

APPENDICES

Appendix I: Human Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms KD Amoils

CLEARANCE CERTIFICATE

M110134

PROJECT

Breast Cancer-Control of Clonal Proliferation by
Hormonal and Growth Factors
(Previously M980307 Prof AP MacPhail)

INVESTIGATORS

Ms KD Amoils.

DEPARTMENT

Department of Internam Medicine

DATE CONSIDERED

28/01/2011

DECISION OF THE COMMITTEE*

Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE

02/02/2011

CHAIRPERSON

PE Cleaton-Jones
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof AP MacPhail

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

KD Amoils

Appendix II: Buffers and Solutions

Buffer/Solution	Working Solution (1x)	Concentrated stock solution (per litre)
Tris-acetate (TAE)	1 x : 0.04M Tris-acetate 0.001M EDTA	50 x : 242g Tris base 57.1ml glacial acetic acid 100ml 0.5M EDTA (pH 8.0)
Phosphate Buffered Saline (PBS) [0.15M]	8g NaCl 0.2g KCl 1.15g Na ₂ HPO ₄ 0.2g KH ₂ PO ₄ Make up to 1 litre with distilled water.	
Sodium acetate (3M) pH 5.2	408.1g sodium acetate.3H ₂ O 800ml H ₂ O Adjust to pH 5.2 with glacial acetic acid. Adjust volume to 1 litre with H ₂ O	

Appendix III: Serum preparation and Stains for *in vitro* experiments.

A. Preparation of Stripped foetal calf serum using Dextran Coated Charcoal (DCC).

Physiological concentrations of steroid hormones are present in serum-containing media in which cells are usually grown (Esber *et al*, 1973). Oestradiol concentrations as high as 10^{-8} M have been found in some foetal calf sera (Esber *et al*, 1973). Serum is therefore stripped of endogenous steroid prior to experiments involving response to steroidal compounds. Dextran coated charcoal is used to strip endogenous hormones from foetal calf serum. This method is a modification of that of Korenman (1968).

Mix 0.02g Dextran (BDH Laboratory Supplies, Poole, England) with 2g activated charcoal (Merck, Darmstadt, Germany) in 50 ml sterile water. Centrifuge for 1 hour on a benchtop centrifuge.

Pour off liquid.

Mix pellet with 20ml FCS (Delta Bioproducts, Kempton Park, South Africa).

Vortex and allow to stand at 4°C for 1 hour with occasional shaking.

Centrifuge and pour off FCS.

Filter FCS through a 0.22 μ m Millipore filter (Millipore, Bedford, MA, USA).

B. Preparation of May and Grunwald's Stain.

0.3g May and Grunwald's powdered dye (BDH Chemicals Ltd,

Poole, England)

100ml methanol (SAARCHEM, Muldersdrift, South Africa)

Mix and warm to 50°C.

Cool to room temperature, shaking occasionally

Stand for 24 hours and filter through Whatman No. 1 filter paper

(Clifton, NJ, USA) before use.

C. Preparation of Giemsa Stain.

1g Giemsa powdered dye (BDH Chemicals Ltd)

Add 66ml glycerol (SAARCHEM, Muldersdrift, South Africa) and heat at 56°C for 90 to 120 mins.

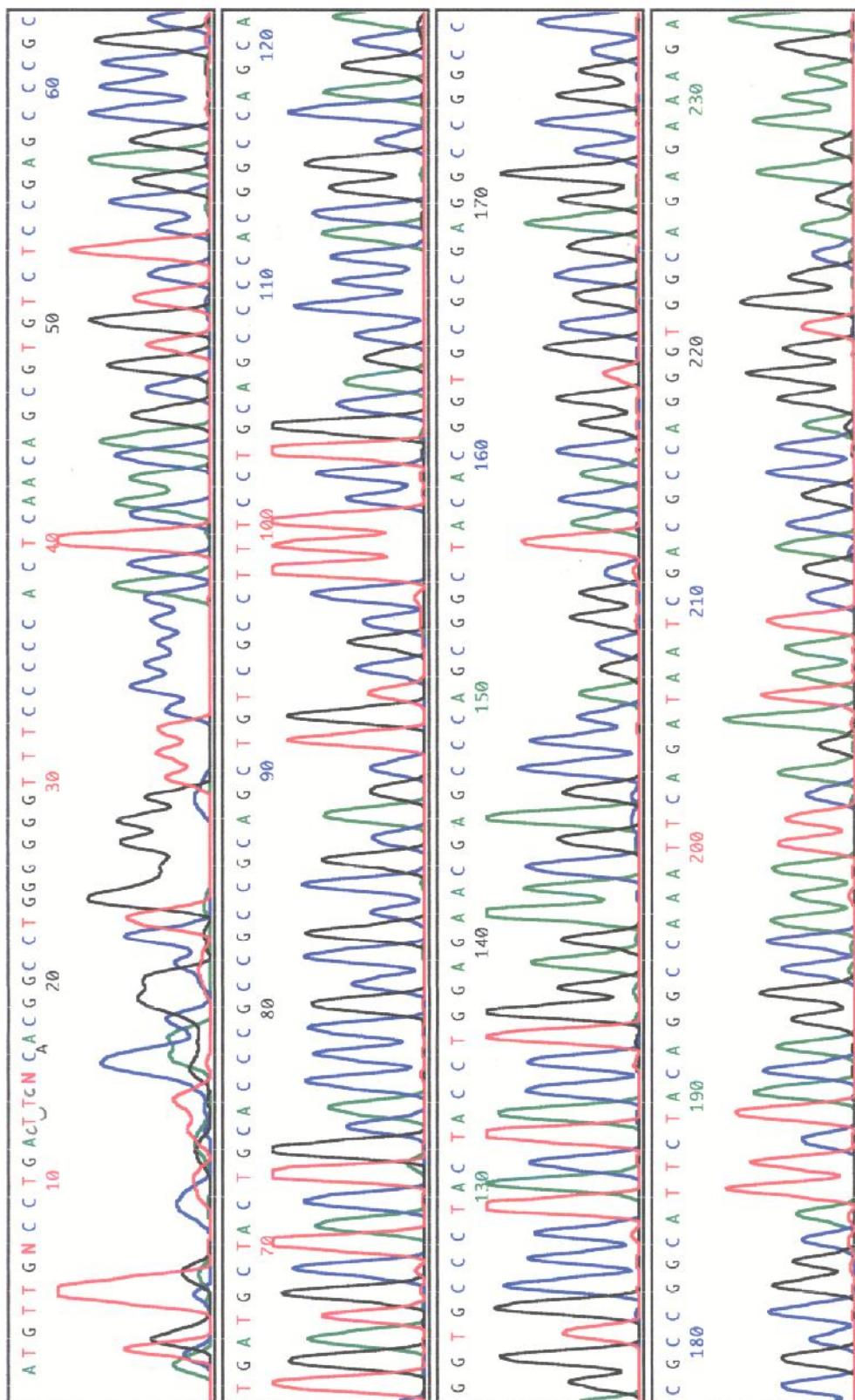
Add 66ml methanol (SAARCHEM) and mix thoroughly.

Stand for 7 days at room temperature and then filter through Whatman No. 1 filter paper (Clifton, NJ, USA) prior to use.

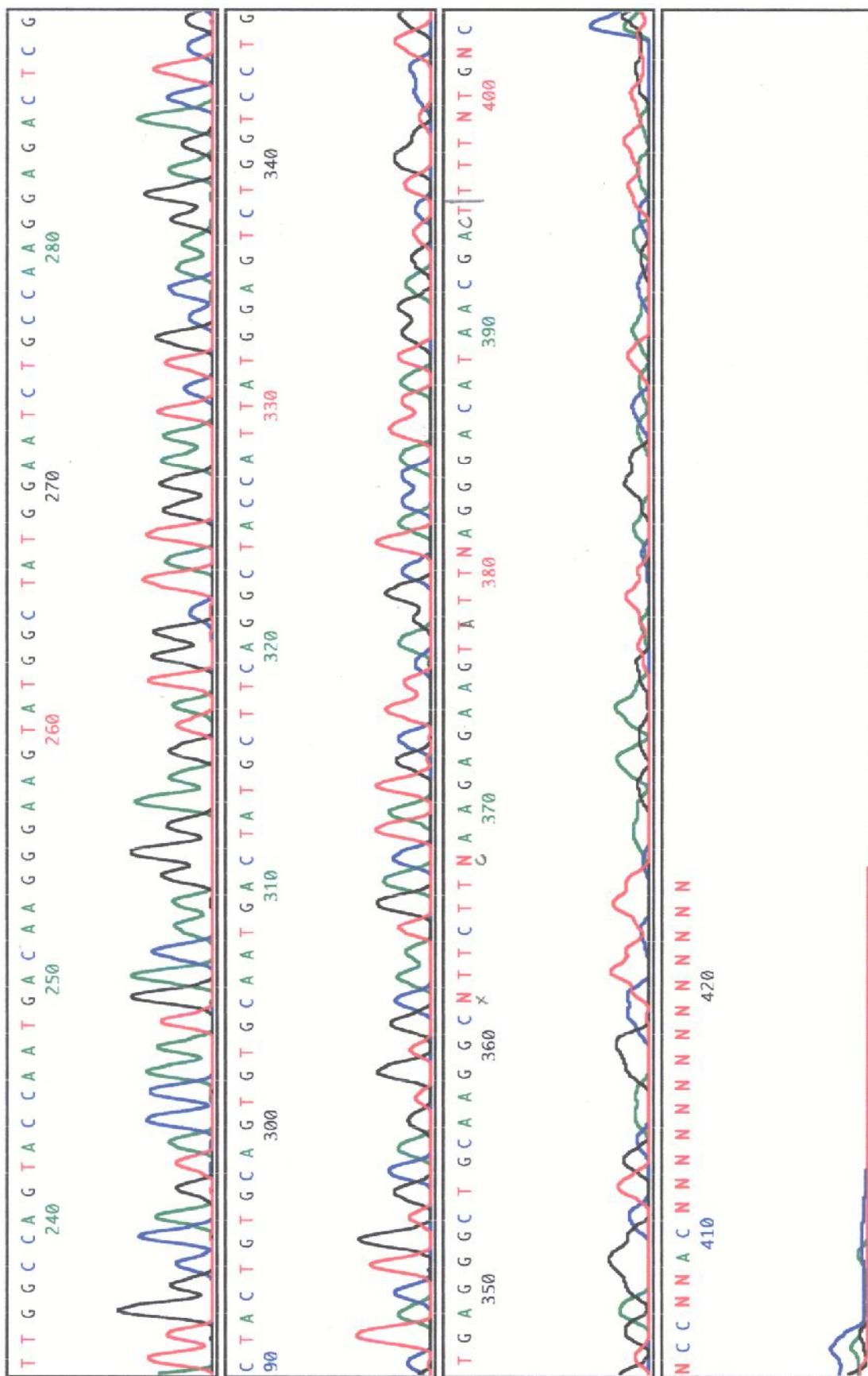
APPENDIX IV: Sequencing spectrographs of ER α exons

A. ER α exon 2

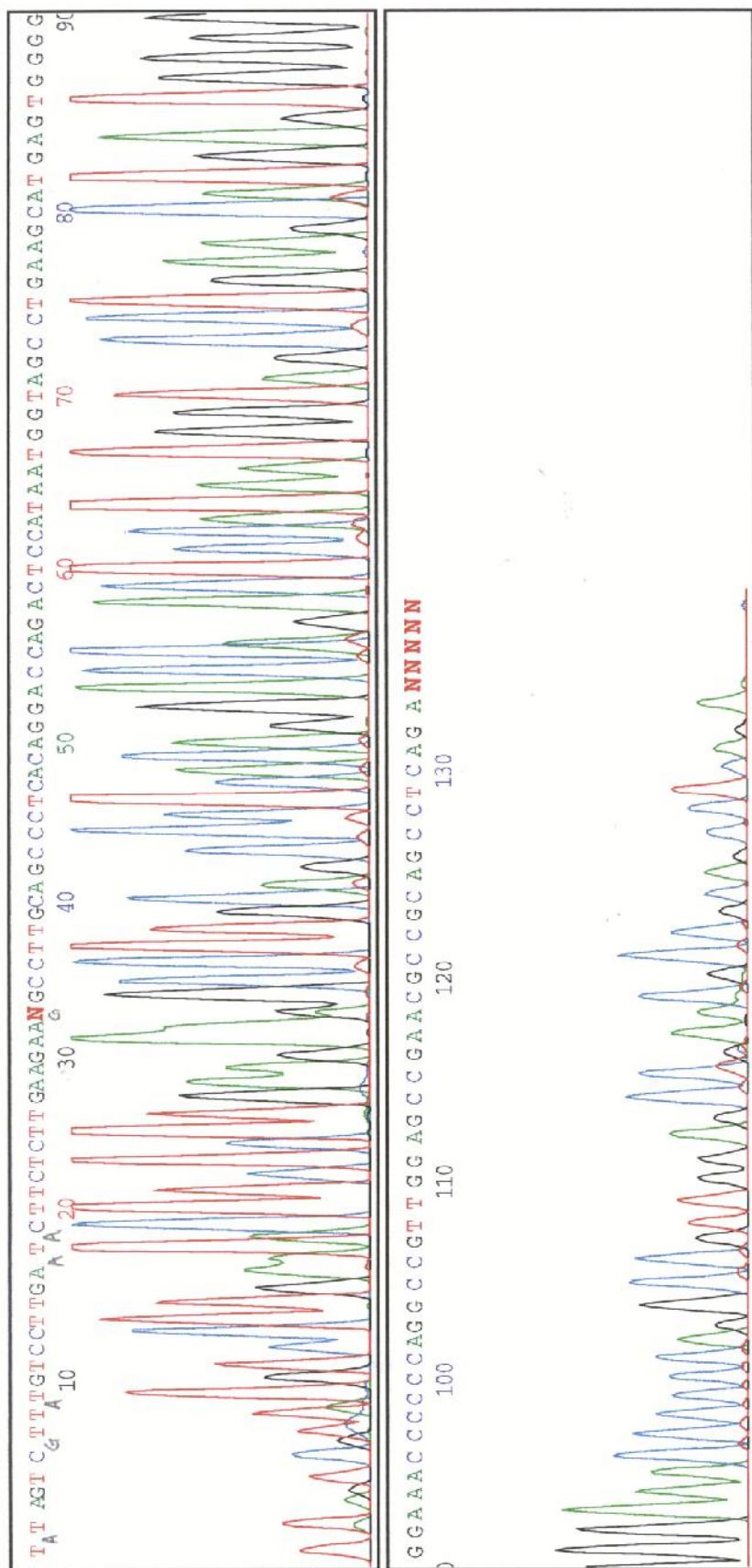
A1. ER α 2 (427bp band) spectrograph (forward primer)



A1. (contd). ER α 2 (427bp band) spectrograph (forward primer) continued

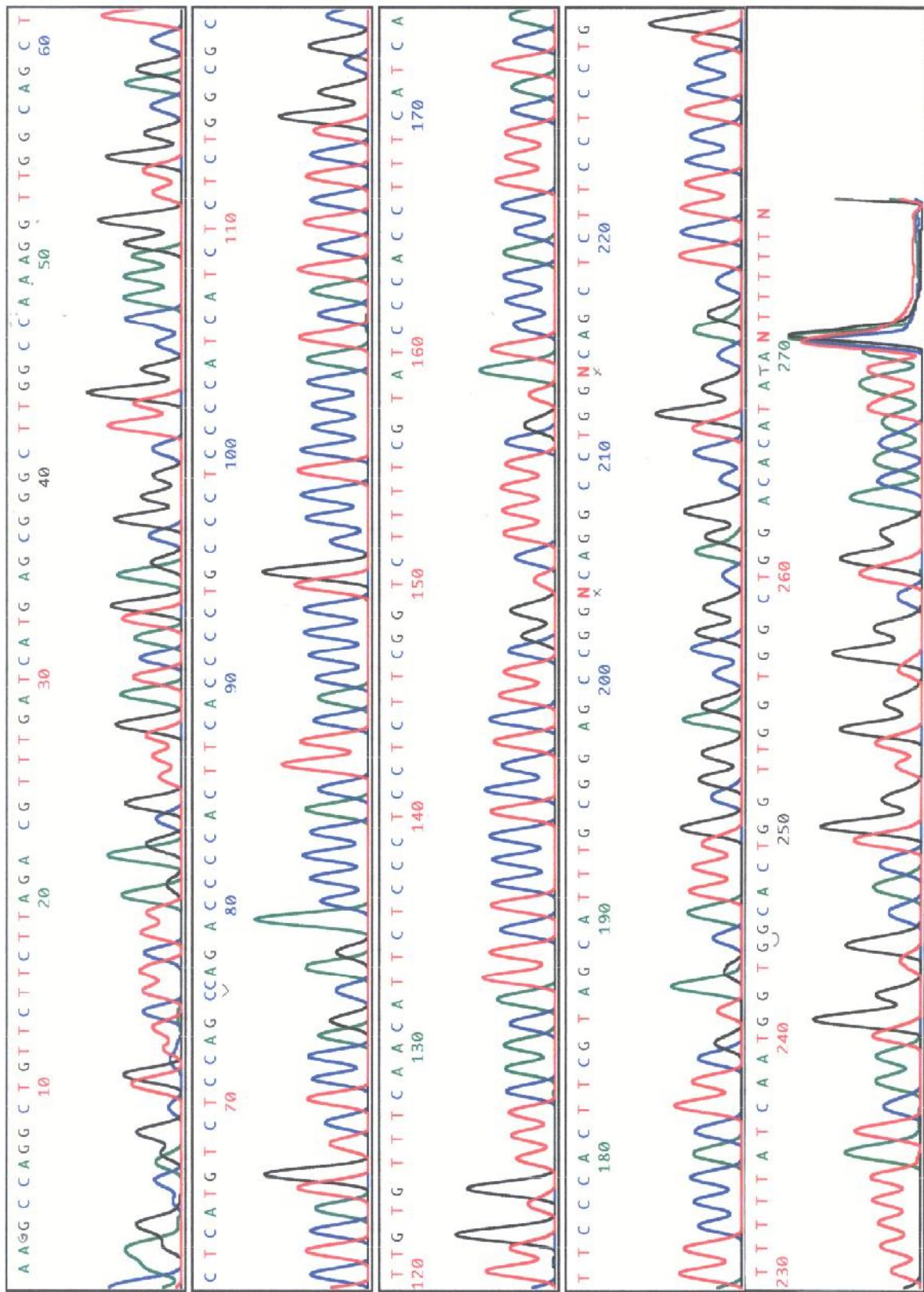


A2. ER α 2 (236bp band) spectrograph (complementary primer)



