCTCAGGTCTGAGCTGGTCCTAAAGA
ATCGTAAATAAATAATATGGTAAACTTACTGCAGGTGAAGCTGACAACCTGCCCACCGTGGAGCCATATCGACTGGCTGACATTCA
CAAAATGTACTTTGGCAAAACACTGAGTTCAGTCTGTCATGTCCAGGGCAGGTAAACCTAACCCAGGGTCAACATAACTTTTTCTG
CCTCTTTTTATTCTGTCTTACTATATATCTTCAAGCTATTTGTAAAGATTTTTTAATATAATTTGTTTTCAGAAAATGTGTCTGTA
TTTATCTCATCCCATAAGAAATAAAATGTAAATTGAGCTTTCAGTCAACAGCTACATTTTATTGGCTACAGCTAGGGCTTACGAGT
CTGCTGAGGGATGGCCAACTGAAGGGGCGGGGAAGTAATGAAAATAAACAGGTTCACAGCGTTATAAAAGTAGAAGCAAGTTCCCA
CCACTCTTACAGCACCTTGAGAATCTCTCAGATCGCTTGTCACTGTGGAGCCATGGTGATGAAGTGGACTGCTGCTTGCCTTGTGG
CACTGGCTCTATTTGCCAGCGTCTGTGGTGCTCAGTGGGGAGAGTACACGCCTTCAAAATATCAGAAACCTGCACCTCCTGTGAAG
CAGGAGCCCAAACAAGTGCCTCAG

chg-L D12-D20

CGACGGCCCGGGCAGGTAAATTAGATCCAGGGTGTCAGAACTGAGATCTGAAAATCCCCATTTGAGCTCAGAGCAGAATAAACACA
GTCGTTGTTGGTGCCCTGATGAAACAAAGGTTCAAAGGTTTTACTTGCTTTCACAACCTGGATATCTTTTGGCTATTTGGTTATTT
TTACAGTAATGAT*TGTTCT*TCATATATATGTATATATACAGTGATCAGGCATAACATTATGACCACTTGCCTAATATTGTGTAAGT
CTCCATTGTGCCTCAGTTGTGGCTCATCAGAGAGTGAACATGGGCCCTCTGAGGGTGCCCTGTGGTGTCTGTCAATATAATGTTGT
TGGGGGGGGAGGGGGCTTTGGCTCCTAAGGGCTGAAAGGAGGGGCCTCTGTGAATCAGGCTTGTTCCAGTCCATCCCAGAGACACT
TGATCAGATTGGGAAGTTGTGAATTTGGAGGCCAGGTCAACACCTAGTACTGTTTGTCATGTTTTTTTTTAGTTGTTCCTAAACTA
TTTTTGTGTGTTTGTCTGTGTCAGGCTGCATCCTGCTGGGGATGGCTGCTGCTTGCTATGGGGTGATGGTTGGGTTGTGGGGGTA
TCTGGTCTATATATCAACCTTGCTTTGTGCACGTGAAGGATGGTTATGTAGAAAACATACCTACAATGTGTTACTGTAGAGTCTAC
TAATGAAATACTGCAAATATTAAATAAATTAATACAATCTATTATGAAGATTTATGTAATATAGGCTTAAACAAATCAAATAAAGC
TACGATAGAAAACAAACATTTAATGGAATATCAGAAGAGGAGGAGAGGAAGGATCAGCCCAAATTAAAAGTTTTGTCATGAGGTGT
ATGTGGCAGTAATATTATCACAATAATAAAACAAGATCTTCAGTCCAGTGATTTTTCCTCAGGTCTGAGCTGGTCCTAAAGAATCG

