

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 
(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...



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 0.22µm 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.

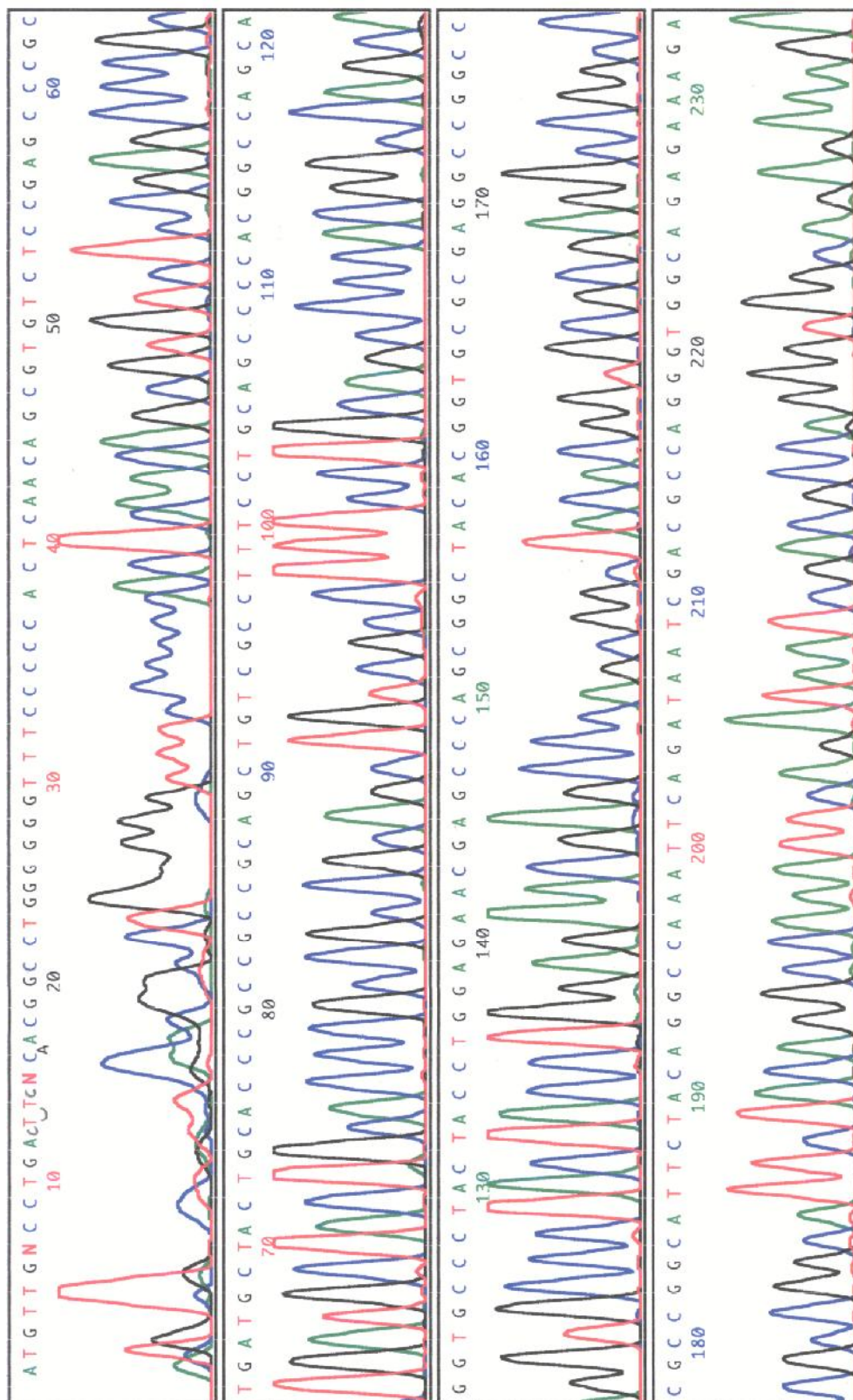
Add 66ml methanol (SAARCHEM) and mix thoroughly.