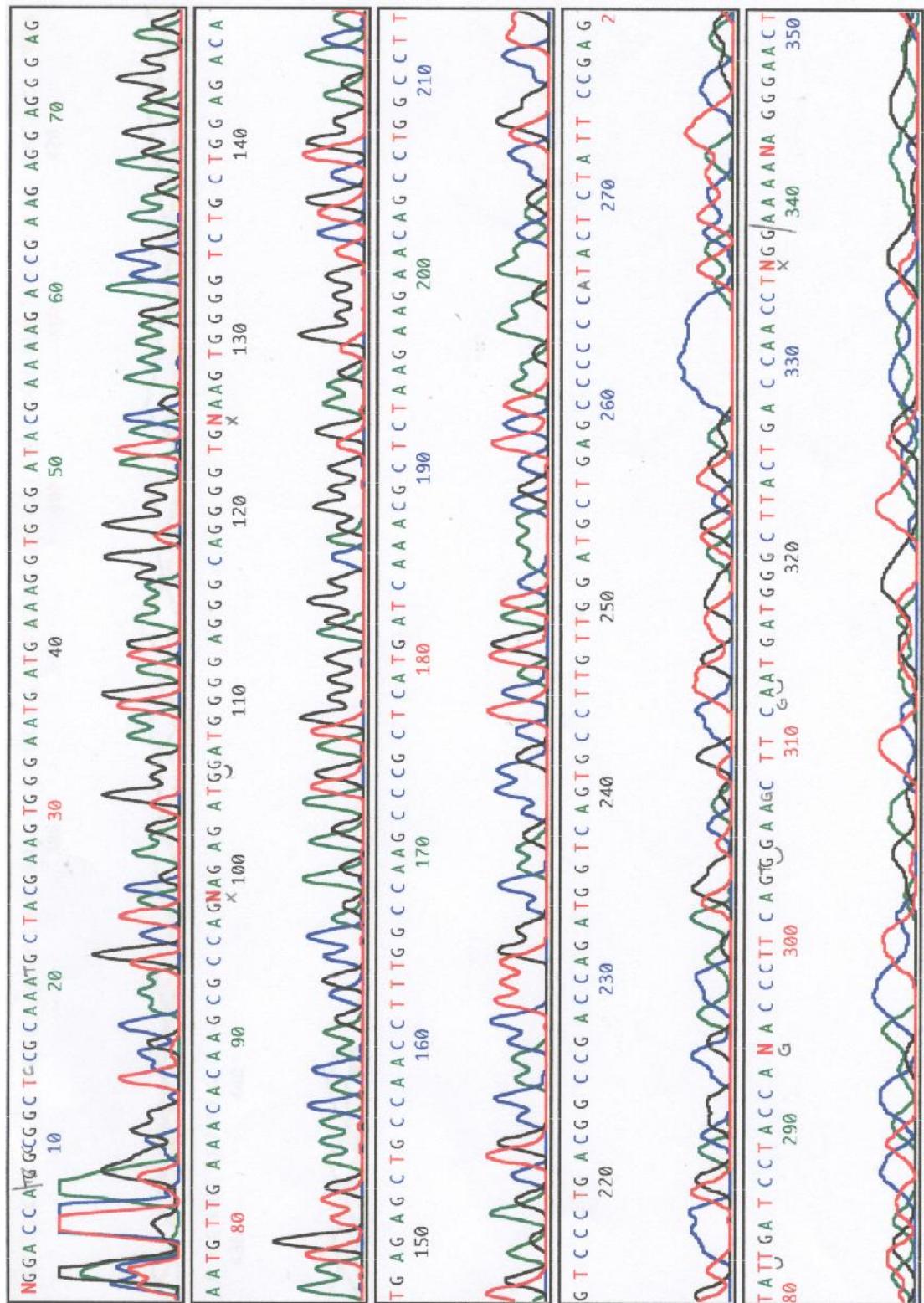
B. ERα exon 3

B1. ERα3 (322bp band) spectrograph (reverse primer)

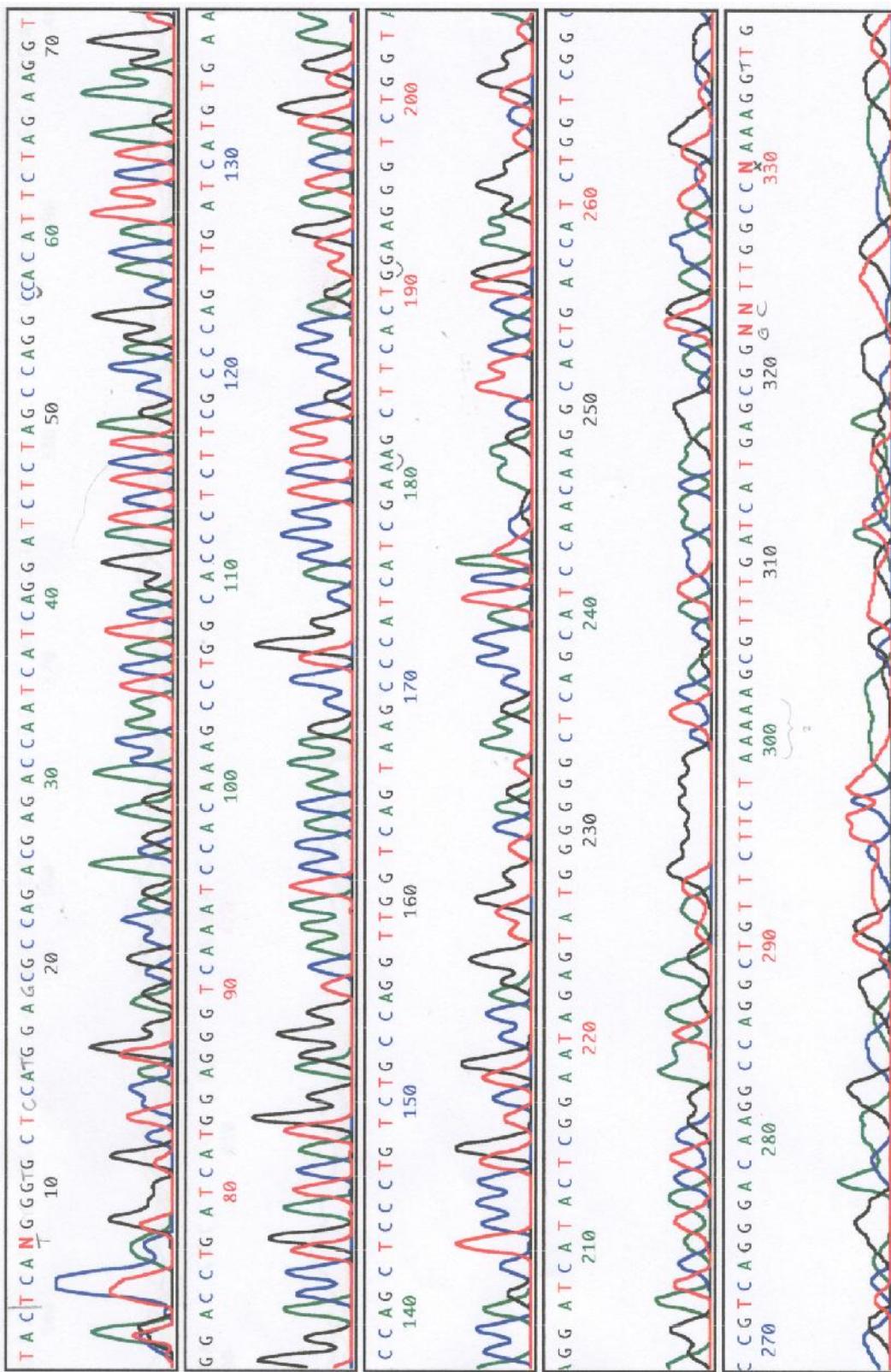


C. ER α exon 4

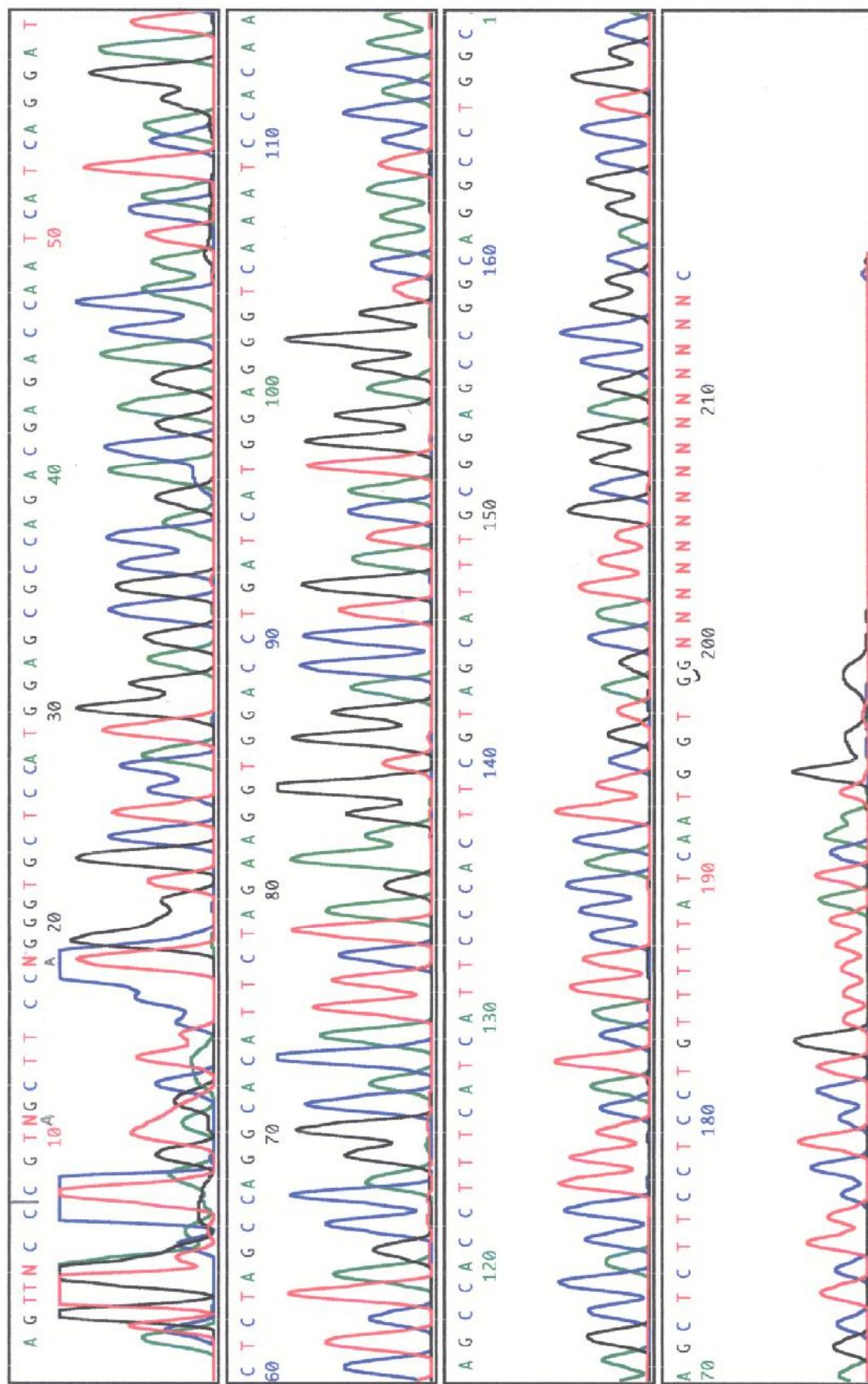
C1a. ER α 4 (555bp band) spectrograph (forward primer)



C1b. ER α 4 (555bp band) spectrograph (complementary primer)

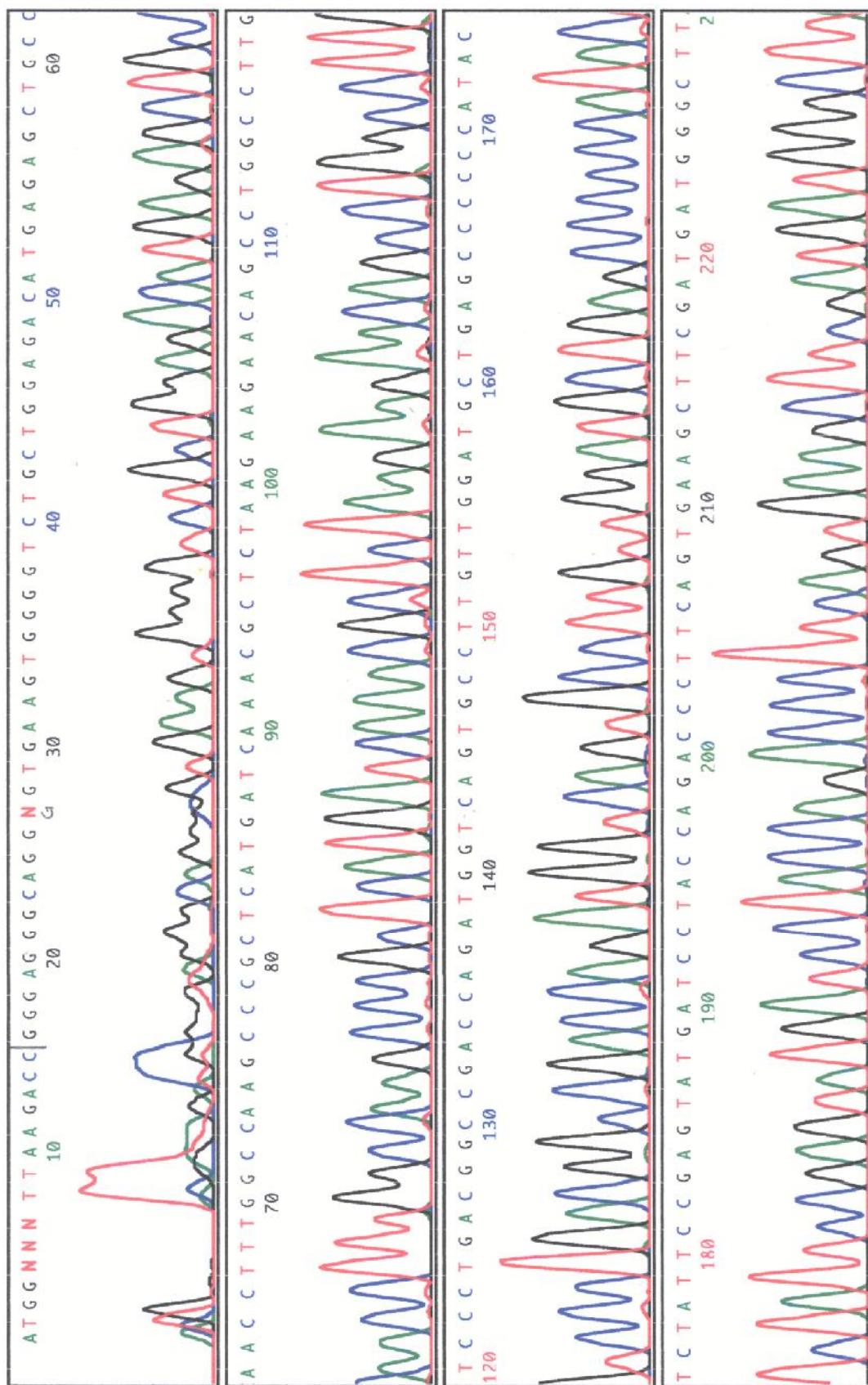


C2a. ER α 4 (219bp band) spectrograph (forward primer)