TAAATAAATAATATGGTAAACTTACTGCAGGTGAAGCTGACAACCTGCCCACCGTGGAGCCATATCGACTGGCTGACATTCACAAA
ATGTACTTTGGCAAAACACTGAGTTCAGTCTGTCATGTCCAGGGCAGGTAAACCTAACCCAGGGTCAACATAACTTTTTCTGCCTC
TTTTTATTCTGTCTTTCTATATATCTTCAAGCTATTTGTAAAGATTTTTTAATATAATTTGTTTTCAGAAAATGTGTCTGTATTTA
TCTCATCCCATAAGAAATAAAATGTAAATTGAGCTTTCAGTCAACAGCTGCATTTTATTGGCTACAGCTAGGGCTTACGAGTCTGC
TGAGGGATGGCCAACTGAAGGGGCGGGGAAGTAATGAAAATAAACAGGTTCACAGCGTTATAAAAGTAGAAGCAAGTTCCCACCAC
TCTTACAGCACCTTGAGAATCTCTCAGATCGCTTGTCACTGTGGAGCCATGGTGATGAAGTGGACTGCTGCTTGCCTTGTGGCACT
GGCTCTATTTGCCAGCGTCTGTGATGCTCAGTGGGGAGAGTACACGCCTTCAAAATATCAGAAACCTGCACCTCCTGTGAAGCAAG
AGCCCAAACAAGTGCCTCAAGACACTCAACAGCATAAGCAGACATTTGAAACACCACTTCAATGGACATACCCTGAACCTCCCCCG
CCTGAACCTGCGCCTGAAATACCATTTGAACCGCTACGTCCTCAACCTGTTGCATCTGTTGCTGTTGAGTGCAGAGAGAATGATGC
TCATGTGGAAGTCAGGAGGGATATGTTTGGG

actin/52

TCCCATCTCCTGCTCAAAGTCCAGTGCAACGTAACACAGCTTCTCCTTGATGTCACGCACGATCTCACGCTCAGCAGTGGTGGTGA
AGCTGTAGCCTCTCTCGGTCAGGATTTTCATGAGGTAGTCTGTGAGGTCCCTGCCAGCCAGGTCCAACCTGAGGATGGCGTGGGGC
AGAGCGTAGCCTTCATAGATGGGCACTGTGTGGGTCACACCATCACCGGAGTCCATGACGATACCAGTGGTACGACCAGAGGCGTA
CAGGGACAGCACAGCCTGGATGGCCACGTACATGG

Figure B.1 Nucleotide sequence of *ER*, *chg*, *vtg*, and β-actin of *L. subviridis*.

APPENDIX C

Table C.1 Results from BLAST*X* analysis of PCR product which studied in this research

Clone or sequence	Insert size (bp)	Homologue (transcript of species)	E-value
ER FR/59	116	estrogen receptor alpha (*Mugil cephalus*)	2e-09
3'*ER*/5	1,403	estrogen receptor alpha (*Oreochromis aureus*)	3e-63
3'*ER*/32	778	estrogen receptor alpha (*Mugil cephalus*)	3e-53
3'*ER*/36	1,769	estrogen receptor alpha (*Micropogonias undulates*)	1e-145
3'*ER*/49 M13F	757	estrogen receptor alpha (*Micropterus salmoides*)	1e-05
3'*ER*/49 M13R	733	estrogen receptor alpha short form (*Kryptolebias marmoratus*)	3e-95
5'*ER*α 1.2/37	974	estrogen receptor alpha (*Dicentrarchus labrax*)	9e-119
*ER*α full/8 M13F	714	estrogen receptor alpha (*Micropterus salmoides*)	4e-20
5'*ER*/23	573	estrogen receptor beta (*Micropterus salmoides*)	2e-80
5'*ER*/36	817	estrogen receptor beta 2 (*Oreochromis niloticus niloticus*)	9e-90
5'*ER*/39	969	estrogen receptor beta (*Micropterus salmoides*)	1e-85
5'*ER* 900/14	816	estrogen receptor beta 2 (*Oreochromis niloticus niloticus*)	2e-90
3'*ER*β 1.3/33	1,310	estrogen receptor beta 2 (*Dicentrarchus labrax*)	5e-124
3'*ER*β 600/38	425	estrogen receptor beta (*Lepisosteus oculatus*)	1e-14
3'*ER*β 600/1	608	estrogen receptor betaB variant X (*Fundulus heteroclitus*)	3e-08
chg-L 900	634	chorion protein (*Sparus aurata*)	2e-83
5'L 500/12	379	chorion protein (*Sparus aurata*)	3e-14
5'L 500/ 18	424	chorion protein (*Sparus aurata*)	4e-22
3'L 500/19	431	choriogenin L (*Oryzias javanicus*)	1e-04
3'L 500/ 37	335	choriogenin L (*Oryzias javanicus*)	1e-04
chg-L full length/19	1,293	chorion protein (*Sparus aurata*)	7e-145
5'*chg*-H 1.5/10-M13F (*chg*-L)	723	chorion protein (*Liparis atlanticus*)	5e-62
chg-H/11 M13F	705	zona pellucida protein (*Pseudopleuronectes americanus*)	3e-43
chg-H/11 M13R	699	zona pellucida protein Bb (*Sparus aurata*)	4e-103
3'*chg*H 1.1/13	969	zona pellucida protein Bb (*Sparus aurata*)	2e-62
3'*chg*H 800/51	699	zona pellucida protein Bb (*Sparus aurata*)	7e-62