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

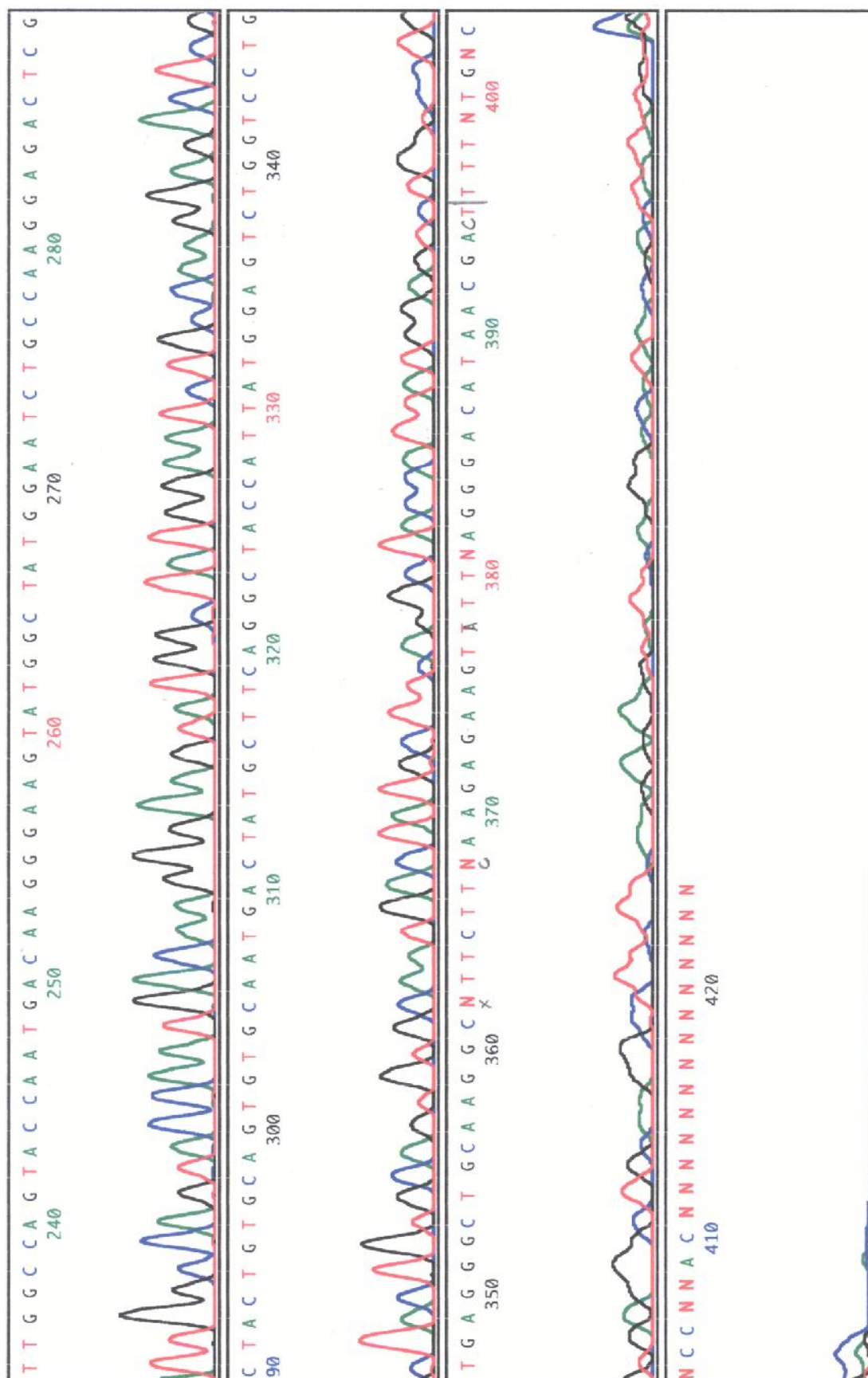
APPENDIX IV: Sequencing spectrographs of ER α exons

A. ER α exon 2

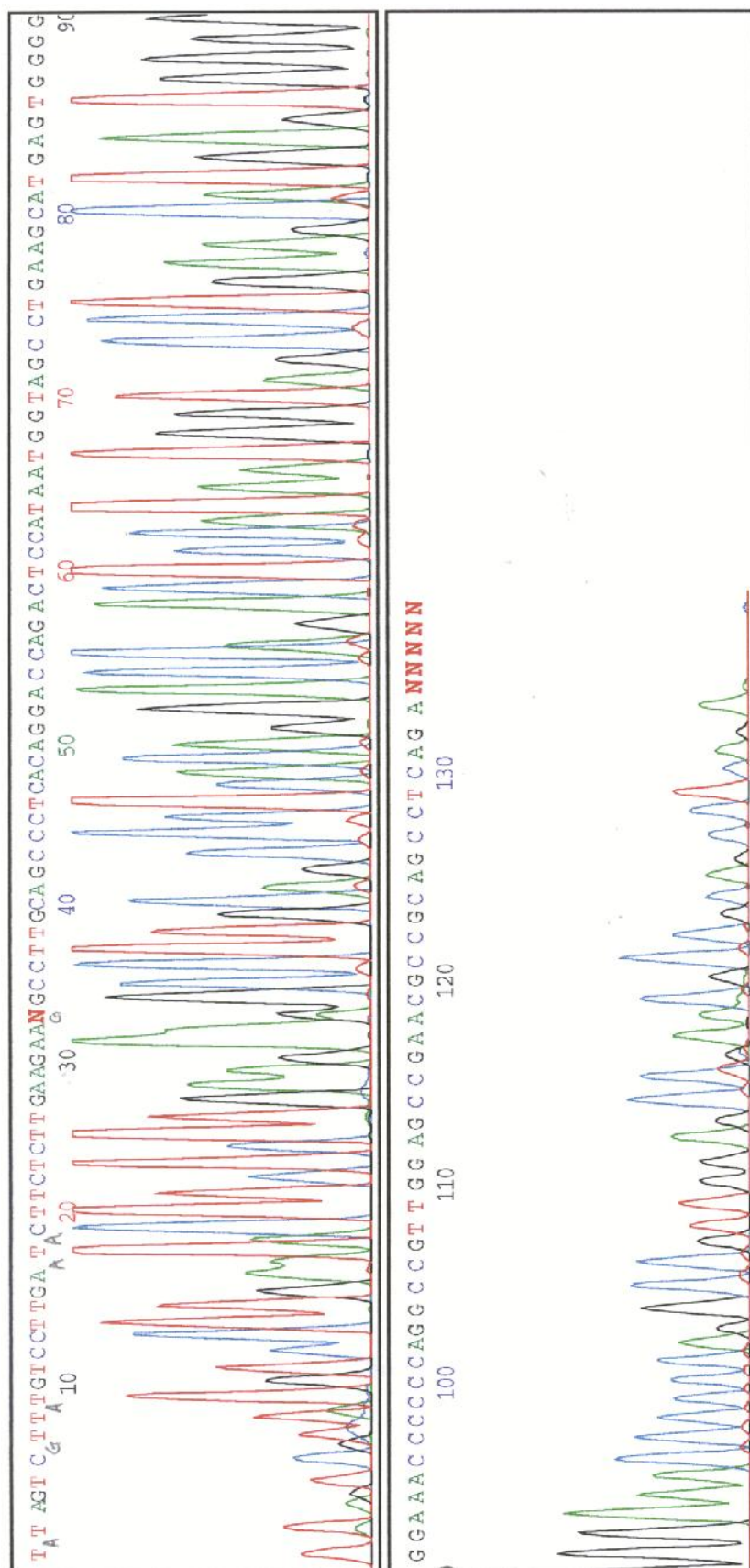
A1. ER α 2 (427bp band) spectrograph (forward primer)