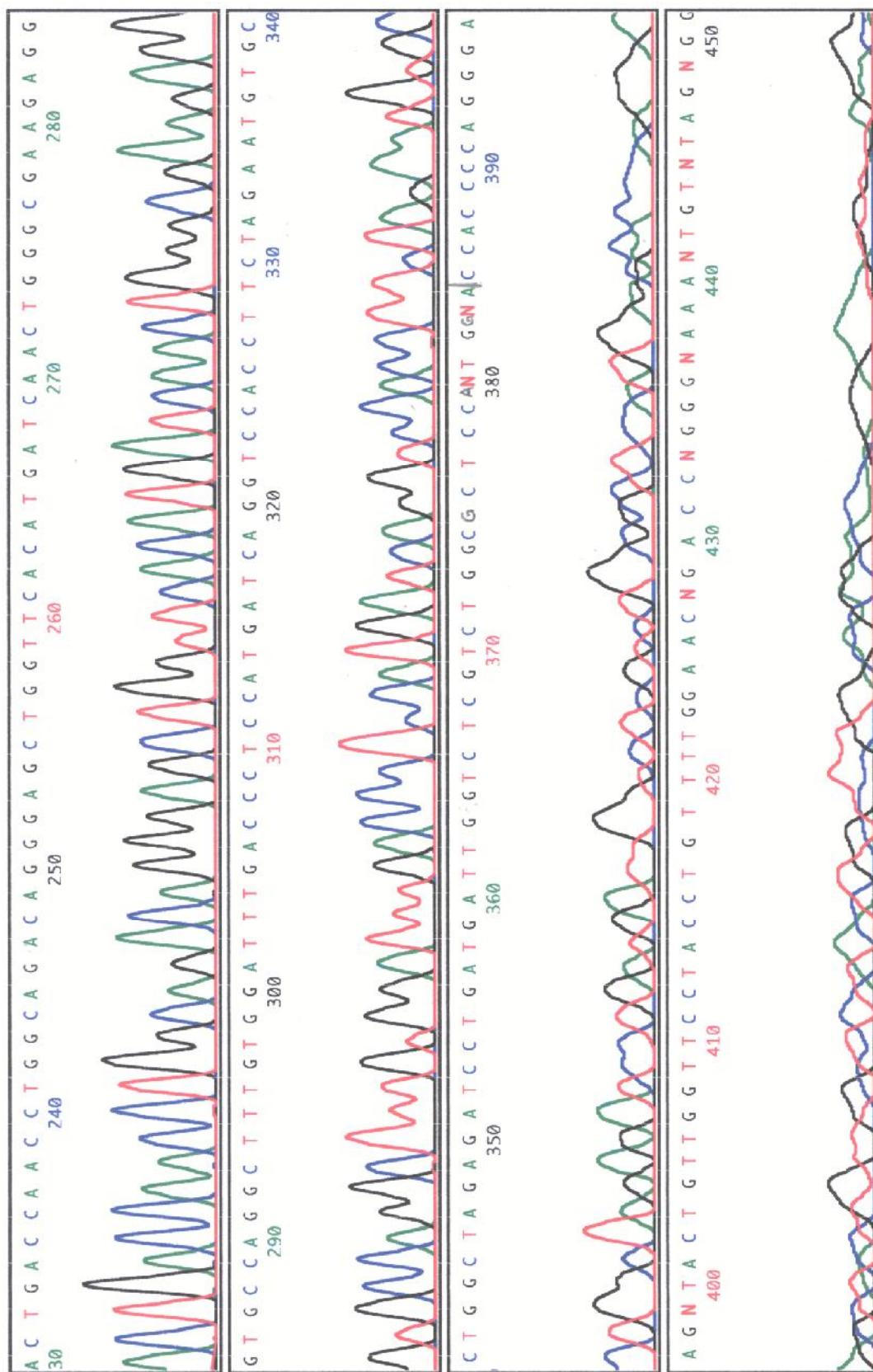


D. ER α exon 5

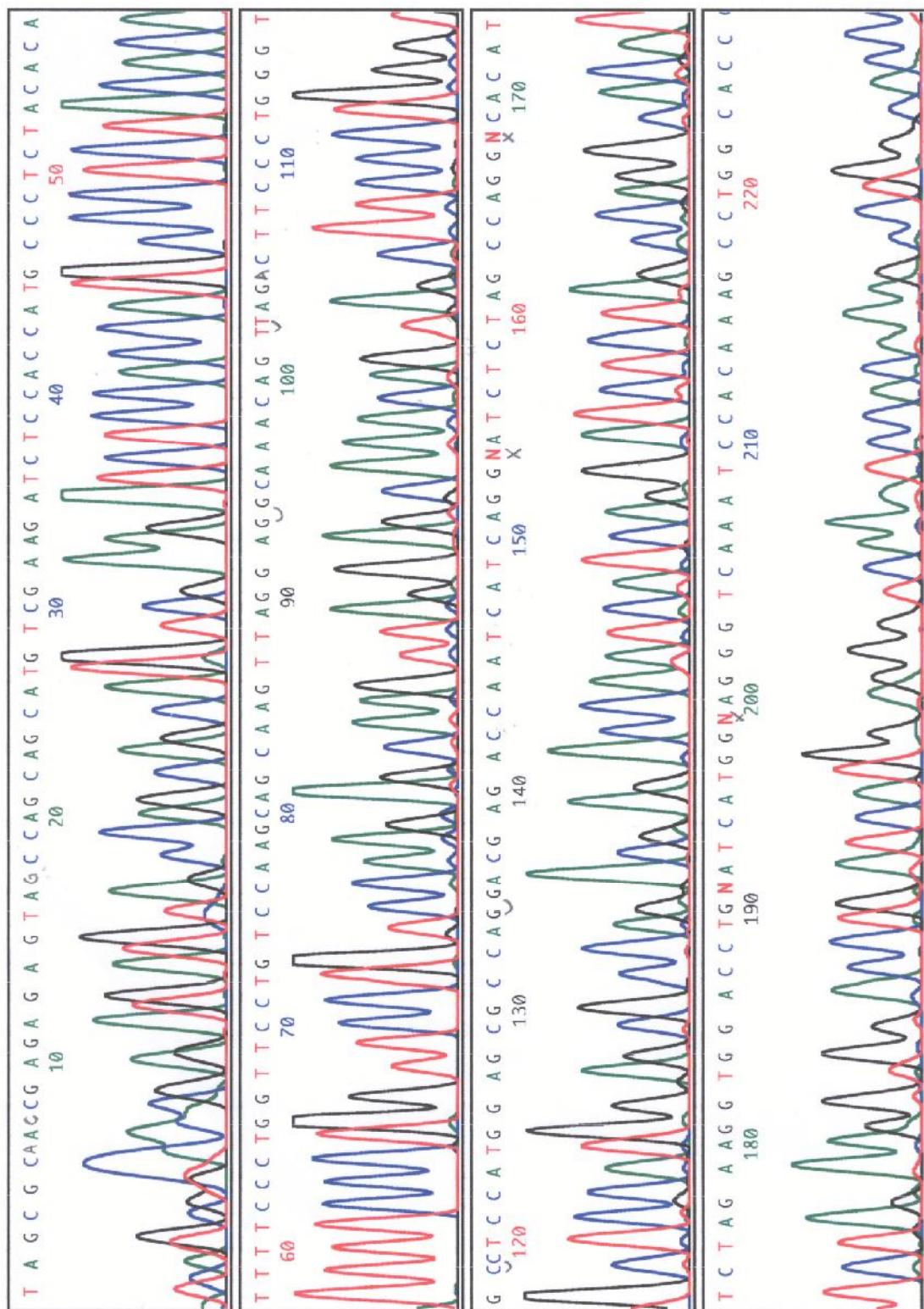
D1a. ER α exon 5 (544bp band) spectrograph (forward primer)



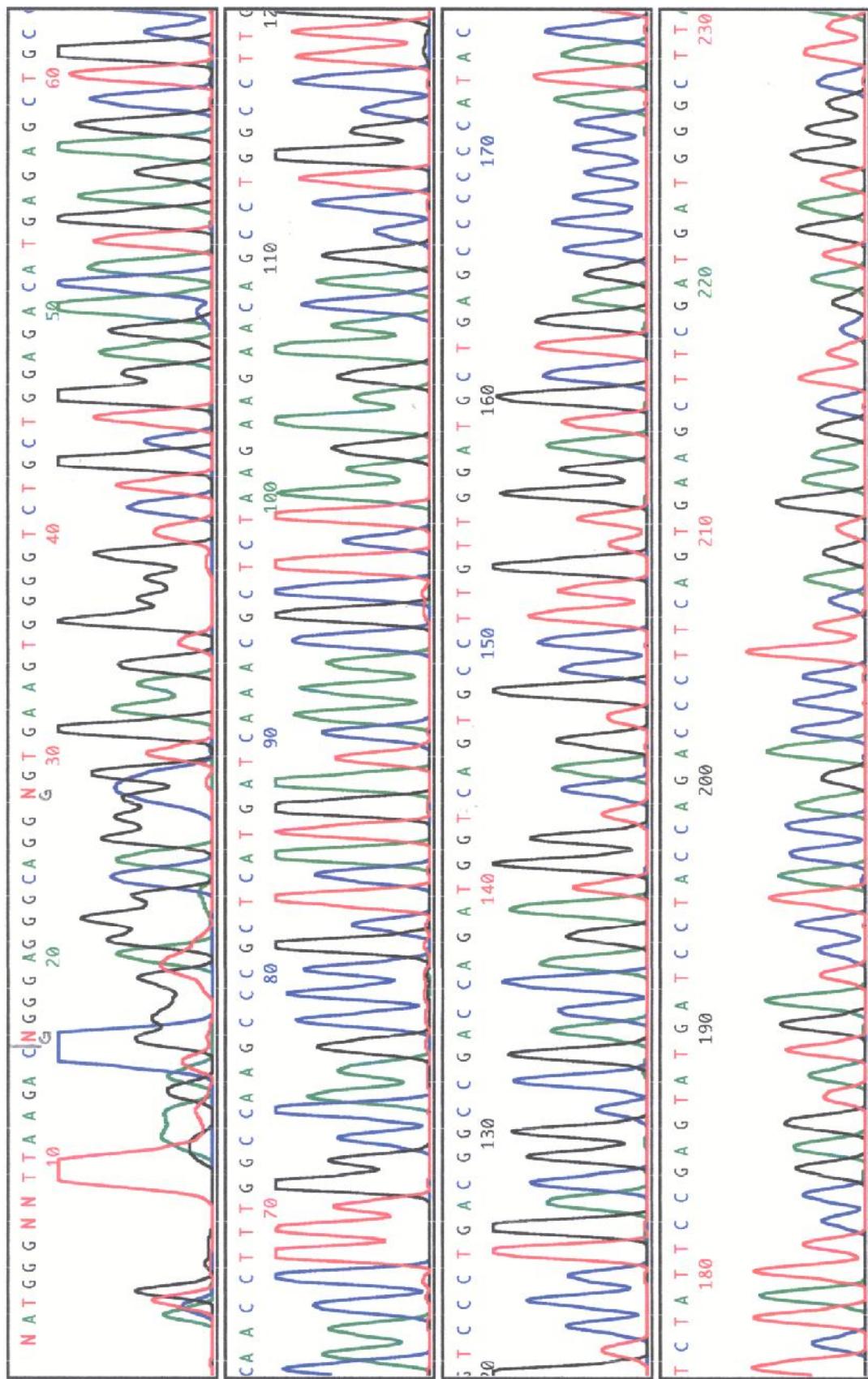
D1a (contd). ER α 5 (544bp band) spectrograph (forward primer) continued



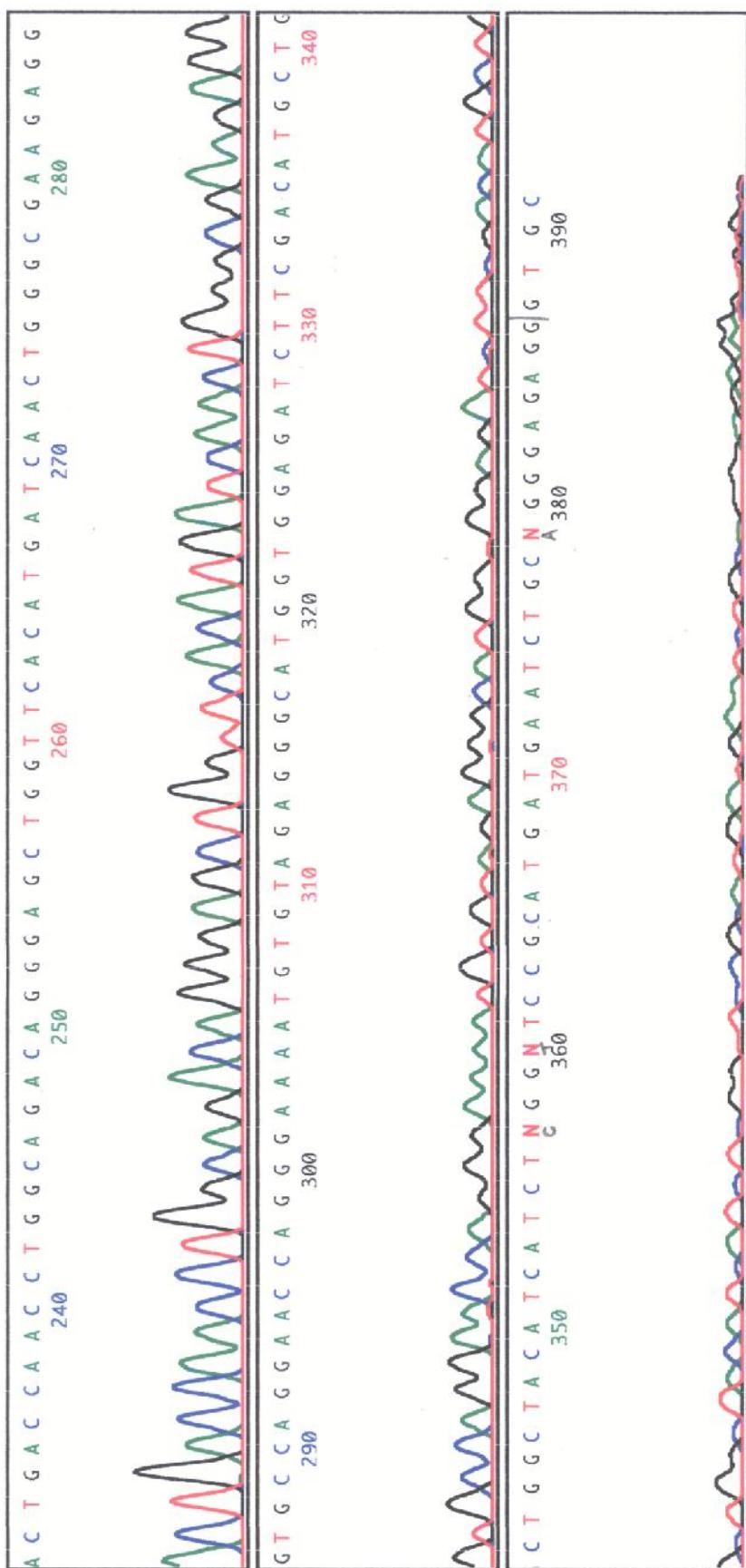
D1b. ER α 5 (544bp band) spectrograph (complementary primer)