Table C.1 Results from BLAST*X* analysis of PCR product which studied in this research (cont.).

Clone or sequence	Insert size (bp)	Homologue (transcript of species)	E-value
vtg-1/28	367	vitellogenin (*Sillago japonica*)	2e-52
5'*vtg*-1 250/16	203	vitellogenin A (*Salvelinus alpinus*)	7e-05
3'*vtg*-1 4.3/51 M13F	737	No significant similarity found	-
3'*vtg*-1 4.3/51 M13R	732	vitellogenin (*Epinephelus coioides*)	3e-112
3'*vtg*-1 4.3/51 3'v1 i F1	910	vitellogenin (*Pagrus major*)	5e-110
3'*vtg*-1 4.3/51 3'v1 i F2	835	vitellogenin (*Pagrus major*)	2e-105
3'*vtg*-1 4.3/51 3'v1 i R1	907	vitellogenin (*Pagrus major*)	1e-123
3'*vtg*-1 4.3/51 3'v1 i R2	847	vitellogenin (*Sillago japonica*)	6e-99
3'GW v1-H-33-M13F	783	vitellogenin (*Epinephelus coioides*)	6e-66
3'GW v1-H-33-M13R	814	vitellogenin (*Epinephelus coioides*)	2e-64
3'GW v1-H-45	493	vitellogenin (*Sillago japonica*)	1e-35
vtg-3 F1-alpha 500	374	phosvitinless vitellogenin (*Pagrus major*)	2e-53
vtg-3 F1-*Sal*I 1kb/7 M13F	784	phosvitinless vitellogenin (*Pagrus major*)	1e-37
vtg-3 F1-*Sal*I 1kb/7 M13R	714	phosvitinless vitellogenin (*Pagrus major*)	6e-83
5'V3-1-5-10-M13F	736	phosvitinless vitellogenin (*Pagrus major*)	5e-98
5'V3-1-5-10-M13R	689	phosvitinless vitellogenin (*Pagrus major*)	8e-06
5'v3 2kb/1 M13F	757	phosvitinless vitellogenin (*Pagrus major*)	2e-118
5'v3 2kb/1 M13R	732	phosvitinless vitellogenin (*Pagrus major*)	1e-112
5'v3 1.2kb/15 M13F	760	phosvitinless vitellogenin (*Pagrus major*)	3e-122
5'v3 1.2kb/15 M13R	723	phosvitinless vitellogenin (*Pagrus major*)	3e-105
5'v3 900/44 M13F	731	phosvitinless vitellogenin (*Pagrus major*)	6e-59
5'v3 900/44 M13R	717	phosvitinless vitellogenin (*Pagrus major*)	4e-111
chg-L D12	643	chorion protein (*Liparis atlanticus*)	6e-33
chg-L S32	391	choriogenin L (*Oryzias latipes*)	3e-12
chg-L D12-S4 M13F	880	chorion protein (*Sparus aurata*)	8e-13
chg-L D12-S4 M13R	712	chorion protein (*Liparis atlanticus*)	1.3

Table C.1 Results from BLAST*X* analysis of PCR product which studied in this research (cont.).