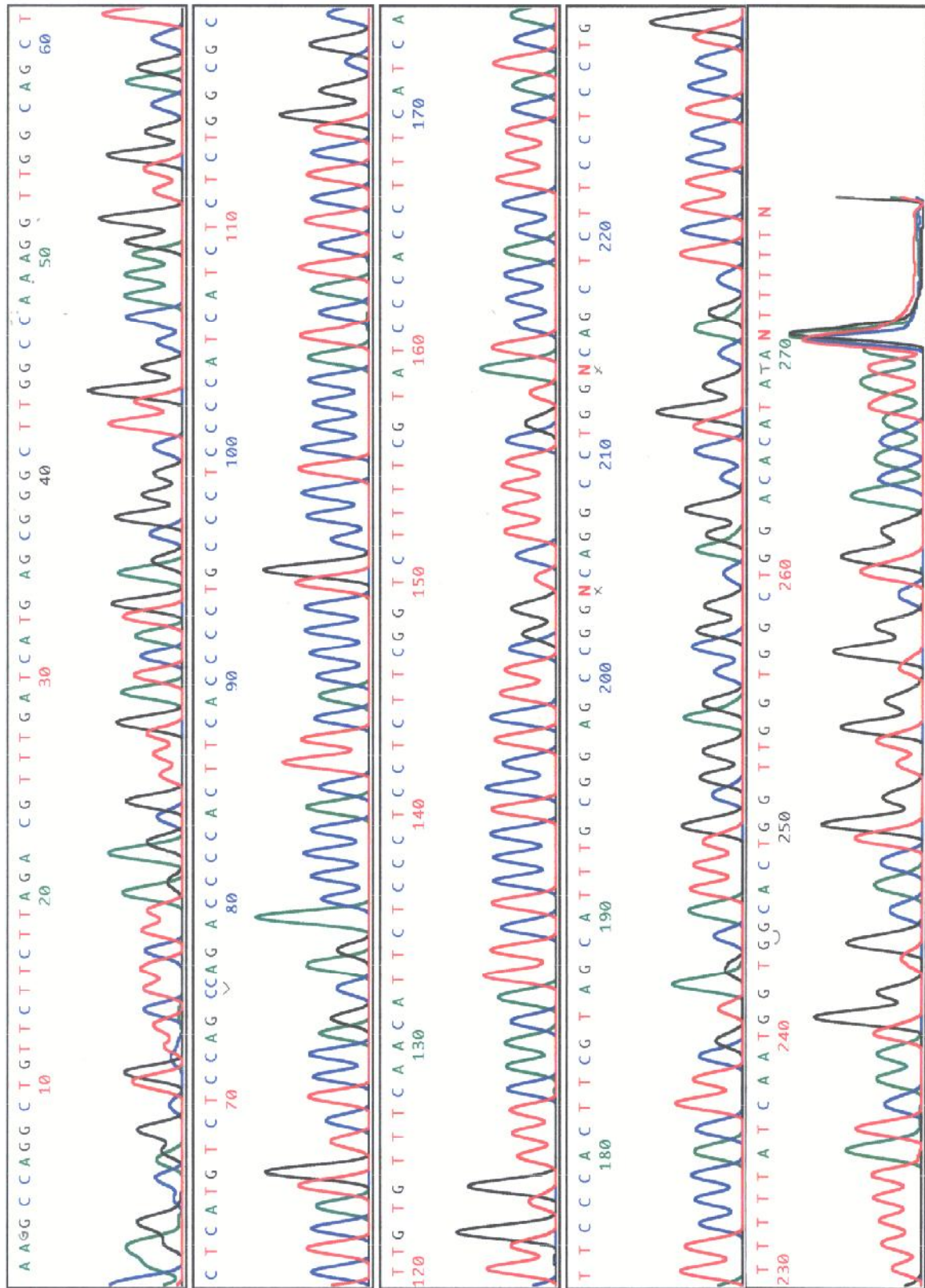
A1. (contd). ER α 2 (427bp band) spectrograph (forward primer) continued