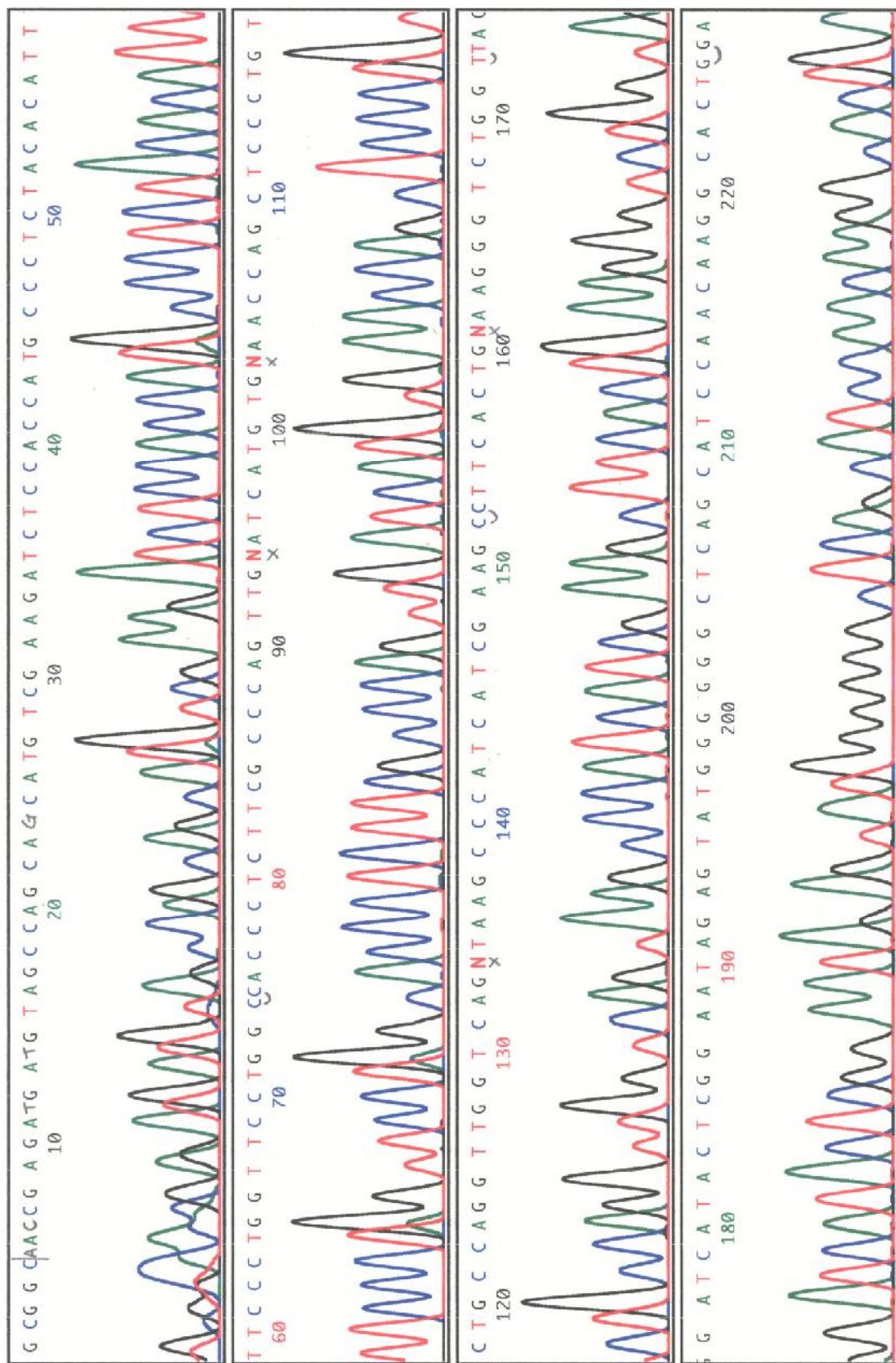
D2a. ERa5 (405bp band) spectrograph (forward primer)



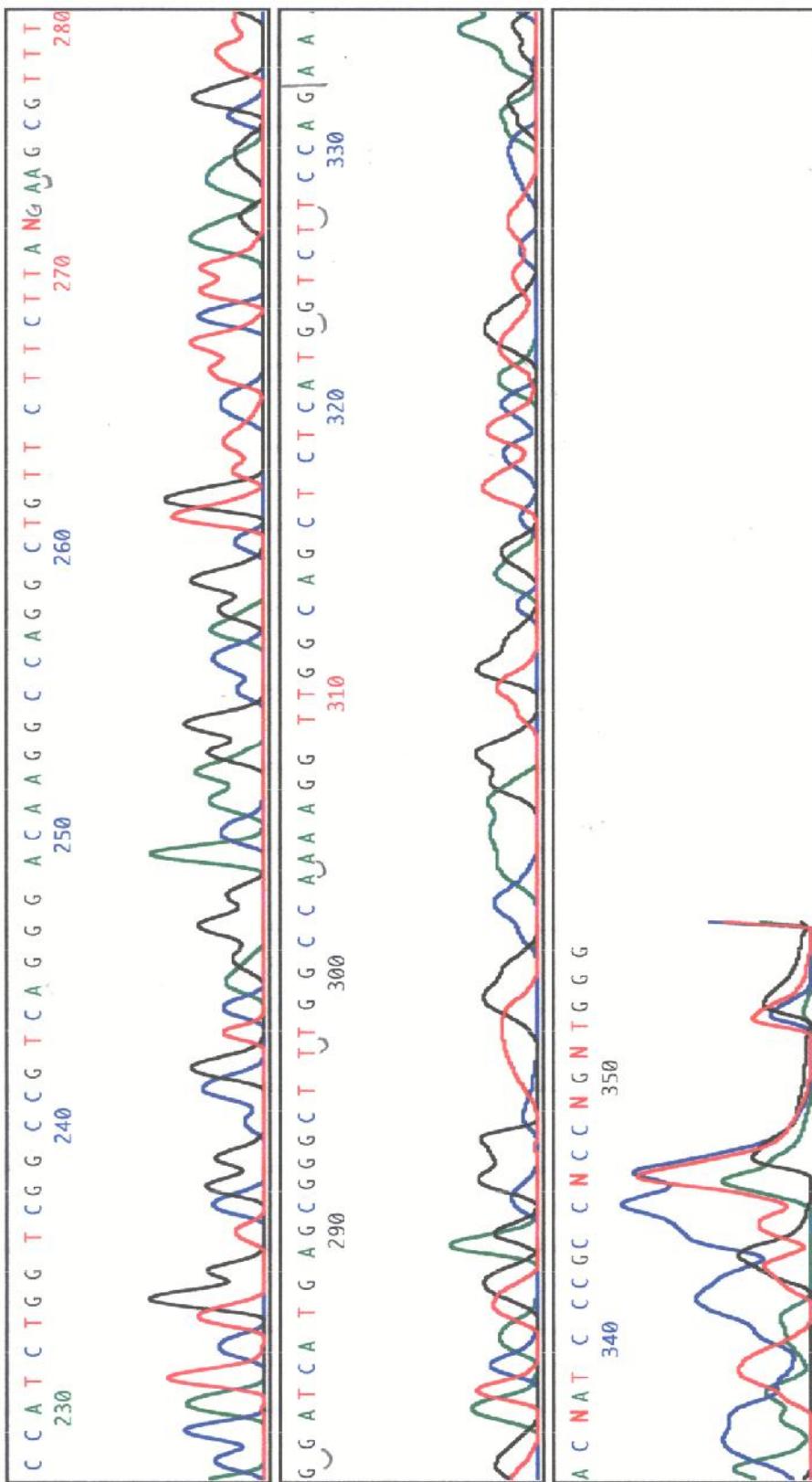
D2a (contd). ERα5 (405bp band) spectrograph (forward primer) continued



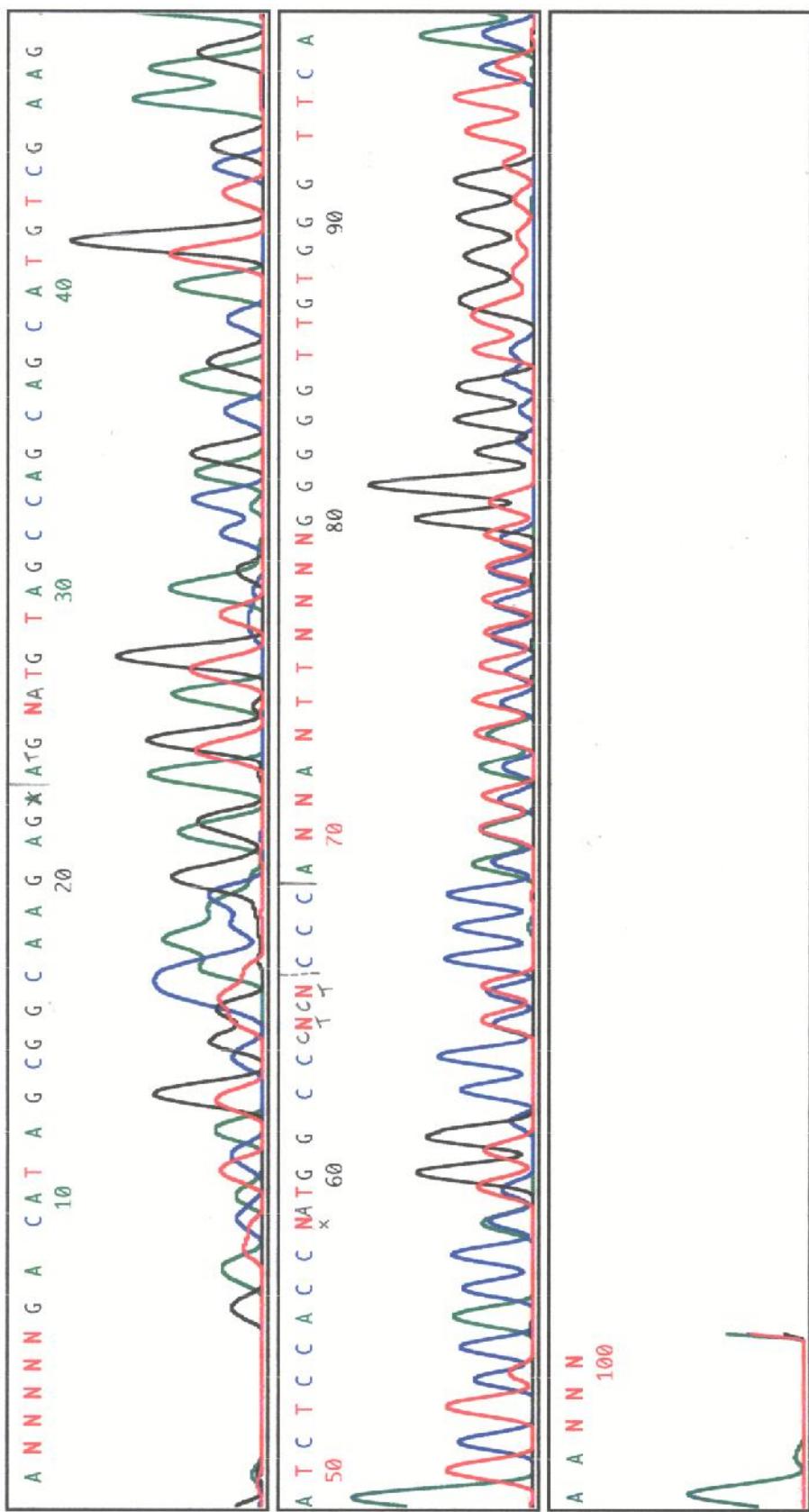
D2b. ER α 5 (405bp band) spectrograph (complementary primer)



D2b (contd). ERa5 (405bp band) spectrograph (complementary primer)
continued

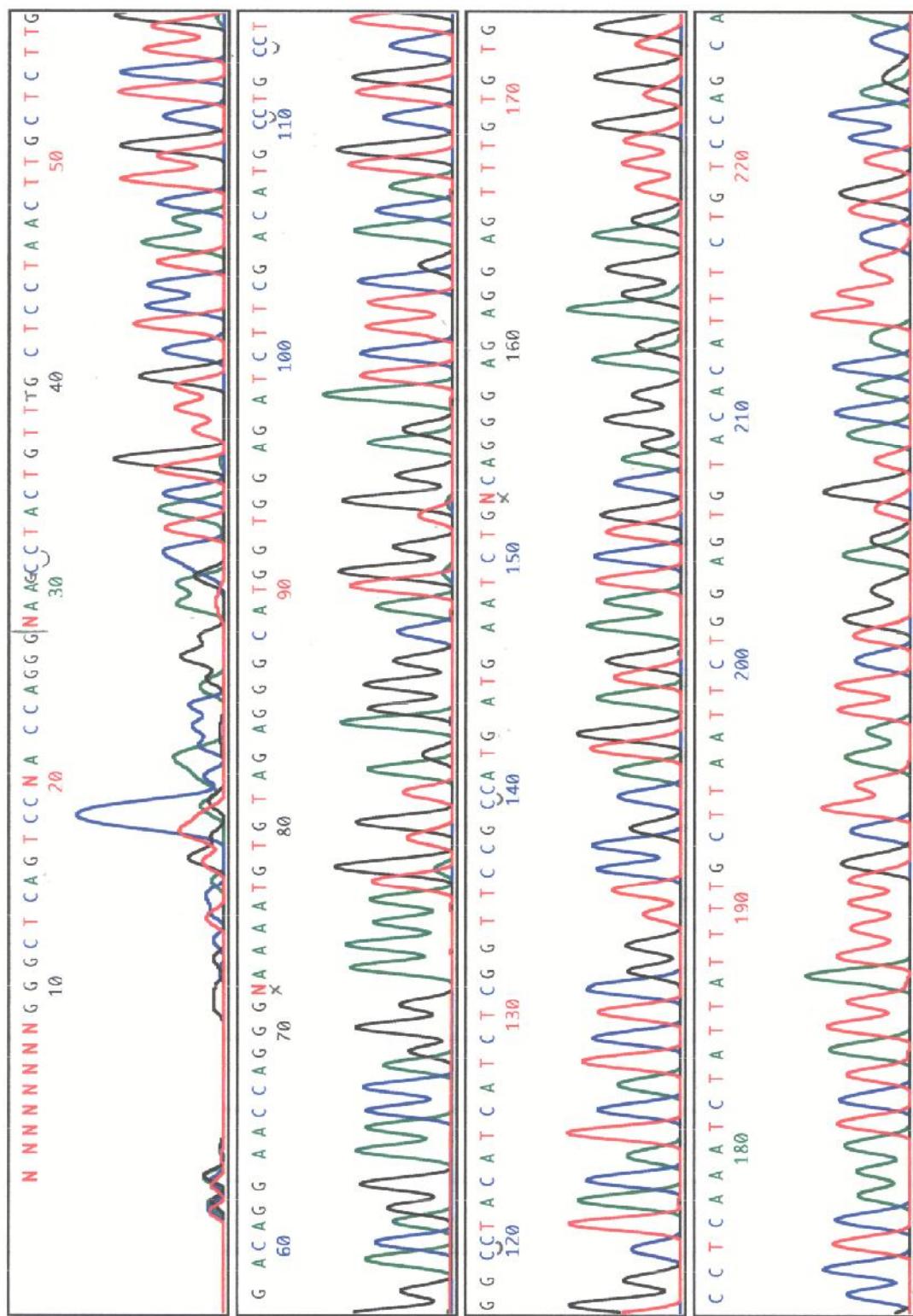


D3. ERa5 (80bp band) spectrograph (complementary primer)