Clone or sequence	Insert size (bp)	Homologue (transcript of species)	E-value
chg-L D12-D20	1,751	chorion protein (*Sparus aurata*)	2e-13
actin/52	293	beta-actin (*Sebastes schlegeli*)	1e-48

Table C.2 Results from BLAST*N* analysis of PCR product which studied in this research

Clone	Insert size (bp)	Homologue (transcript of species)	E-value
ER FR/59	116	estrogen receptor alpha (*Mugil cephalus*)	6e-36
3'*ER*/5	1,403	estrogen receptor alpha (*Dicentrarchus labrax*)	7e-101
3'*ER*/32	778	estrogen receptor alpha (*Mugil cephalus*)	0.0
3'*ER*/36	1,769	estrogen receptor alpha (*Chelon labrosus*)	0.0
3'*ER*/49 M13F	757	estrogen receptor alpha (*Dicentrarchus labrax*)	1e-14
3'*ER*/49 M13R	733	estrogen receptor alpha (*Chelon labrosus*)	3e-160
5'*ER*α 1.2/37	974	estrogen receptor alpha (*Oreochromis niloticus niloticus*)	0.0
*ER*α full/8 M13F	714	estrogen receptor alpha (*Micropogonias undulatus*)	5e-11
5'*ER*/23	573	estrogen receptor beta (*Micropterus salmoides*)	7e-111
5'*ER*/36	817	estrogen receptor beta (*Micropterus salmoides*)	3e-145
5'*ER*/39	969	estrogen receptor beta (*Micropterus salmoides*)	5e-141
5'*ER* 900/14	816	estrogen receptor beta (*Micropterus salmoides*)	1e-138
3'*ER*β 1.3/33	1,310	estrogen receptor beta (*Micropogonias undulatus*)	0.0
3'*ER*β 600/38	425	estrogen receptor beta (*Micropterus salmoides*)	3e-32
3'*ER*β 600/1	608	estrogen receptor beta b (*Haplochromis burtoni*)	1e-26
chg-L 900	634	chorion protein (*Sparus aurata*)	2e-108
5'L 500/12	379	chorion protein (*Sparus aurata*)	5e-06
5'L 500/ 18	424	chorion protein (*Sparus aurata*)	7e-09
3'L 500/19	431	DNA sequence from clone RP23-272C14 on chromosome 2 (*Mus musculus*)	0.090

Table C.2 Results from BLAST*N* analysis of PCR product which studied in this research (cont.)