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



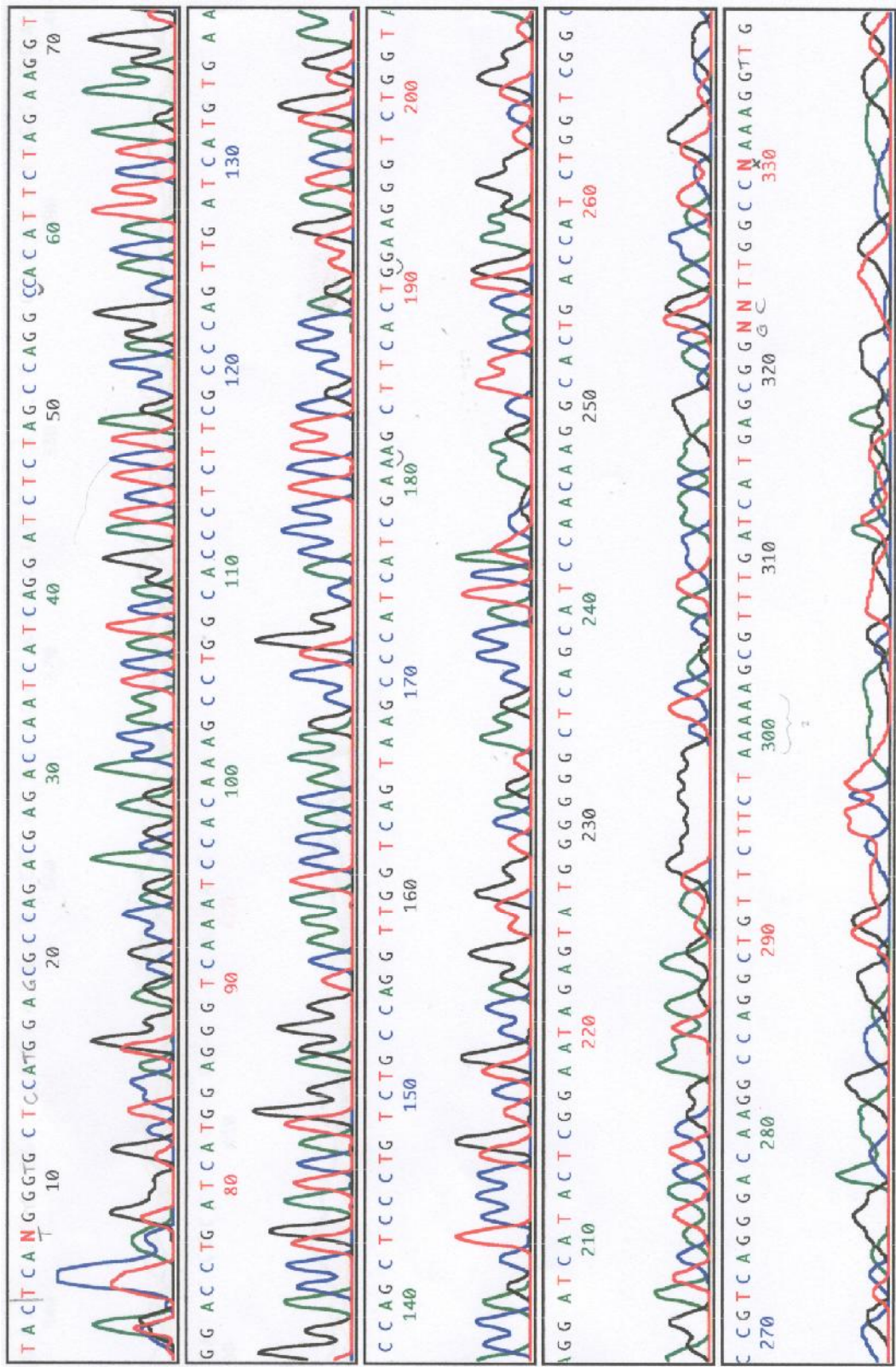
B1. ER α 3 (322bp band) spectrograph (reverse primer)



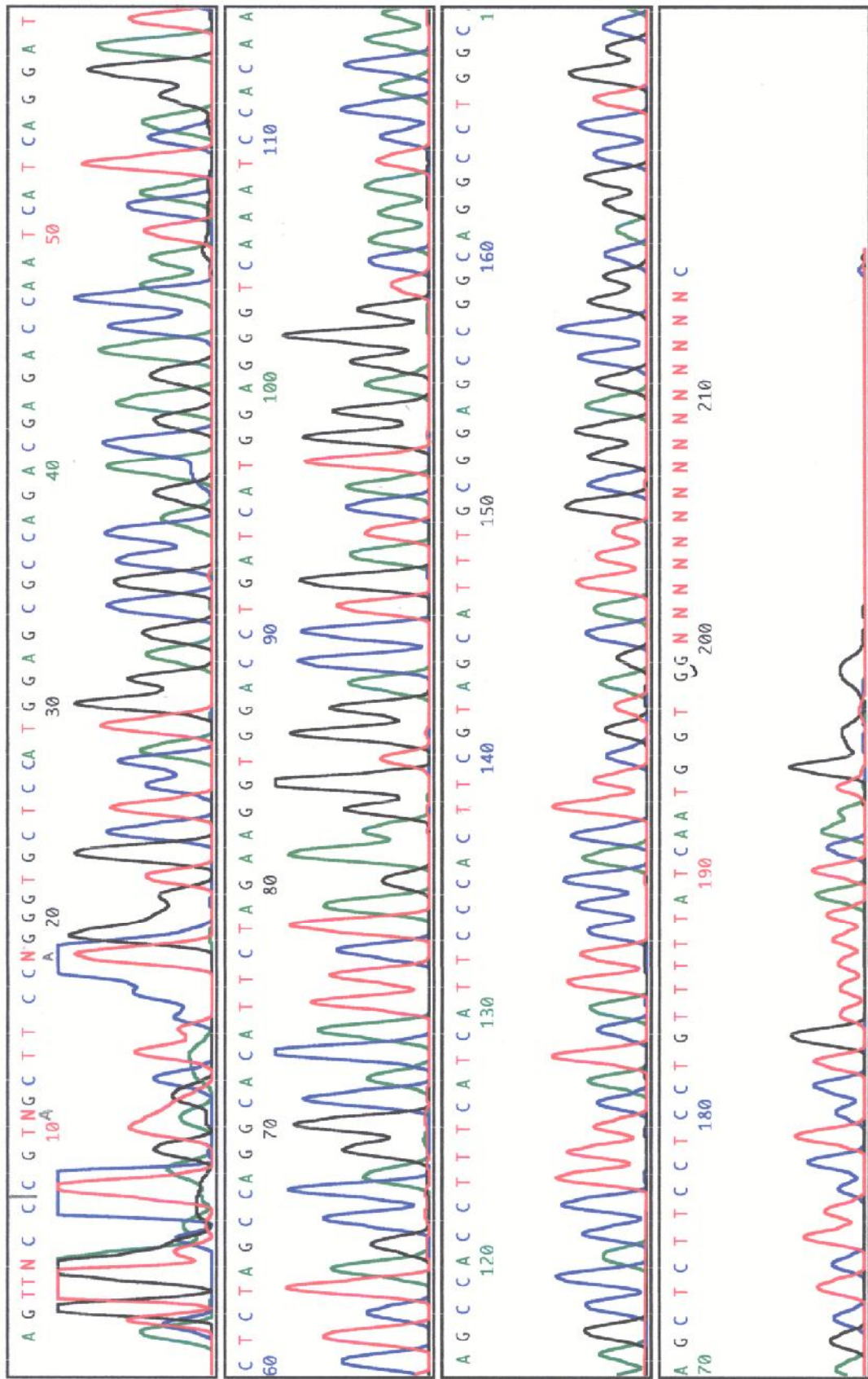
C1a. ER α 4 (555bp band) spectrograph (forward primer)