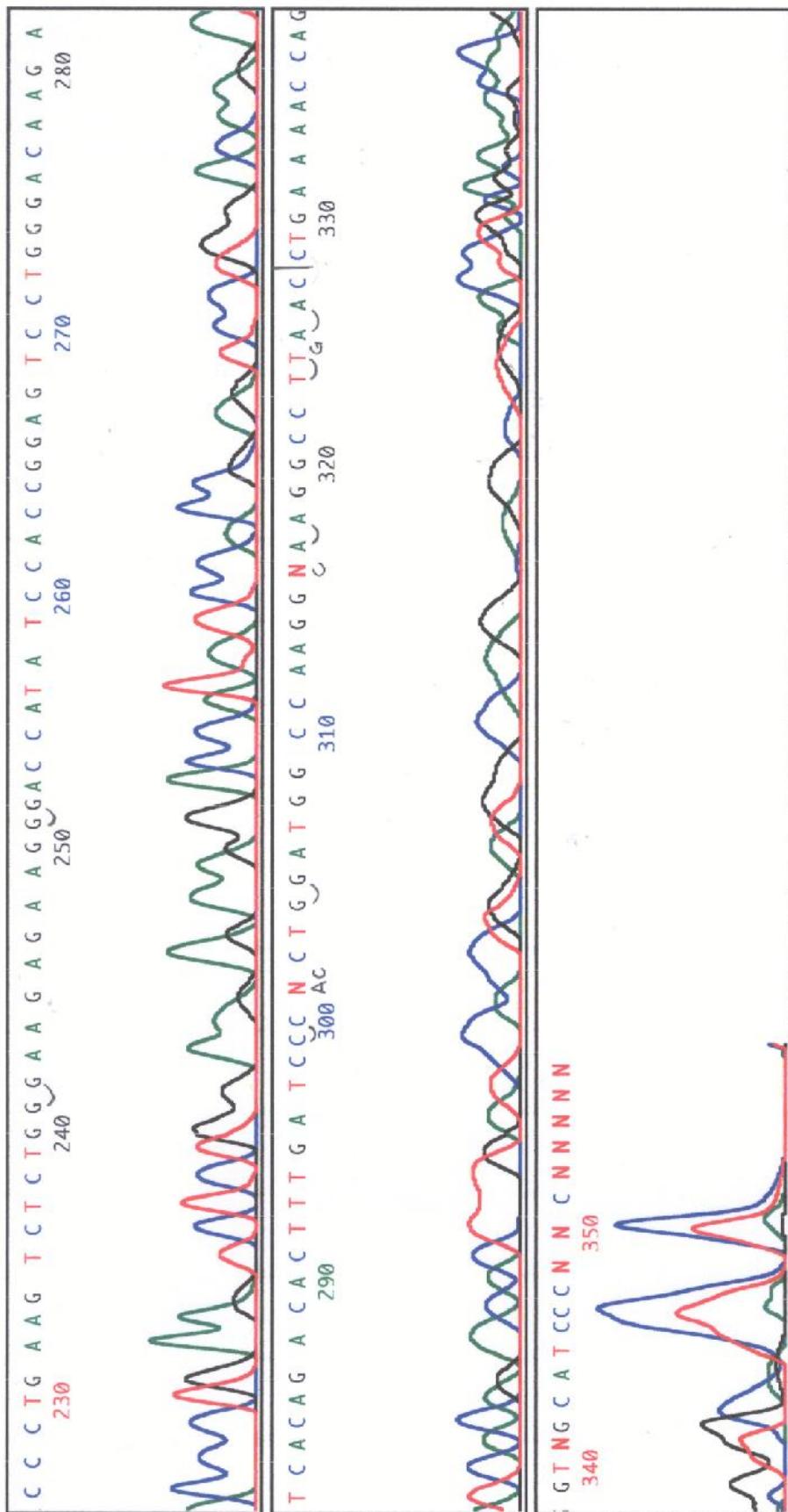


E. ER α exon 6.

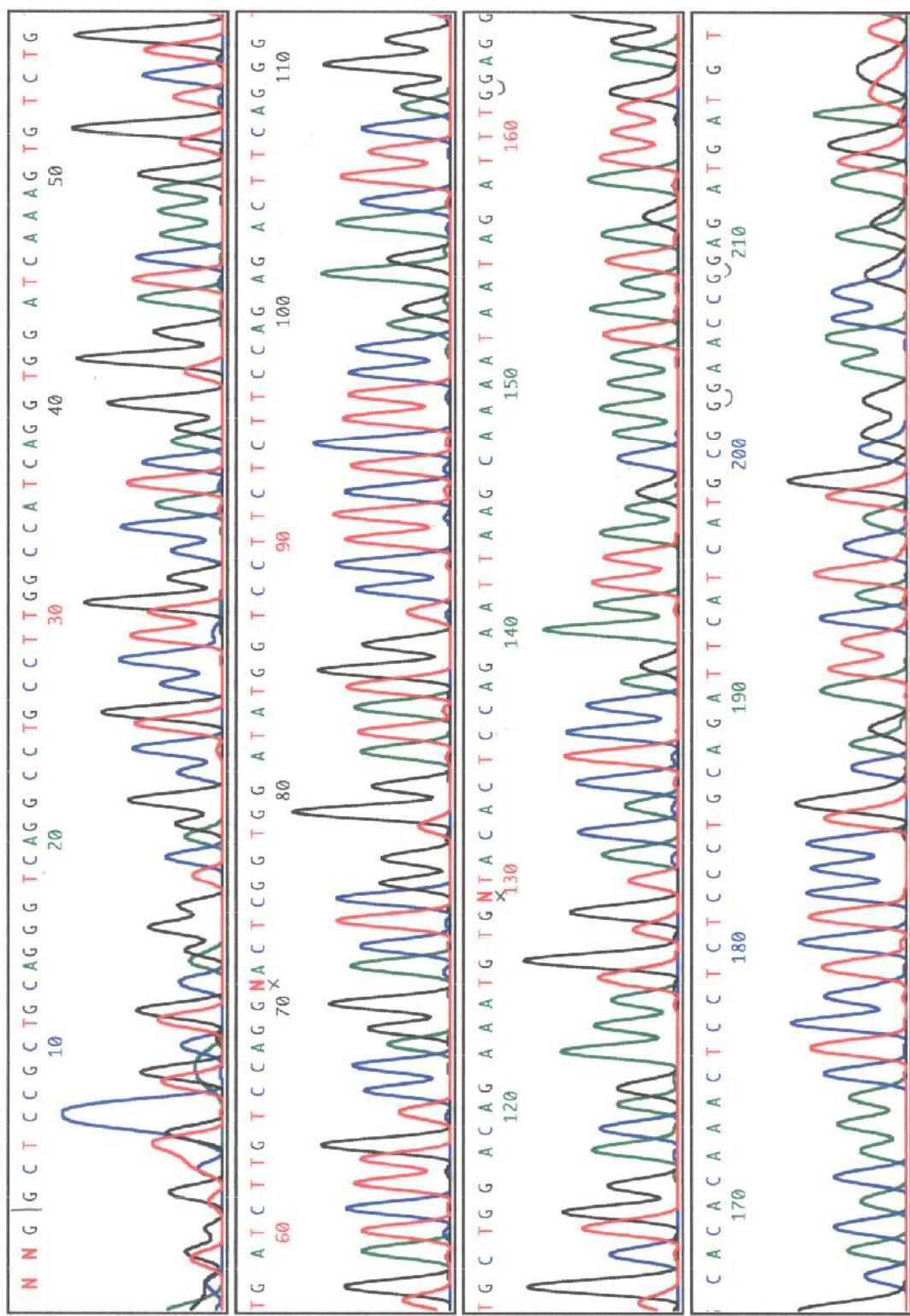
E1a. ER α 6 (388bp band) spectrograph (forward primer)



E1a. (contd). ER α 6 (388bp band) spectrograph (forward primer) continued

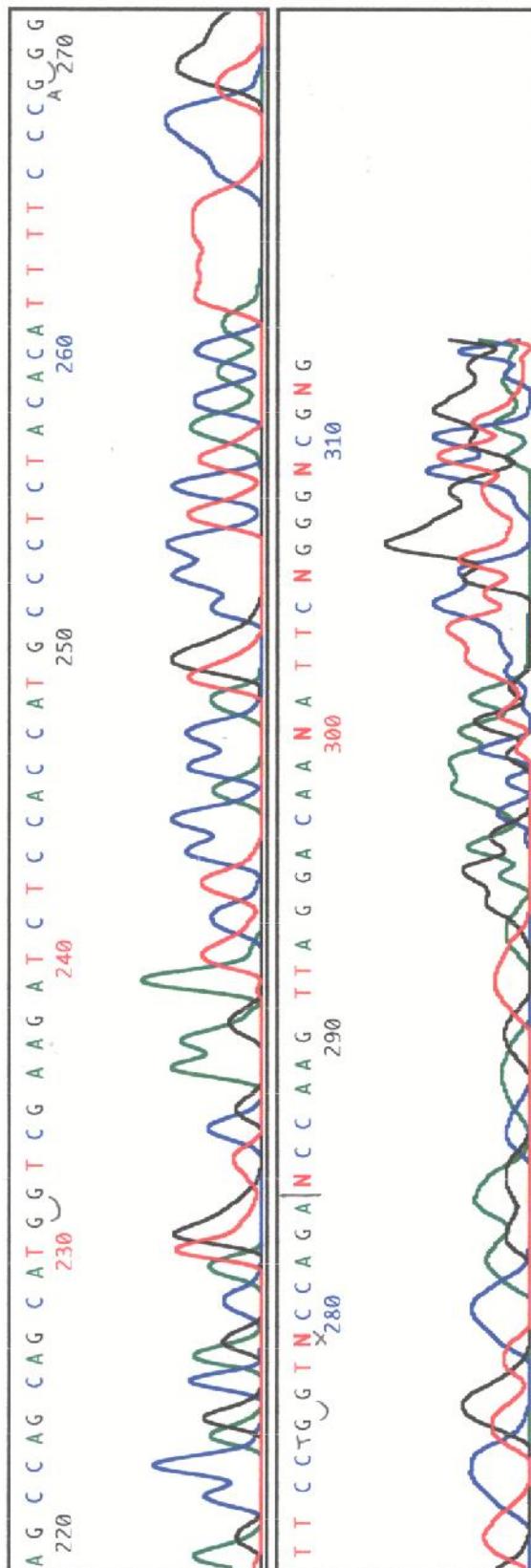


E1b. ERα6 (388bp band) spectrograph (complementary primer)



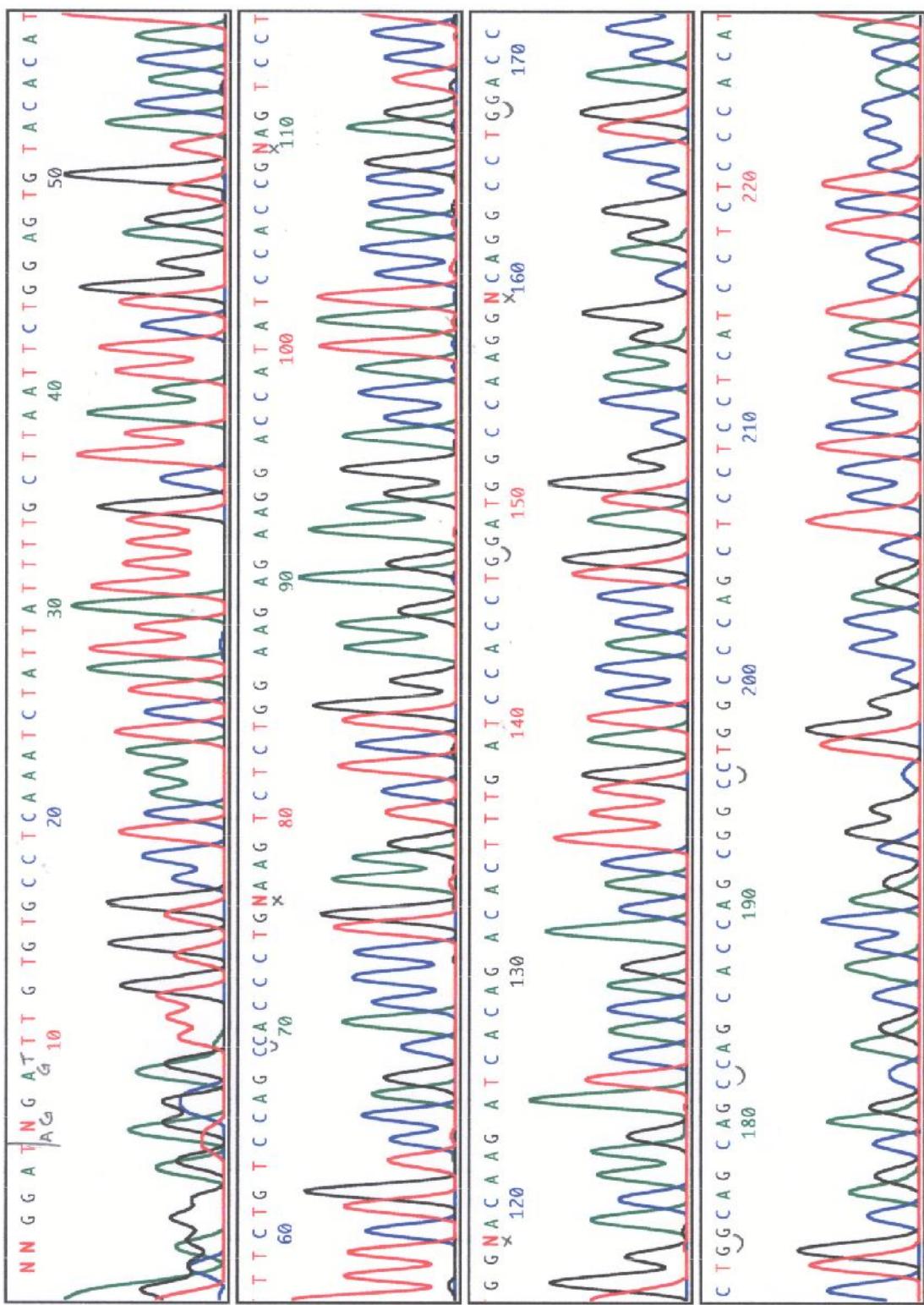
E1b. (contd). ER α 6 (388bp band) spectrograph (complementary primer)

continued

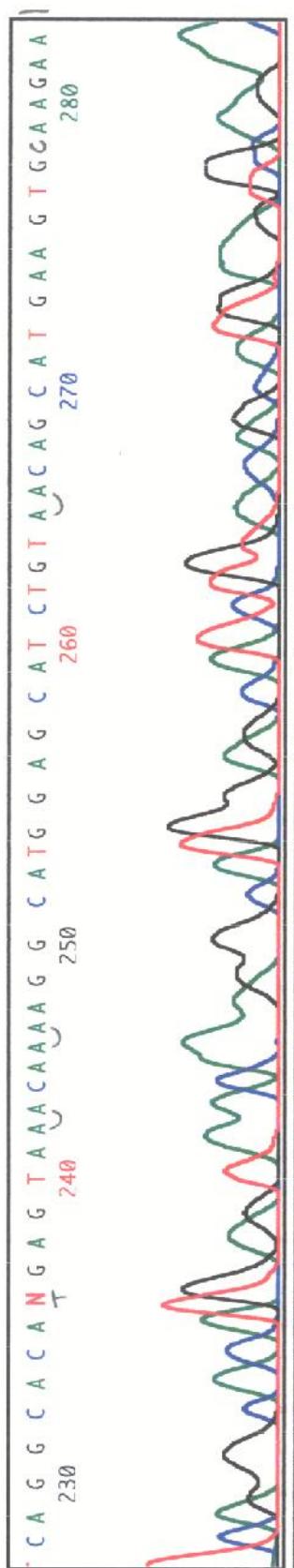


F. ER α exon 7.

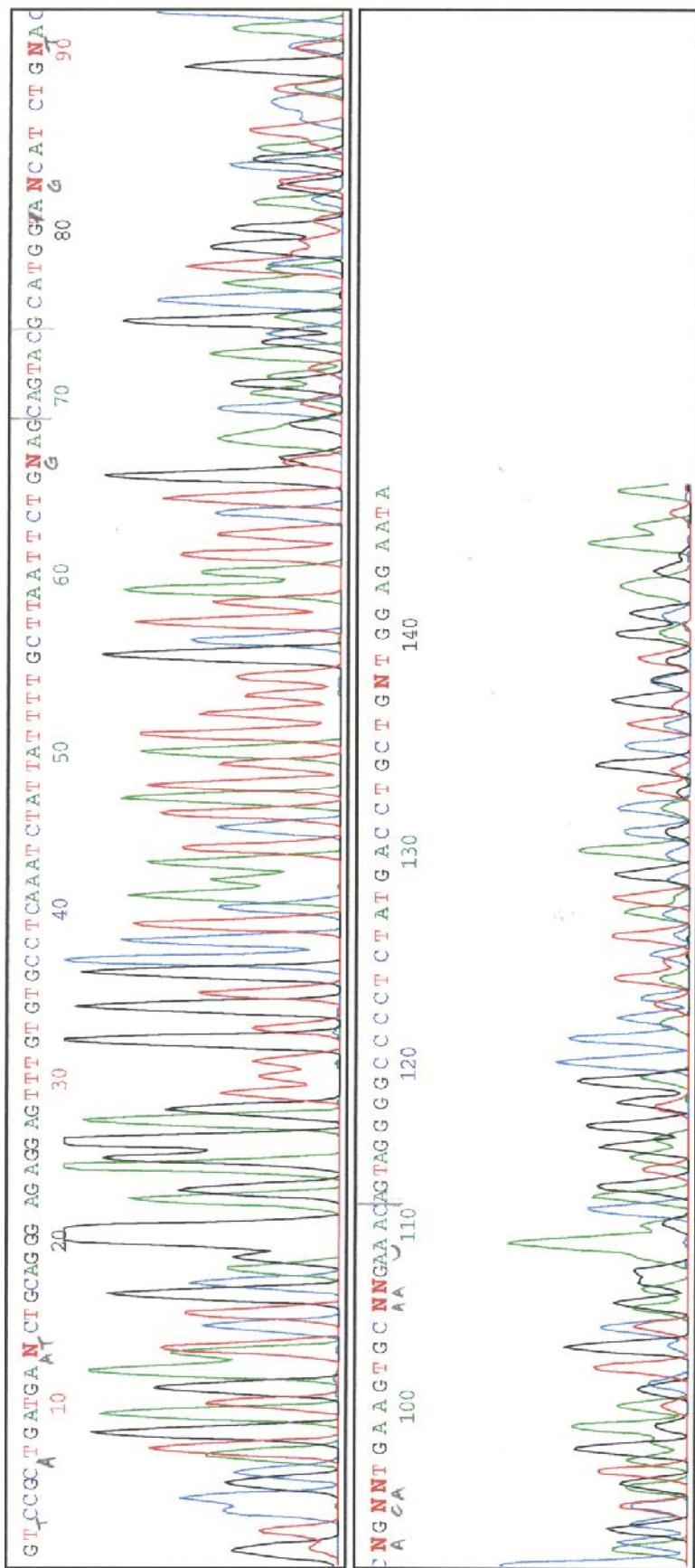
F1a. ER α 7 (345bp band) spectrograph (forward primer)