Clone	Insert size (bp)	Homologue (transcript of species)	E-value
3'L 500/ 37	335	DNA sequence from clone RP11-211B4 on chromosome 1 Contains part of the LPHN2 gene for latrophilin 2 (*Homo sapiens*)	1.1
chg-L full length/19	1,293	chorion protein (*Sparus aurata*)	7e-110
5'*chg*-H 1.5/10-M13F (*chg*-L)	723	chorion protein (*Liparis atlanticus*)	2e-47
chg-H/11 M13F	705	vitelline envelope protein alpha (*Oncorhynchus mykiss*)	0.009
chg-H/11 M13R	699	chorigenin H (*Cichlasoma facetum*)	9e-62
3'*chg*H 1.1/13	969	zona pellucida protein Bb (*Sparus aurata*)	2e-122
3'*chg*H 800/51	699	zona pellucida protein Bb (*Sparus aurata*)	7e-120
vtg-1/28	367	Vitellogenin 1 (*Mugil curema*)	1e-126
5'*vtg*-1 250/16	203	Vitellogenin A (*Gambusia affinis*)	1e-14
3'*vtg*-1 4.3/51 M13F	737	DNA sequence from clone DKEY-25L21 in linkage group 17 (*Danio rerio*)	3e-68
3'*vtg*-1 4.3/51 M13R	732	vitellogenin (*Epinephelus coioides*)	1e-156
3'*vtg*-1 4.3/51 3'v1 i F1	910	Vitellogenin A (*Pagrus major*)	8e-174
3'*vtg*-1 4.3/51 3'v1 i F2	835	Vitellogenin A (*Pagrus major*)	8e-180
3'*vtg*-1 4.3/51 3'v1 i R1	907	Vitellogenin A (*Pagrus major*)	8e-171
3'*vtg*-1 4.3/51 3'v1 i R2	847	Vitellogenin A (*Pagrus major*)	3e-59
3'GW v1-H-33-M13F	783	vitellogenin (*Epinephelus coioides*)	3e-43
3'GW v1-H-33-M13R	814	vitellogenin (*Epinephelus coioides*)	3e-43
3'GW v1-H-45	493	vitellogenin (*Epinephelus coioides*)	6e-31
vtg-3 F1-alpha 500	374	phosvitinless vitellogenin (*Pagrus major*)	9e-82
vtg-3 F1-*Sal*I 1kb/7 M13F	784	phosvitinless vitellogenin (*Pagrus major*)	7e-07
vtg-3 F1-*Sal*I 1kb/7 M13R	714	phosvitinless vitellogenin (*Pagrus major*)	3e-120
5'V3-1-5-10-M13F	736	phosvitinless vitellogenin (*Pagrus major*)	7e-41
5'V3-1-5-10-M13R	689	clone mth2-143b6 (*Medicago truncatula*)	2.7
5'v3 2kb/1 M13F	757	phosvitinless vitellogenin (*Pagrus major*)	0.0
5'v3 2kb/1 M13R	732	phosvitinless vitellogenin (*Pagrus major*)	2e-149

Table C.2 Results from BLAST*N* analysis of PCR product which studied in this research (cont.)

5'v3 1.2kb/15 M13F	760	phosvitinless vitellogenin (*Pagrus major*)	1e-178
5'v3 1.2kb/15 M13R	723	phosvitinless vitellogenin (*Pagrus major*)	1e-125
5'v3 900/44 M13F	731	phosvitinless vitellogenin (*Pagrus major*)	1e-92
5'v3 900/44 M13R	717	phosvitinless vitellogenin (*Pagrus major*)	3e-154
chg-L D12	643	choriogenin L (*Oryzias javanicus*)	6e-13
chg-L S32	391	chorion protein (*Liparis atlanticus*)	3e-04
chg-L D12-S4 M13F	880	chorion protein (*Sparus aurata*)	1e-05
chg-L D12-S4 M13R	712	BAC clone RP23-193M8 from 13 (*Mus musculus*)	0.16
chg-L D12-D20	1,751	chorion protein (*Sparus aurata*)	3e-34
actin/52	293	beta-actin (*Monopterus albus*)	1e-117

APPENDIX D

Publication from this thesis

1. Arttasit Tangserisuksan, Narongsak Puanglarp and Piamsak Menasveta (2005). Molecular cloning and characterization of choriogenin and vitellogenin genes in greenback mullet (*Liza subviridis*). 31^{st} Congress on Science and technology of Thailand (Oral presentation).

2. Arttasit Tangserisuksan, Narongsak Puanglarp and Piamsak Menasveta (2006). Molecular cloning and characterization of estrogen receptor genes in greenback mullet (*Liza subviridis*). 32^{nd} Congress on Science and technology of Thailand (Oral presentation).

Biography

Mr. Arttasit Tangserisuksan was born on August 25, 1981 in the province Bangkok, Thailand. He graduated with the degree of Bachelor of Science (2^{nd} Class Honours) in Biochemistry from faculty of Science at Chulalongkorn University in 2003. In 2003, he entered the Master program of Biotechnology at Chulalongkorn University.