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

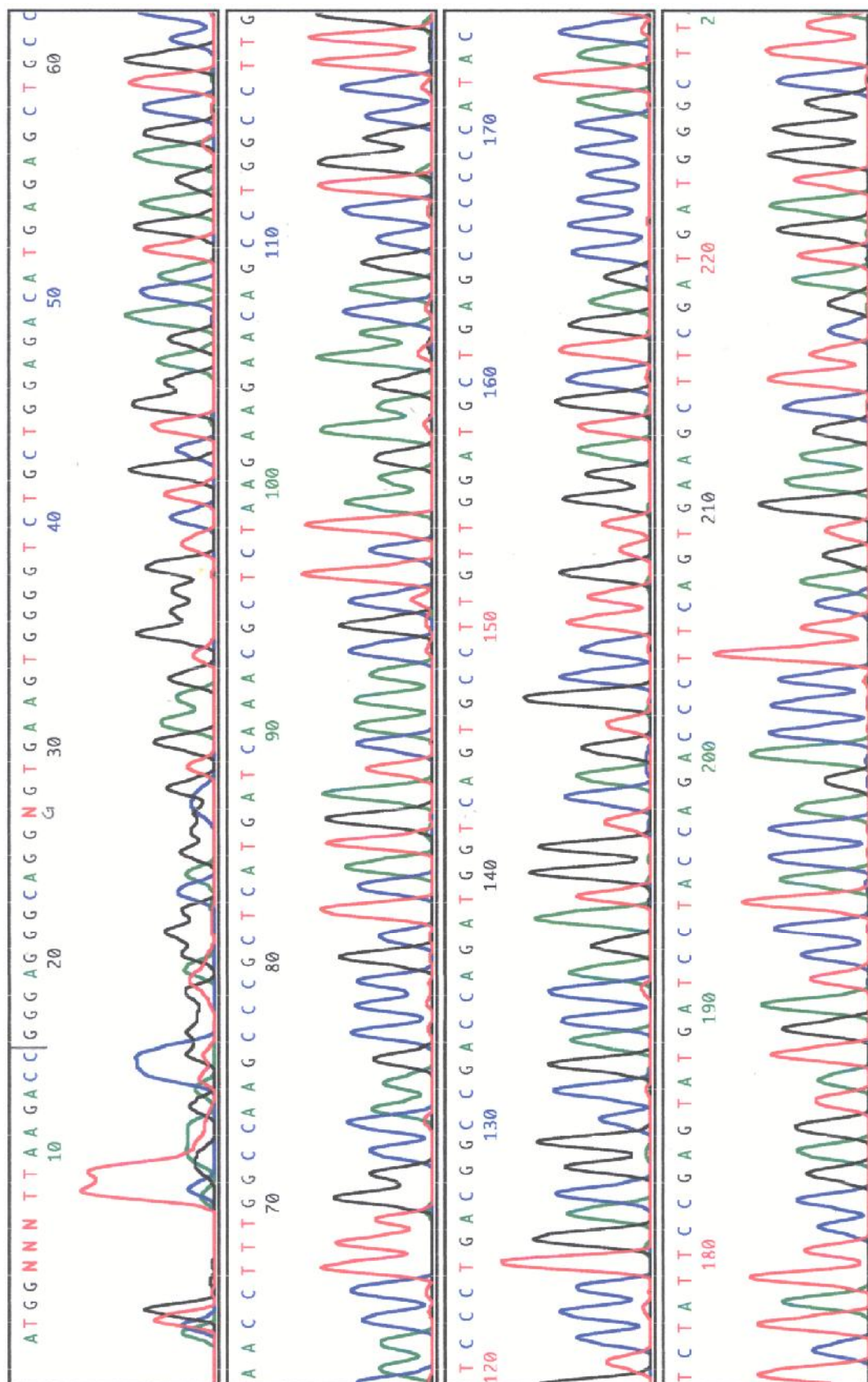


C2a. ER α 4 (219bp band) spectrograph (forward primer)