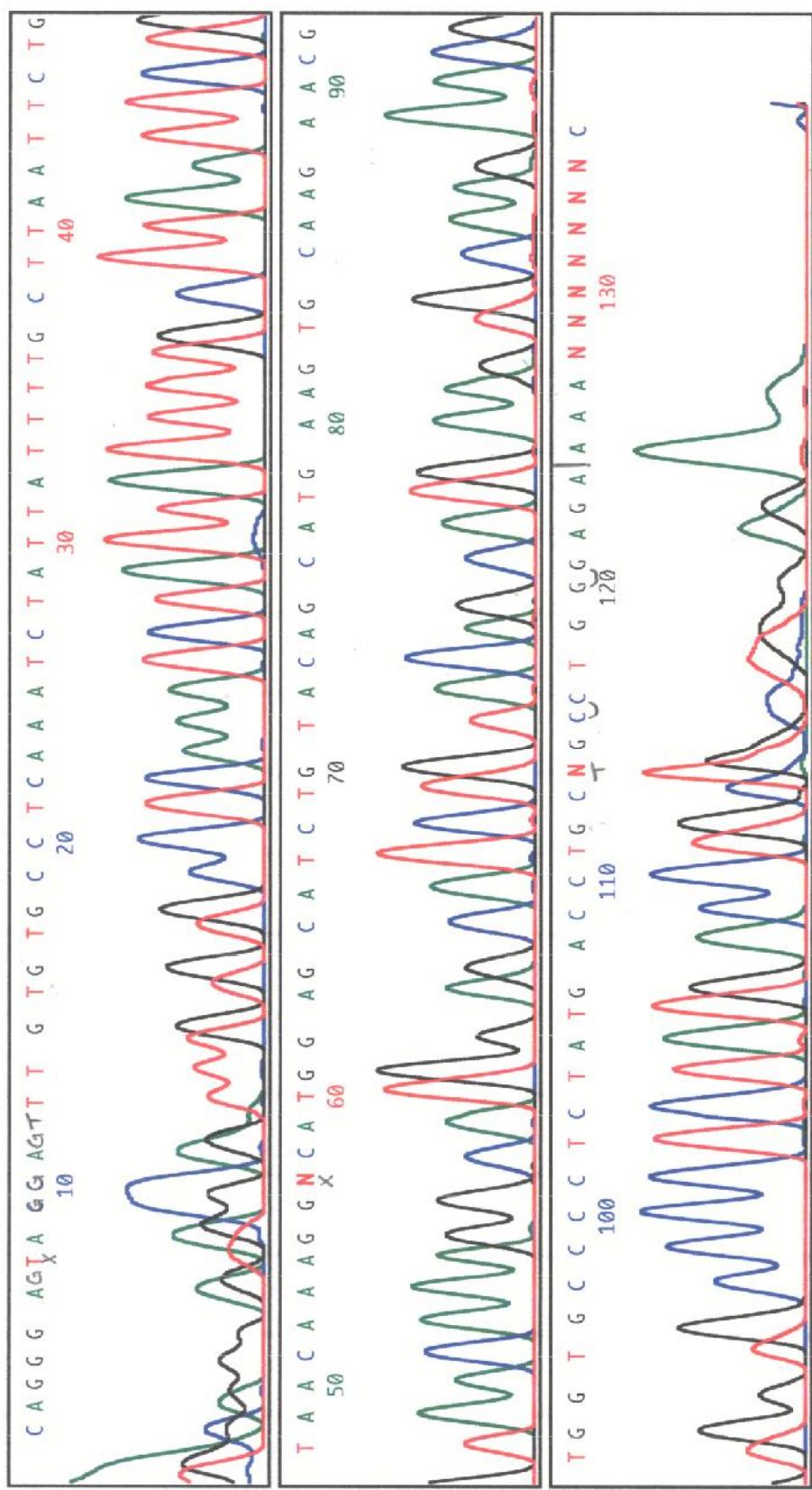
F1a. (contd). ER α 7 (345bp band) spectrograph (forward primer) continued



F2a. ER α 7 (310bp band) spectrograph (forward primer)



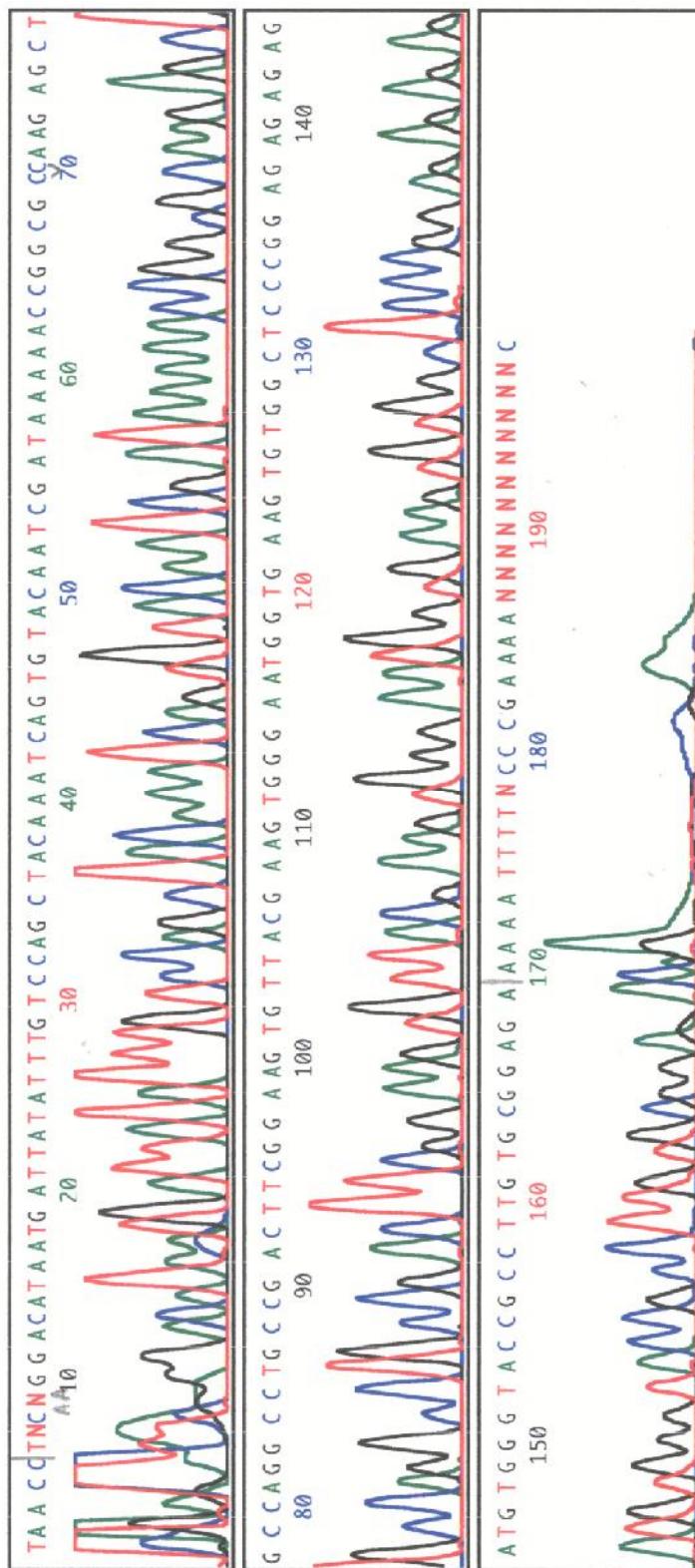
F3a. ER α 7 (161bp band) spectrograph (forward primer)



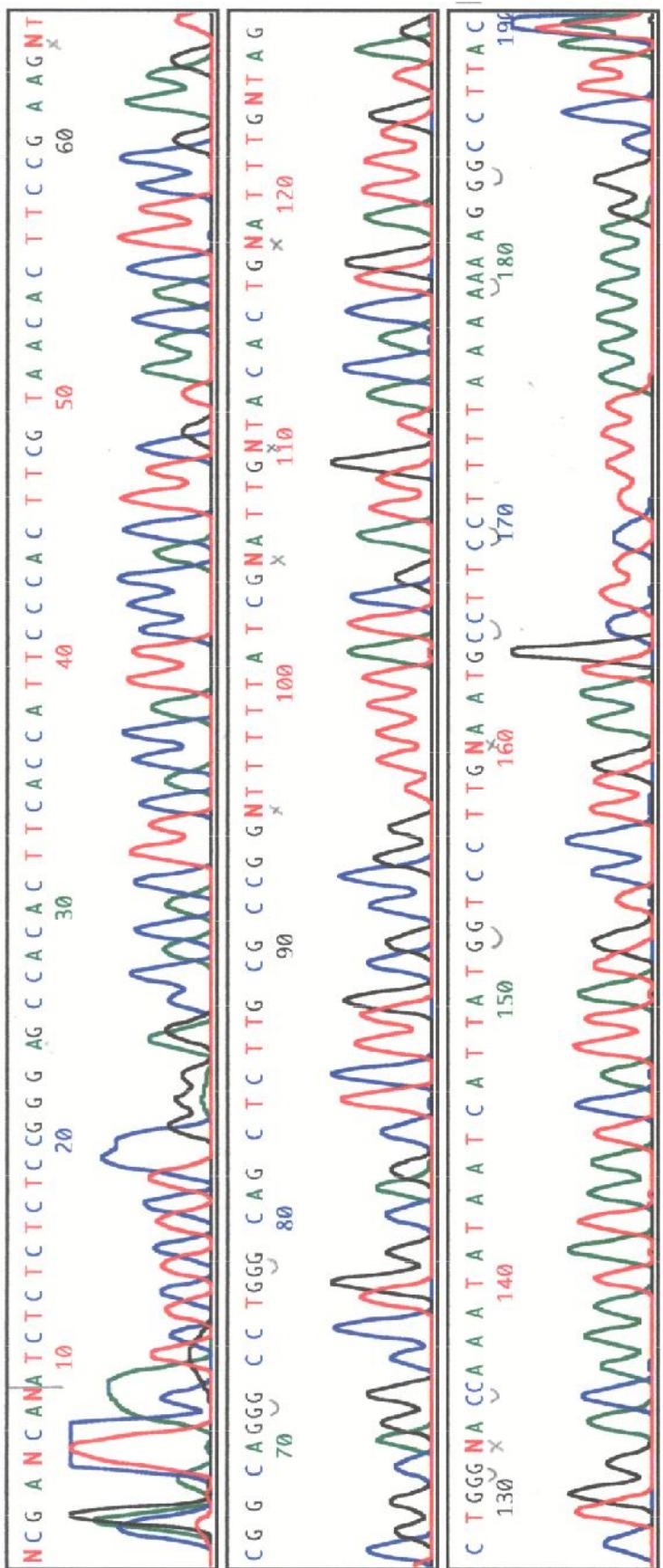
APPENDIX V: Sequencing spectrographs of ER β exons

A. ER β DBD

A1a. ER β DBD (214bp band) spectrograph (forward primer)

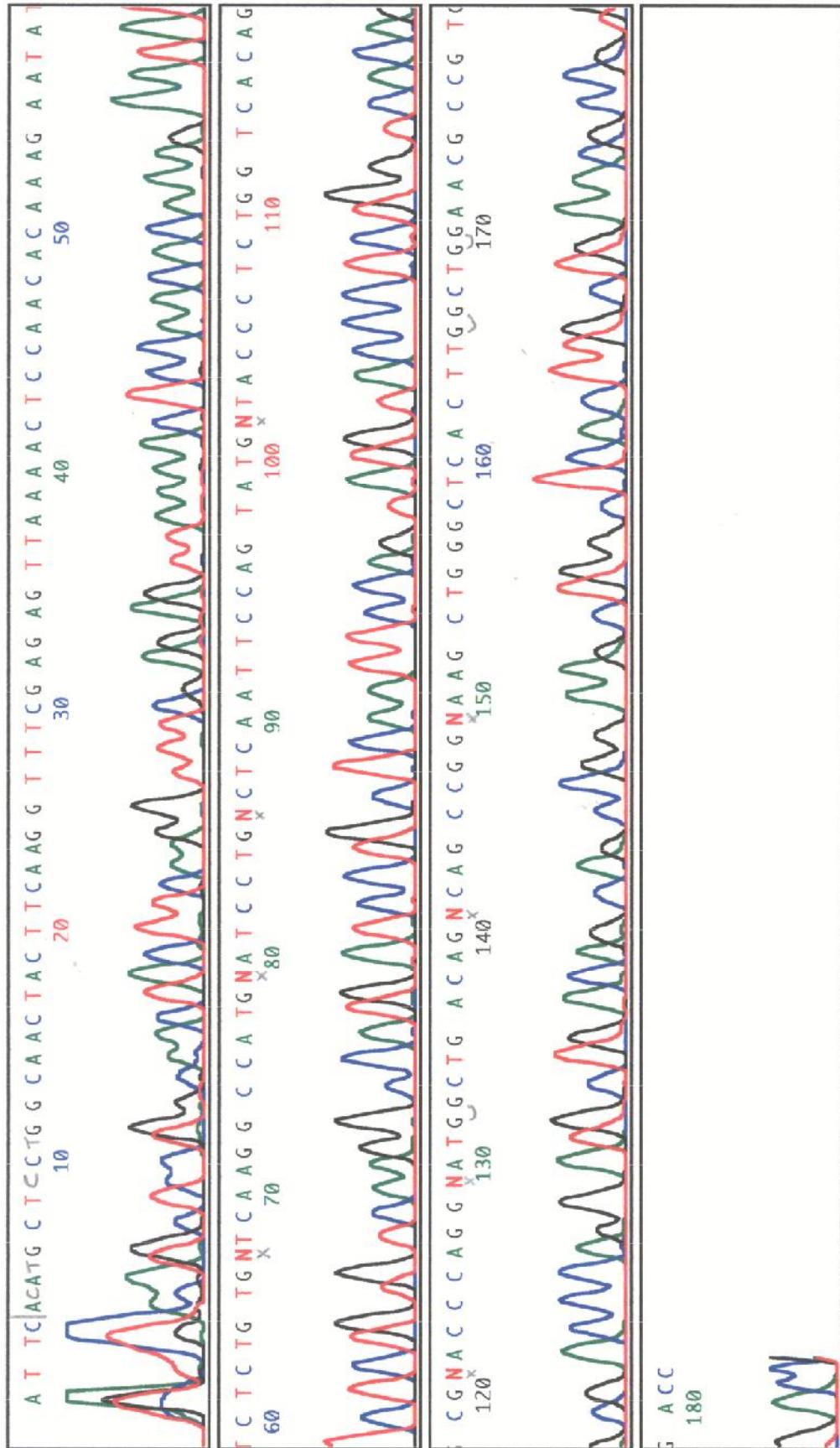


A1b. ER β DBD (214bp band) spectrograph (complementary primer)

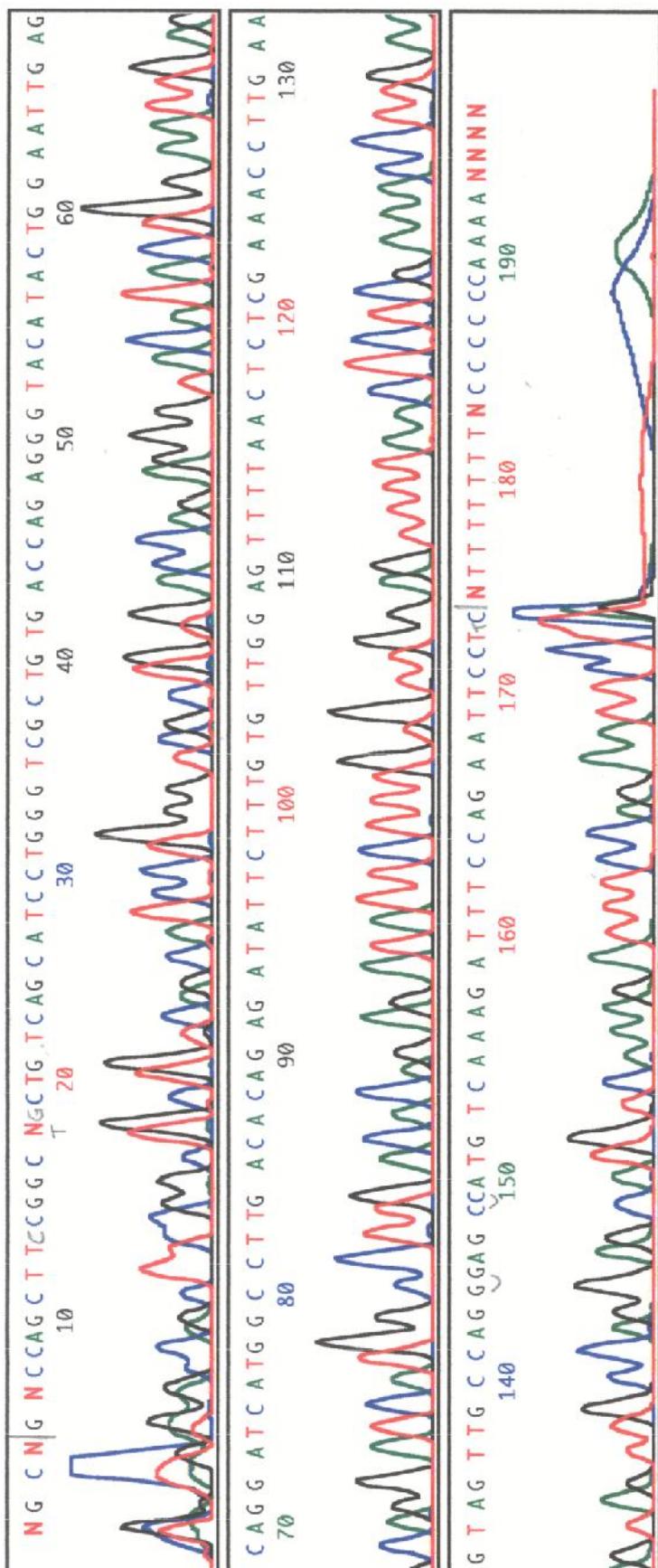


B. ERB LBD

B1a. ER β LBD (222bp band) spectrograph (forward primer)



B1b. ER β LBD (222bp band) spectrograph (complementary primer)



APPENDIX VI: Positions of primers and PCR products within the ER α gene

A. ER α exon 2

A1. Sequence and position of the 427bp band from nested PCR of ER α exon 2

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gcaaaagtattacatcacggggaggcagagggttccctgccacagtctgagagactccctggc

_____ primer position

bold sequence

italic exon 2 position (according to Ponglikitmonkol *et al.*, 1988)

A2. Sequence and position of the 236bp band from nested PCR of ERα exon 2

gagttgtgcctggagtgtatgttaagccaatgtcaggcaaggcaacagtccctggccgtcctccagcaccttg
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_____ primer position

bold sequence

italic exon 2 position (according to Ponglikitmonkol *et al.*, 1988)

B. ERα exon 3

B1. Sequence and position of the 322bp band from nested PCR of ERα exon 3

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atcacggggaggcagagggttccctgcccacagtctgagagctccctgg

_____ primer position

bold sequence

italic exon 3 position (according to Ponglikitmonkol *et al.*, 1988)

C. ERα exon 4

C1. Sequence and position of the 555bp band from nested PCR of ERα exon 4

gagttgtgcctggagtgtatgttaagccaatgtcaggcaaggcaacagtccctggccgtcctccagcaccttgc
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ttatggagtctggcctgtgagggtctgcaaggccttcaagagaagtattcaaggacataacgactatatgtc
cagccaccaaccagtgc**caccattgataaaaacaggagaagagctgccaggcctggctccgc**
aatgtctacgaagtggaaatgtgaaagggtggatacggaaaagaccgaagaggaggagaatgttg
aaacacaagcgcacagagatgtggggagggcaggggtaagtggtctgtggagacatga
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ctgacggccgaccagatggtcagtgcccttgtggatgtcagccccatactctattccgagatgtat
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tttgtctaattctggagtgtacacattctgtccagcaccctgaagtctgtggagaggaaggaccatccacc
tcctggacaagatcacagacacttgcgtccacatcgtggccaaaggcaggcgtaccctgcagcagcacc
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cgcccaactagccgtggaggggcatccgtggaggagacggaccaaggccacttggccactgcgggctactt
catcgattcctgcaaaagtattacatcacggggaggcagagggttccgtccacagtctgagagctccctg
gc

_____ primer position

bold sequence

italic exon 4 position (according to Ponglikitmonkol *et al.*, 1988)

C2. Sequence and position of the 219bp band from nested PCR of ER α exon 4

gagttgtgcctggaggagtatgttaagccaatgtcaggcaaggcaacagtccctggccgtcctccagcaccttgc
aatgcataatgagctcgggagaccagtacttaaagtggaggcccggagcccaggagctggcggagggcgt
tcgtcctggagactgcactgtccgtcggtcgccggctcaccggaccgcaggctccggggcaggccgg
ggccagagactcgcgtcgccggacatgcgtcgccctctaaccctgggtgtctttccaggtggcc
cgccggttctgagcctctgcccgtggggacacggctgtgcaccctgcccggccacggaccatgaccatg
accctccacaccaaagcatctggatggccactgtcatcagatccaagggaaacgagctggagccctgaac
cgtccgcagctcaagatccccctggagcggccctggcgagggtacactggacagcagcaagccgcgt
gtacaactaccccgagggcgccgtacgagttcaacgcgcggccgcacgcgcaggctacggtc
agaccggcctcccacggccccgggtctgaggctgcggcgtcggctccaacggcctgggggttcccccc
actcaacagcgtctccgagccgctgtactgcacccgcgcgcagctgcgccttcctgcagcccc
acggccagcagggtccctactacctggagaacagcagccagcggctacacggcgcgcaggccggcc
ggcattctacaggccaaattcagataatcgcacgcgcagggtggcagagaaaagattggccagttaccaatgaca
gggaagtatggatggaaatctgccaaggagactcgctactgtgcagtgtcaatgactatgttcaggctacca
ttatggagtctggccctgtgagggctgcaaggcccttcagagaatgttcaaggacataacgactatatgtc
cagccaccaaccagtgc**caccattgataaaaacaggagagactgccaggcctggcgtccgc**
aatgtctacgaagtggaaatgtgaaaggtggat*acgaaaagaccgaagagaggaggagaatgttgc*
cacaagcgccagagagatgtggggagggcaggggtgaagtggggctgtggagacatgagagactgcca
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gtggcagtgcccttgtggatgtcgagccccccatactctattccgaglatgtatccctaccagacccttcagtgaa
cttcgatgtggctactgaccaacctggcagacaggagctggtcacatgtcaactggcgaagaggg
gccaggcttggatttgaccctcatgtcaggccacccatgttgcctggcttagaatgtgcctggctagagatcctga
tgattggtctgtctggcgtccatggcaccacccagtgaagactactgttgccttaactgtctggacag
gaaccaggaaaaatgttagagggcatggggatctcgacatgtctggctacatcatctgggtccgc
gatgaatctgcaggagaggatgtgtgcctcaaatttgcattttgcattctggagtgatcacatctgtcca
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tatgacactgtgtggagatgtggaccccacccatgtgcggccactagccgtggagggcatccgtgg
aggagacggaccaaaggccactggccactgcggctacttcatgcattcgtcaaaagtattacatc
ggggaggcagagggttccctgcccacagtctgagagactccctggc

_____ primer position

bold sequence

italic exon 4 position (according to Ponglikitmonkol *et al.*, 1988)

D. ERα exon 5

D1. Sequence and position of the 544bp band from nested PCR of ER α exon 5

gagttgcctggagtgtatgttaaggccaatgtcagggcaaggcaacagtccctggccgtcccagcaccttg
aatgcataatgagctcggagaccagtacttaaagtggaggccccggagcccaggagctggcggagggcgt
tcgtcctggagctgcactgctccgtcggtcgccgctcaccggaccgcaggctccggggcagggccgg
ggccagagctcgctgtcgccggacatgcgtcgctcaccctcgccgcccacggaccatgaccatg
accctccacaccaaagcatctggatggccctactgcatcagatccaaggaaacgagctggagccctgaac
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gtacaactaccccgagggcgcgcctacgagttcaacgcgcggcccaacgcgcaggctacggc
agaccggcctccctacggcccccgggtctgaggctggcgttgcgtccaaacggcctgggggttcccccc
actcaacagcgtgtcccgagccgcgtatgtactgcacccgcgcgcagctgcgccttgcgcagcccc
acggccagcaggtgcctactacctggagaacgagccagcggctacacggcgcgcaggccggccgc
ggcattctacaggccaaattcagataatcgcgcagggtggcagagaaagattggccagttaccaatgacaa
gggaagtatggctatggaatctgccaaggagactcgctactgtgcgtgcattactatgtcaggctacca
ttatggagtctggcctgtgaggctgcaaggccttcaagagaagtattcaaggacataacgactatatgtc
cagccaccaaccagtgcaccattgataaaaaacaggaggaagagactgcgcaggcctggcgtccgc
ctacgaagtggaaatgtgaaagggtggatacgaaaagaccgaagaggagggagaatgttgaacacaaa
gcqcccaqagagatgtggggggcagggtgaagtgggtctgctggagacatgagactgtgc
acctttggcaagccgcgtcatgtcaaacgcgtctaagaagaacagcctggcctgtccgtacggcc
gaccagatggcagtgccttgtggatgtcgagccccctactctattccgcgtatgtatccaccagac
ccttcagtgaagctcgatgtggctactgaccaacctggcagacaggagactggtcacatgatc
aactggcgaagagggtgccag **gcttggatttgcacctccatgtcaggtcacccatgttagaatgt**
gctggctagagatcctgtatggtctcgctggcgtccatggagacccagtgaaagactgtt
gctccataacttgcttggacag gaaccaggaaaatgttagagggcatgtggagatctcgacat
gctgctggctacatcatctcggttccgcgtatgtaaatctgcagggagaggagttgtgcctcaaattatta
tttgcttaattctggagtgtacacattctgtccgcaccctgaagtcgtggagaggaaggaccatccaccga
gtcctggacaagatcacagacacttgcgtccgcaccctgtggccaaaggcaggcgtaccctgcgcgc
cagcggctggcccgctccatcctccatcaggcaccatcaggcgtatgttagaaacaggcatggagcatgt
cagcatgttagtgcagaacgtggtgcgcctctatgcacccgtcgctggagatgtggacgcaccgc
gcgcggactagccgtggagggcatccgtggaggagacggaccacaaagccacttggccactgcgggctctac
ttcatcgcatccctgcaaaagtattacatcacggggaggcagagggttccctgccacagtctgagagctccct
ggc

_____ primer position

bold sequence

italic exon 5 position (according to Ponglikitmonkol *et al*, 1988)

D2. Sequence and position of the 405bp band from nested PCR of ER α exon 5

gagttgcgcctggaggatgtttaagccaatgtcaggcaaggcaacagtccctggccgtccagcaccttg
aatgcataatgagctcgggagaccagtactaaagtggaggccccggagcccaggagctggcggagggcgt
tcgtccctggagactgcacttgccgtcggtcgccgttccaccggaccgcaggctccggggcaggggccgg
ggccagagctcgctgtcgccggacatgcgtcgccctaaacctcggtctgctttccagggtggcc
cgccgggttctgagcctctgcccgtggggacacggctgcaccctgcccggccacggaccatgaccatg
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cgtccgcagactcaagatccccctggagcggccctggcgagggtgtacctgacagcagcaagcccccgt
gtacaactaccccgagggcgccgcctacaggtcaacgcgcggccccaacgcgcaggctacggc
agaccggcctccctacggccccgggtctgaggctgcggcgttcggctcaacggctgggggttcccccc
actcaacagcgttcccgagccgcgtatgtactgcacccgcgcgcagctgtgccttcgtcagcccc
acggccagcaggtgccctactacctggagaacgagccagcggctacacggtgtcgcgaggccggccgc
ggcattctacaggccaaattcagataatcgaacgcgcagggtggcagagaaaagattggccagtaccaatgacaa
ggaaagtatggctatggaatctccaaggagactcgtactgtcagtgtcaatgactatgttcaggctacca
ttatggagtctggcctgtgaggctgcaaggccttcagaagagaagtattcaaggacataacgactatgtgc
cagccaccaaccagtgcaccaattgataaaaaacaggaggaagagactgcccaggccgtccgcaccaatg
ctacgaagtggaaatgtgaaaggatgtggatacggaaaagaccgaagaggaggagaatgttgaacacaa
qcqccqaqagatgtatggggagggcagggtgaagtgggtctgtggagacatgagagactgcca
acctttgccaagccgcctcatgtcaaaacgcgtctaagaagaacacgcctggccttgcacatgacggcc
gaccagatggcagtgccttggatgtcgagccccccatactctattccgagatgtatgcctaccagac
ccttcagtgaagctcgatgtggctactgaccaacctggcagacaggagctggttcacatgatc
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gcttagagatcctgtatgttgcgtcgccatggagcaccaggtaagactactgtttgtcctaacttgc
cttggacaggaaaccaggaaaatgttagagggcatggtgaggatctcgacatgtcgtggctacat
catctcggtccgcatgtgaatgtcgaaggagaggatgtgcctcaaatctattttgtctaattctgg
agtgtacacattctgtccagcaccctgaagtctctggagagagaaggaccatatccaccgagtcctggacaaga
tcacagacacttgcattccacctgtatggccaaggcaggcgtaccctgcagcagcagcaccagcggctggccc
agctccctcatcctccacatcaggcacatgagtaacaaaggcatggagcatgtacagcatgtacatgt
aagaacgtggccctctatgacctgtctggagatgtggacgccccaccgcctacatgcgcactagcc
gtggagggcatccgtggaggagacggaccaagccacttggccactgcggctctacttgcattcccttg
aaaaatgttacatcacggggaggcagagggttccctgcccacagtctgagagactccctggc

_____ primer position

bold sequence

italic exon 5 position (according to Ponglikitmonkol *et al*, 1988)

D3. Sequence and position of the 80bp band from nested PCR of ER α exon 5

gagttgcctggagtatgtttagccaatgtcgaggcaaggcaacagtccctggccgtcccacccat
aatgcataatgagctcggagaccagtacttaaagtggaggcccggagccaggagctggcggaggcgt
tcgtccctggagactgcactgtccgtggcggctcaccggaccgcaggctccggggcaggggccgg
ggccagagctcgctgtcgccggacatgcgtgcgtccctaaacctcggctgtctttccagggtggcc
cgccgggttctgagcctctgcccgtggggacacggctgcaccctgcccggccacggaccatgaccatg
accctccacaccaaagcatctggatggccctactgcatacagatccaaggaaacgagctggagccctgaac
cgtccgcagactcaagatccccctggagcggccctggcgaggtgtacctgacagcacaagcccccgt
gtacaactaccccgagggcgccgcctacagttcaacgcgcggccccaacgcgcaggtctacggtc
agaccggcctccctacggcccccgggtctgaggctgcggcgttcggctccaacggctgggggtttcccccc
actcaacagcgtctccgagccgcgtatgtactgcacccgcgcgcagctgtgccttcctgcagcccc
acggccagcaggtgccctactacctggagaacgagccagcggctacacggtgtcgagggccggccgc
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ggaaagtatggctatggaatctccaaggagactcgtactgtcagtgtcaatgactatgctcaggctacca
ttatggagtctggcctgtgagggtgcaggccttcataagagaagtattcaaggacataacgactatgtgc
cagccaccaaccagtgcaccattgataaaaacaggaggaagagactgcccaggctggcgtccgc
ctacgaagtggaaatgtgaaagggtggatacggaaaagaccgaagaggaggagaatgttgaacacaa
qcgcacqagqagatgtggggaggcagggtgaagtgggtctgtggagacatgagactgcaccc
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tcagtgcctgtggatgtcgagccccccatactctattccgagttgtatgtatccaccagaccctc
tgcagggttgcctccatgtatcggcggcggcggcggcggcggcggcggcggcggcggcggcggcgg
gtttgtggatttgaccctccatgtatcggcggcggcggcggcggcggcggcggcggcggcggcggcgg
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tgcaggqaqagqagtttgtgcctcaaatctattttgttaattctggagttgtacacattctgtccagcacc
ctgaagttctggagaggaaggaccatattccaccgcgtccgtggacaagatcacagacacttgc
ggccaaaggcaggcctgaccctgcagcagcagcaccagcggctggccagctccctcatcctccacat
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cctgctgtggagatgtggacgcccaccgcctacatgcgtccactagccgtggagggcattccgtggagga
gacggaccaagccacttgccactgcggctacttcatgcattcctgcaaaagtattacatcacggggg
aggcagagggttccctgcccacagtctgagagactccctggc

_____ primer position

bold sequence

italic exon 5 position (according to Ponglikitmonkol *et al*, 1988)

E. ERα exon 6

E1. Sequence and position of the 388bp band from nested PCR of ER α exon 6

gagttgcgcggaggatgtttaagccaatgtcaggcaaggcaacagtccctggccgtcccagcaccttg
aatgcataatgagctcggagaccagtacttaaagtggaggcccggagcccaggagctggcgagggcgt
tcgtcctggagctgcactgctccgtcgggtcgccgctcaccggaccgcaggctccggggcagggccgg
ggccagagctcgctgtggggacatgcgtcgctcgctctaaccctgggtgtgtctttccagggtggcc
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accctccacaccaaagcatctggatggccactgtcatcagatccaaggaaacgagctggagcccctgaac
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gtacaactaccccgagggcgccgcctacgagttcaacgcgcggccgcacgcgcagggtacggc
agaccggcctccctacggccgggtctgaggctgcggcgttcggctccaacggcctgggggttcccccc
actcaacagcgtctccgagcccgtatgcgtactgcaccgcgcgcgcgcgcgcgcgcgcgcgc
acggccagcagggtccctactacctggagaacgcggccagcggctacacggcgcgcggccgcgc
ggcattctacaggccaaattcagataatcgcacgcgggtggcagagaaagattggccagttaccaatgacaa
gggaagtatggctatggaatcgcacaggagactcgctactgtgcagtgtcaatgactatgtcaggctacca
ttatggagtctggcctgtgagggtgcacaggccttcagagaatgttcaaggacataacgcactatgtc
cagccaccaaccagtgcaccattgataaaaaacaggaggaagagctgccaggcctggcgtccgc
ctacgaagtggaaatgtgaaagggtggatacgaaaaagacccaagaggaggagaatgtgaaacacaa
gcgcacaggatgtgggagggcagggtgaagtgggtctggagacatgagagctgcaccc
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tgcgtggatgtgaccctccatgtcaggccacccatgttagatgtgcctggctagagatcctqatqattqgtcqt
ctggcgcctccatggagcaccaggatgtactgttgccttaacttgtcttggacag **gaaccagg**
aaaaatgttagagggcatggtagatctcgacatgtctggcatacatctcggttccgcatgt
tgaatctcgaggagaggatgttgcctcaaatctattatgtgccttaattctg **gagtgtacacatttc**
gtcccagcaccctgaagtcttggagaggaaggaccatatccaccgcgtcctggacaagatcacaga
cactttgtccacccatgtggccaaggcaggcctgaccctgcagcagcagcaccagcggctggccca
gctcctcctcatccctccacatcaggcacatgagaacaaaggcatggagcatgtacagcatgaagt
caagaacgtgtggcccttatgacctgtcggagatgtggacgcggccaccgcctacatgcgcactagc
cgtggagggcatccgtggaggagacggaccacaaagccacttggccactgcggcgtacttcatgcattcct
gcaaaagtattacatcacggggaggcagagggttccctgcccacagtctgagagctccctggc