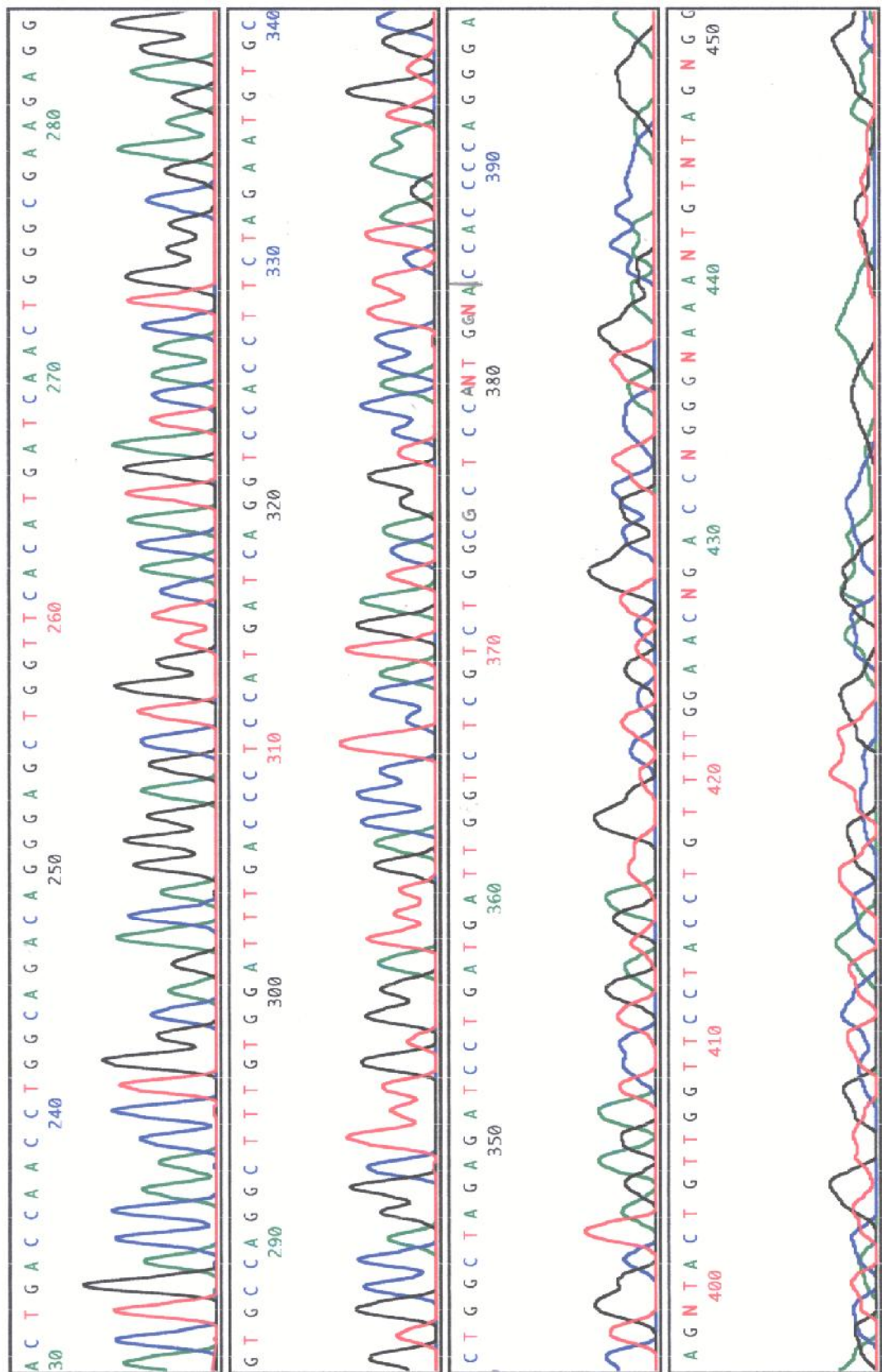


D. ER α exon 5

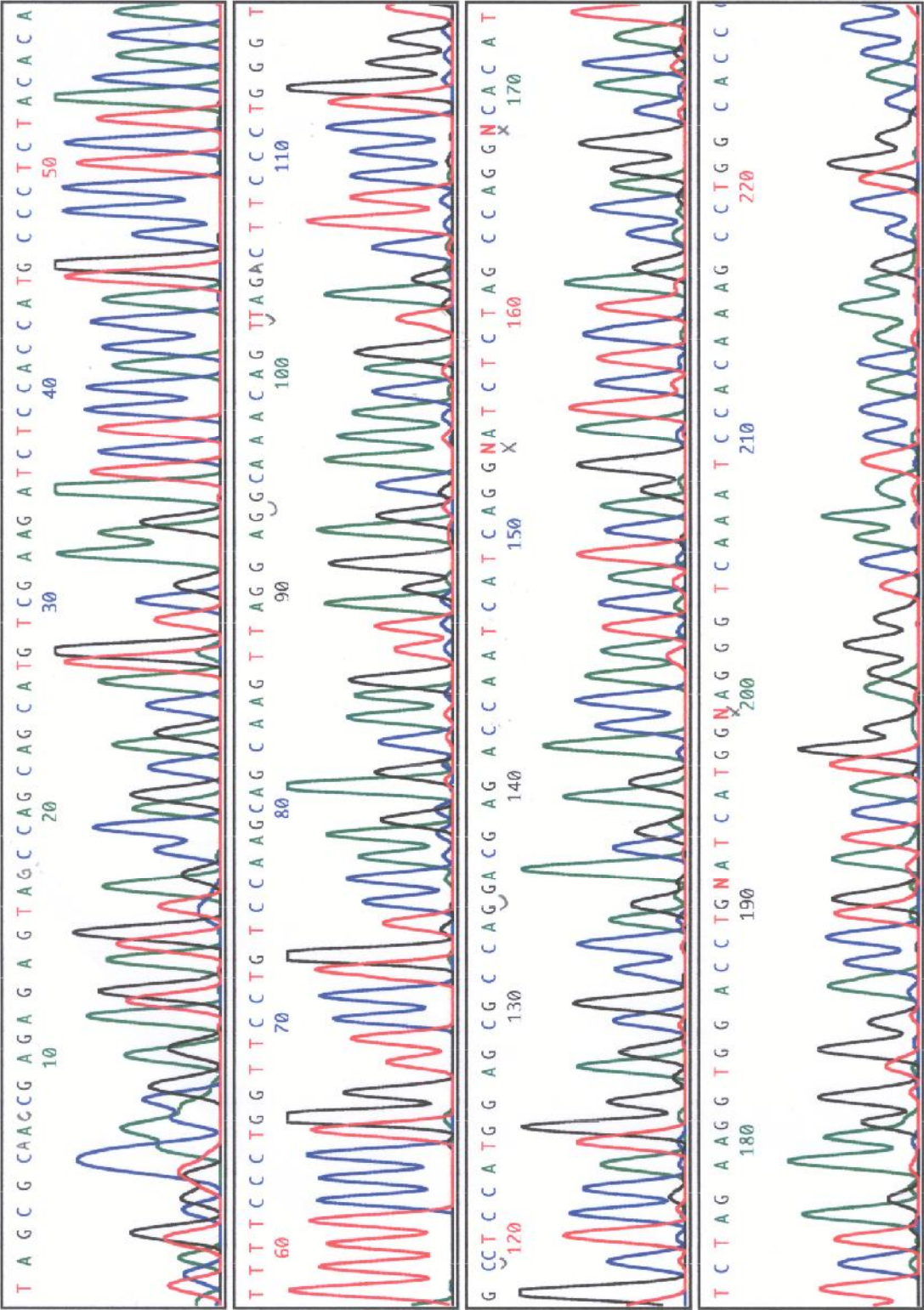
D1a. ER α exon 5 (544bp band) spectrograph (forward primer)