_____ primer position

bold sequence

italic exon 6 position (according to Ponglikitmonkol *et al*, 1988)

F. ERα exon 7

F1. Sequence and position of the 345bp band from nested PCR of ER α exon 7

gagttgcctggagtgtttaagccaatgtcaggcaaggcaacagtccctggccgtcccaccc
aatgcataatgagctcggagaccagtacttaaagtggaggcccggagcccaggagctggcgagggcgt
tcgtcctggagctgcactgctccgtcggcgtccaccggaccgcaggctccggggcagggccgg
ggccagagctcgctgtggggacatgcgtcgctcaaccctggctgtgtctttccagggtggcc
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gtacaactaccccgagggcgccgcctacgagttcaacgcgcggcccaacgcgcaggctacggc
agaccggccctccctacggcccccgggtctgaggctgcggcgttgcgtccaaacggcctgggggttcccccc
actcaacagcgtctccgagccgctgtactgcaccgcgcgcagctgcgccttgcgcagcccc
acggccagcaggtgcctactacctggagaacgcagccagcggctacacggcgcgcaggccggccgc
ggcattctacaggccaaattcagataatgcacgcagggtggcagagaaagattggccagttaccaatgacaa
ggagaagtatggctatggaatctgccaaggagactcgctactgtgcagtgtcaatgactatgctcaggctacca
ttatggagtctggcctgtgagggctgcaaggccttcaagagaagtattcaaggacataacgactatatgtgc
cagccaccaaccagtgcaccattgataaaaacaggaggaagagctgccaggcctggcgtccgc
ctacgaagtggaaatgatgaaagggtggatacgaaaaagacccaagaggagggagaatgttgaacacaa
gcgcagagagatgaaaaaggcggcagggtgaagtgggtctgctggagacatgagagctgccaacctt
ggccaagcccgctcatgatcaaacgcgtctaagaagaacagcgtggcctgtccctgacggccgaccagatgg
tcagtgccttgtggatgctgagccccccatactctattcgagttgtatgcctaccagaccctcagtgaagcttcga
tgatggctactgaccaacctggcagacaggagctggtcacatgtcaactggcgaagagggtgccag
gcttgcgtgatttgcacctccatgatcaggccacccatgttagatgtgcgttagagatccatgtgatgttgc
ctggcgtccatggagccacccaggtaagactactgtttgccttaactgtctggacaggaaccaggaaaatg
tgtagagggcatgtggagatctcgacatgtcgtgctacatcatcggccatgtcatgtgaatctgcagg
gagaggagtttgtgcctcaaatttatttgcataattctg **gagtgtacacatttgcgtcccagcaccct**
gaagtctctggagaggaaggaccatatccaccgcaggcgtccgtggacaagatcacagacacttgcaccc
ctgatggccaaggcaggcctgaccctgcagcagcagcaccagcggctggccagctccctcattc
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tggtgccctctatgacccgtctggagatgctggacgcccaccgcctacatgcgcaccactagccgtggaggg
gcatccgtggaggagacggaccaaaagccacttggccactgcggcgtacttcatgcattcattgcataaaagta
ttacatcacggggaggcagagggttccctgcccacagtctgagagctccctggc

_____ primer position

bold sequence

italic exon 7 position (according to Ponglikitmonkol *et al.*, 1988)

F2. Sequence and position of the 310bp band from nested PCR of ERα exon 7

gagttgtgcctggagtgtatgttaagccaatgtcaggcaaggcaacagtccctggccgtcctccagcaccttg
aatgcataatgagctcgggagaccagtacttaaagtggaggcccggagcccaggagctggcggagggcgt
tcgtcctggagactgcactgtccgtcggtcgccggcttaccggaccgcaggctccggggcaggccgg
ggccagagactcgcgtcgccggacatgcgtcgccctctaaccctgggtgtcttccaggtggcc
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cgtccgcagctcaagatccccctggagcggccctggcgagggtacactggacagcagcaagccgcgt
gtacaactaccccgagggcgccctacgagttcaacgcgcggccgcacgcgcaggctacggtc
agaccggcctccctacggccccgggtctgaggctgcggcgtccaaacggcctgggggttcccccc
actcaacagcgtctccgagccgcgtatgtactgcacccgcgcgcagctgtcccttcgcagcc
acggccagcagggtccctactacctggagaacagcagccagcggctacacggcgcgcaggccggcc
ggcattctacaggccaaattcagataatcgcacgcagggtggcagagaaaagattggccagtaccaatgacaa
gggaagtatggatggaaatctgccaaggagactcgctactgtcagtgtcaatgactatgttcaggctacca
ttatggagtctggccctgtgagggctgcaaggcccttcagaagagaagtattcaaggacataacgactatatgtc
cagccaccaaccagtgcaccattgataaaaacaggaggaagagctgccaggcctgcggctccgcaaatg
ctacgaagtgggaatgtgaaagggtggatacgaaaaagaccgaagaggagggagaatgttcaaaca
gcccgcagagagatgtgggagggcaggggtgaagtgggtctgtggagacatgagagctgccaaccc
ggccaaagccgcctcatgatcaaacgcctaagaagaacagcgcctggcctgtccctgacggccgaccagatgg
tcagtgccttgtggatgctgagccccccatactctatccgagatgtatgcctaccagacccttcagtgaaagctcga
ttagggcttactgaccaacctggcagacaggagctgggtcacatgtatcaactggcgaagagggtgc
gttttggttgcatttgcacccatgtcgttgcacccatgtcgttgcacccatgtcgttgcacccatgtcgt
ctggcgtccatggagcacccagtgaagctactgtttgcctctaactgtcttgcacccatgtcgttgcacccatgtcgt
tgttagggcatgtggatgttgcacatgtcgttgcacccatgtcgttgcacccatgtcgttgcacccatgtcgt
agaggagtttgtgcctcaatcttattttgttgcataattctg*gagtgtacacattctgtccagcaccctg*
aagtctctggagaggaaggaccatatccaccgcgtccgtggacaagatcacagacacttgcacccatct
gtggccaaaggcaggcctgcaccctgcagcagcagcaccagcggctggccagctccctcatct
cctccatcaggcacatgagtaacaaaaggcatggagcatgtacagcatgaagtgcagaacgtgggtgc
cctctatqacctgtgtggagatgtggacgcccaccgcctacatgcgcactagccgtggaggggcatcc
gtggaggagacggaccaaagccactggccactgcgggcttacttcatcgcattccctgcaaaagtattacatc
acggggaggcaggggttccctgcccacagtctgagagctccctggc

— primer position

bold sequence

italic exon 7 position (according to Ponglikitmonkol *et al.*, 1988)

F3. Sequence and position of the 161bp band from nested PCR of ERα exon 7

gagttgtgcctggagtgtatgttaagccaatgtcaggcaaggcaacagtccctggccgtcctccagcaccttg
aatgcataatgagctcgggagaccagtacttaaagtggaggcccggagcccaggagctggcggagggcgt
tcgtcctggagactgcactgtccgtcggtcgccggcttaccggaccgcaggctccggggcaggccgg
ggccagagactcgcgtcgccggacatgcgtcgccgtctaaccctgggtgtcttccaggtggcc
cgccggttctgagcctctgcccgtggggacacggctgtgcaccctgcccggccacggaccatgaccatg
accctccacaccaaagcatctggatggccactgtcatcagatccaaggaaacgagctggagccctgaac
cgtccgcagctcaagatccccctggagcggccctggcgagggttacctggacagcagcaagccgcgt
gtacaactaccccgagggcgccgttacgagttcaacgcgcggcccaacgcgcagggttacggtc
agaccggcctccctacggccccgggtctgaggctgcggcgtccaaacggcctgggggttccccc
actcaacagcgtctccgagccgcgtatgtactgcaccgcgcgcagctgtcccttcgcagcc
acggccagcagggtccctactacctggagaacgcgcggcttacacgggtgcgcgaggccggcc
ggcattctacaggccaaattcagataatcgcgcagggtggcagagaaaagattggccagtaccaatgacaa
gggaagtatggatggaaatctgccaaggagactcgctactgtgcagtgtcaatgactatgttcaggctacca
ttatggagtctggccctgtgagggctgcaaggcccttcagaagagaagtattcaaggacataacgactatatgtc
cagccaccaaccagtgcaccattgataaaaacaggaggaagagctgccaggcctgcggctccgcaaatg
ctacgaagtgggaatgtgaaagggtggatacgaaaaagaccgaagaggaggagaatgttcaaaca
gcccgcagagagatgtggggagggcaggggtgaagtgggtctgtggagacatgagagctgccaaccc
ggccaaagccgcctcatgatcaaacgccttaagaagaacagcgcctggcctgtccctgacggccgaccagatgg
tcagtgccttgtggatgtcgcggcccccatactctatccgcgtatgatccgcgcgcgcgcgcgcgc
tgcgtggatttgcaccctccatgatcaggccacccatgtcgcgcgcgcgcgcgcgcgcgcgc
ctggcgcctccatggagcaccaggtaagctactgtttgcctcaactgcttggacaggaaccaggaaaaatg
tgttagggcatgtggatgttcgcacatgtcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc
gagaggagtttgtgcctcaaatctattttgtctaattctg *gagtgacacatctgtccagcaccctgaa*
gtctctggagaggaaggaccatccaccgc
aaggcaggcctgaccctgcagcagcagcaccagcggctggccagctccctcatacctctccacatcagg
cacatgagtaacaaaggcatggagcatctgtacagcatgatgcgcgcgcgcgcgcgcgcgcgcgcgc
cctqctqqaqatgtggacgc
gaggcagagggttccctgccacagtctgagagctccctggc
gaggcagagggttccctgccacagtctgagagctccctggc

_____ primer position

bold sequence

italic exon 7 position

APPENDIX VII: Positions of primers and PCR products

within the ER β gene.

A. ER β DBD

A1. Sequence and position of the 214bp band from nested PCR of ER β DBD

gttgacagccattatactgcccacgaatcttggagaacattataatgacccttgtgccttcttgcaggta
tttctcagcttatctcaagacatggatataaaaaactcaccatctagcctaattctccttcctacaa
ctgcagtcaatccatcttacccctggagcacggctccatatacataccttcctcctatgttagacagccacc
atgaatatccagccatgacattctatagccctgtgtatgaattacagcattccagcaatgtcactaact
tgaagggtggcctggcggcagaccacaagccaaatgtgttgccaaacacccctggcaccccttc
tttagtggccatcgccaggatcacatctgtatgcggAACCTCAAAGAGTCCCTGGTGTGAAGCAAGAT
cgctagaacacacccattacctgtaaacag* agagacactgaaaaggaaaggtagtggAACCGTTGCG
ccagccctgttactggccagggtcaaaaggaggatgctcacttctgcgtctgcagcattacgcattcg
ggatcatgactatggagctggcgtqagqatgttaaggccctttaaaagaagcattcaag*gaca
taatgattatatttgcaggactacaatcagtgtacaatcgataaaaaccggcgaagagctgcc
aggcctggcacttccggaaagtgttacgaagtggaaatggtaagtgtg* gctccggagagag
agatgtgggtaccgccttgtgcggagacagagaagtgcgcacgcaggactgcactgtgcggca
aggccaagagaagtggccgcacgcgcggcggactgcggagactgcgtgcggacgcggcggactgcgg
gaggcggactgcgtcaccctcctggaggctgaggccatgtgtatgcgtacgcggccggactgcgg
cttcaccggccctccatgtatgtccctgaccaagtggccgacaaggagttgttacatgtatgc
ctggccaaagaagattcccg* gcttggagactcagcgttgcaccaagtgcggcttggagagctgt
tggatggaggtgttaatgtatgggctgtatgtggcgctcaattgaccacccggcaagctcatcttgc
agatctgttgcacag* ggtatggggaaatgcgtagaaggaaatttgcacatgtcc
gcaactactcaagggttcggagactaaactccaacacaaagaatctgtgtcaaggccatgtatcc
tgctcaattcca* gtatgtaccctctggtcacagcgcacccaggatgcgtacacgcgcggaaagctgg
cacttgcgtacgcgcgtgaccgtcttgggtgtatgcgcacgcggcatctccctccggcaggc
aatccatgcgcctggctaaccctctgtatgcctgtccacgcgtcaggcatgcgag* taacaaggccatg
gaacatctgtcaacatgaagtgcaaaaatgtggccaggatgtatgcacgcgcggaaagctgt
cccacgtgtccgggtgcacgtccctcatcagggtccaggatgcgtacgcgcggcaggagactaa
aagcaaagagggtcccagaacccacagtctcagtgcacgcgcctggccctgaggtaactggccaca
gaggtcacaagactgcgtgaactccaggatgtgtcaggagcgcctggctcatcttgc
cattgg

_____ primer position

bold sequence

* intron/exon borders (according to Enmark *et al*, 1997; Herynk and Fuqua, 2004)

B. ER β LBD

B1. Sequence and position of the 222bp band from nested PCR of ER β LBD

gttgacagccattatacttgcacgaatcttggagaacattataatgacccttgtgcctcttgcagggttttc
tcagctgttatctaagacatggatataaaaaactcaccatctagcctaattccctccctacaactgcagt
caatccatctaccctggagcacggccatatacatacctccctatgttagacagccaccatgaatatcc
agccatgacattctatagccctgtgtatgaattacagcattccagcaatgtcactaacttgaagggtggcc
tggtcggcagaccacaagccaaatgtgttgccaacacctggcaccttcccttagtggccatcgcc
agttatcacatctgtatgcggAACCTCAAAAGAGTCCCTGGGTGAAGCAAGATCGCTAGAACACACCTAC
CTGAAACAG*AGAGACACTGAAAAGGAAGGTTAGTGGGAACCCTGCAGCCCTGTTACTGGTCCAGG
TTCAAAGAGGGATGCTCATTCTGCCTGTGCAGCGATTACGCATCGGGATATCACTGGAGTCGGTCGTG
TGAAGGATGTAAGGCCCTTTAAAAGAACATTCAAG*GACATAATGATTATTTGCCAGCTACAAATCAGT
GTACAATCGATAAAAACCGCGCAAGAGCTGCCAGGCCCTGCCACTCGGAAGTGTACGAAGTGGGAAT
GGTGAAGTGTG*GCTCCCGAGAGAGAGATGTGGTACCGCCTGTGCAGGACAGAGAAGTGCAGCAGA
GCAGCTGCACTGCGGGCAAGGCCAAGAGAAGTGGCGGCCACGCCCCCCGAGTGCAGGAGCTGCTGCT
GGACGCCCTGAGCCCCGAGCAGCTAGTGTCTCACCCCTCCTGGAGGCTGAGCCGCCCATGTGCTGATCAGCC
GCCCGAGTGCCTTACCGAGGCCCTCCATGATGATGTCCTGACCAAGTGGCCACAAGGAGTTGAC
ACATGATCAGCTGGCCAAGAAGATCCCG*GCTTGTGGAGGCTCAGCCTGTCACCAAGTGCAGCTTG
AGAGCTGTGGATGGAGGTTAATGATGGGCTGATGTGGCGCTCAATTGACCACCCGGCAAGCTCATTT
GCTCCAGATCTGTGGACAG*GGATGAGGGAAATQCGTQAAGGAATTCTGAAATCTTGACATG
CTCCTGGCAACTACTCAAGGTTCGAGAGTTAAACTCAACACAAAGAAATCTCTGTGTCAAGGC
CATGATCCTGCTCAATTCCA*GTATGTAACCTCTGGTCACAGCGACCCAGGATGCTGACAGCAGCCG
GAAGCTGGCTCACTTGCTGAACGCCGTGACCQATGCTTGGTTGGQTGATGCCAAGAGCGGCATCTCT
CCCAGCAGCAATCCATGCCCTGGCTAACCTCCTGATGTCCTGTCACGTCAGGATGCGAGTAACAAG
GGCATGGAACATCTGCTCAACATGAAGTGCAAAAATGTGGCCAGTGTATGACCTGCTGGAGATGCTG
AAATGCCACGTGCTCGCGGTGCAAGTCCTCCATCACGGGGTCCAGTGCAGCCGGCAAGAGGACAGTA
AAAGCAAAGAGGGCTCCAGAACCCACAGTCAGTGAACGCCCTGGCCCTGAGGTGAACTGGCCCACAGA
GGTCACAAGCTGAAGCGTGAACTCCAGTGTGTCAAGGACGCCCTCATTTCTGCTGTGGTCCCTATTG
G

_____ primer position

bold sequence

* intron/exon borders (according to Enmark *et al*, 1997; Herynk and Fuqua, 2004)