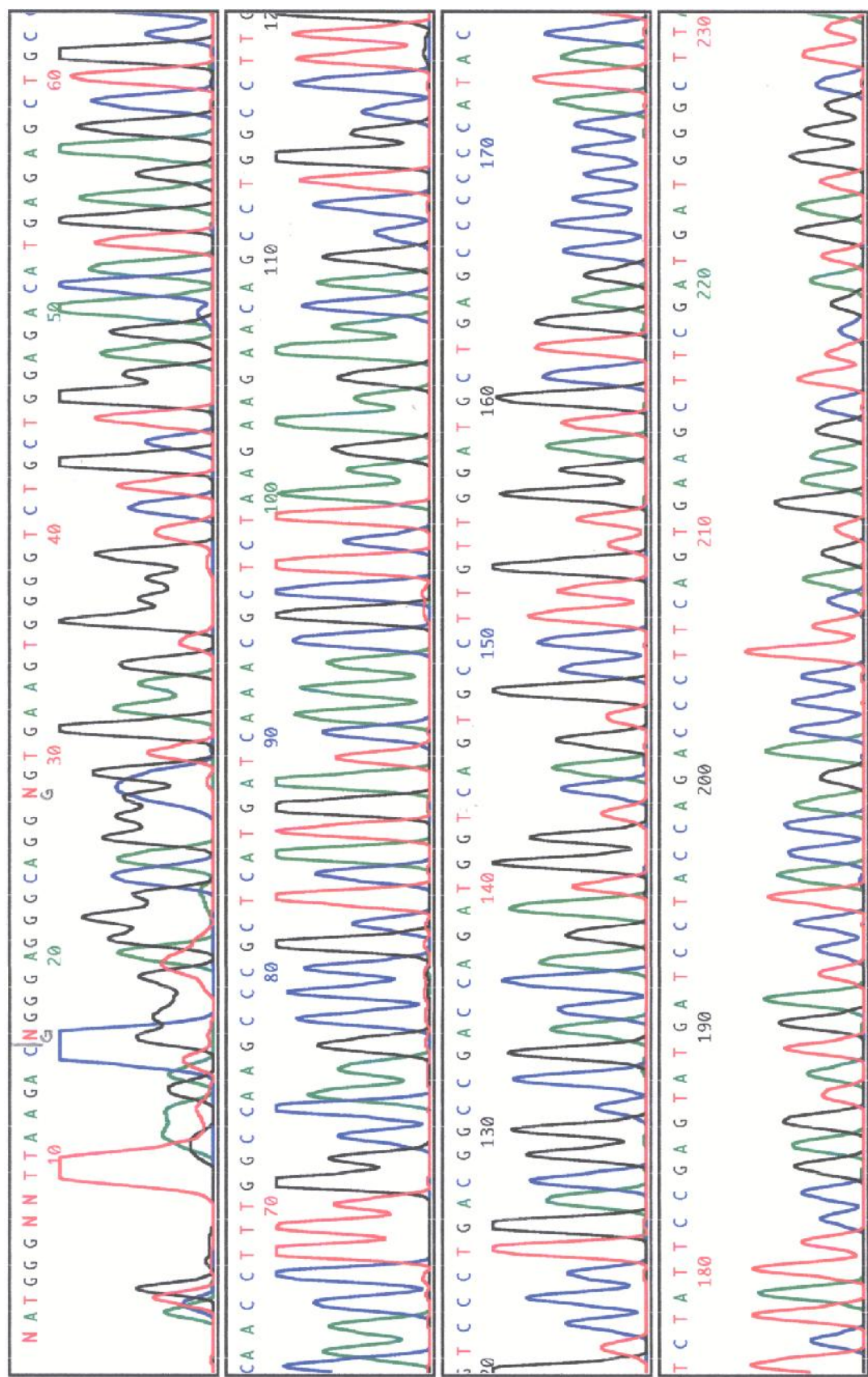
D1a (contd). ERα5 (544bp band) spectrograph (forward primer) continued



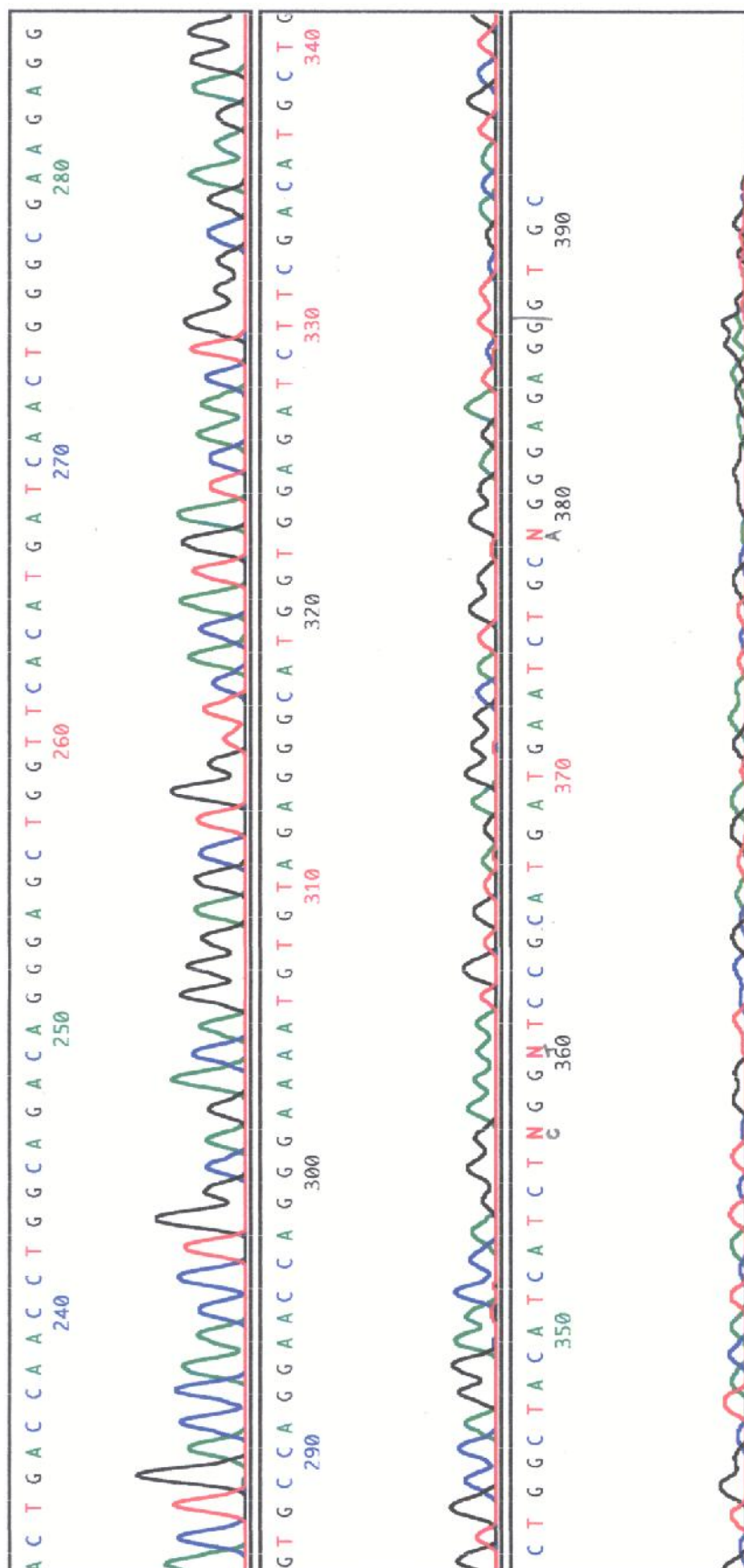
D1b. ERα5 (544bp band) spectrograph (complementary primer)



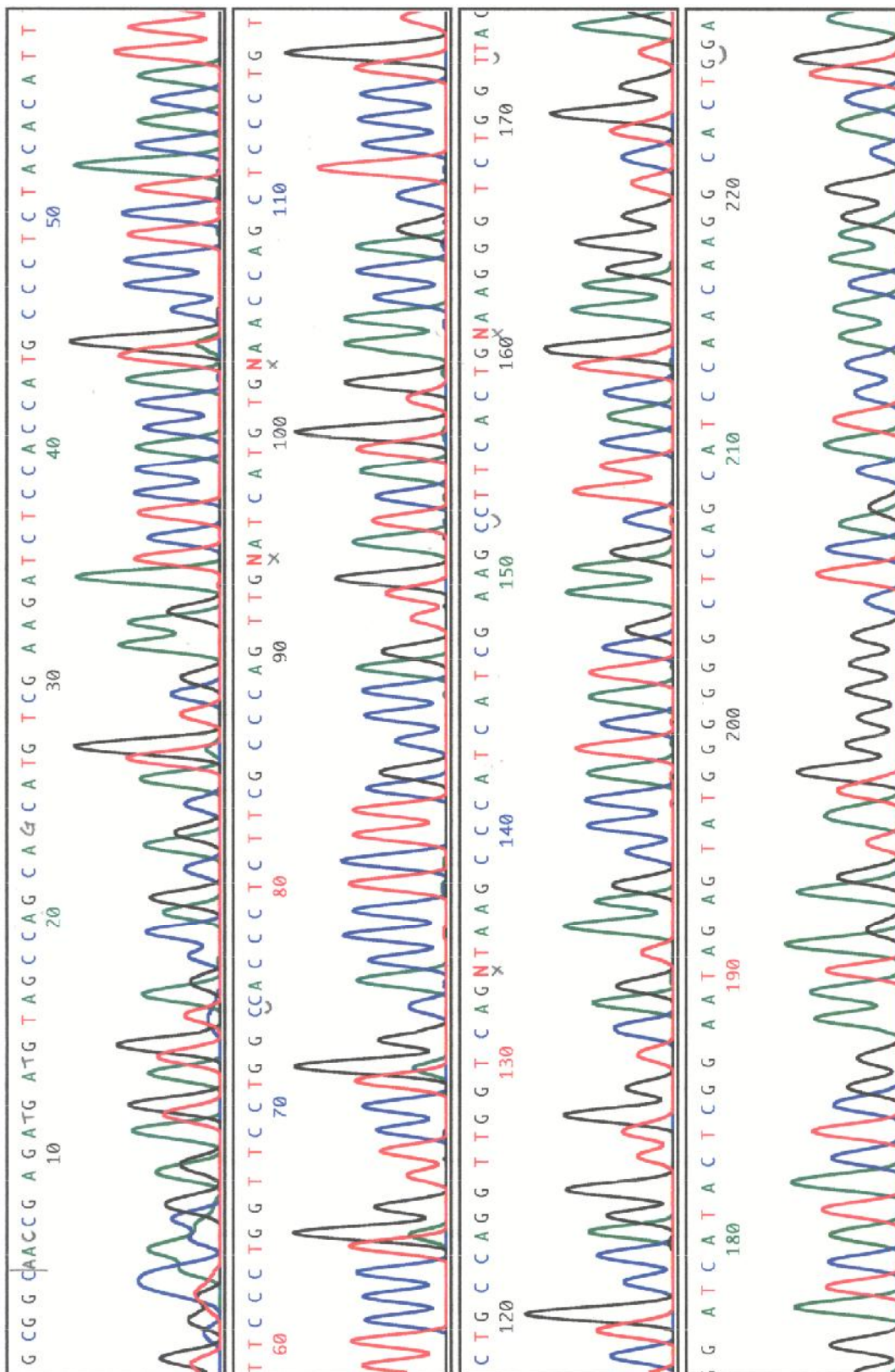
D2a. ERα5 (405bp band) spectrograph (forward primer)



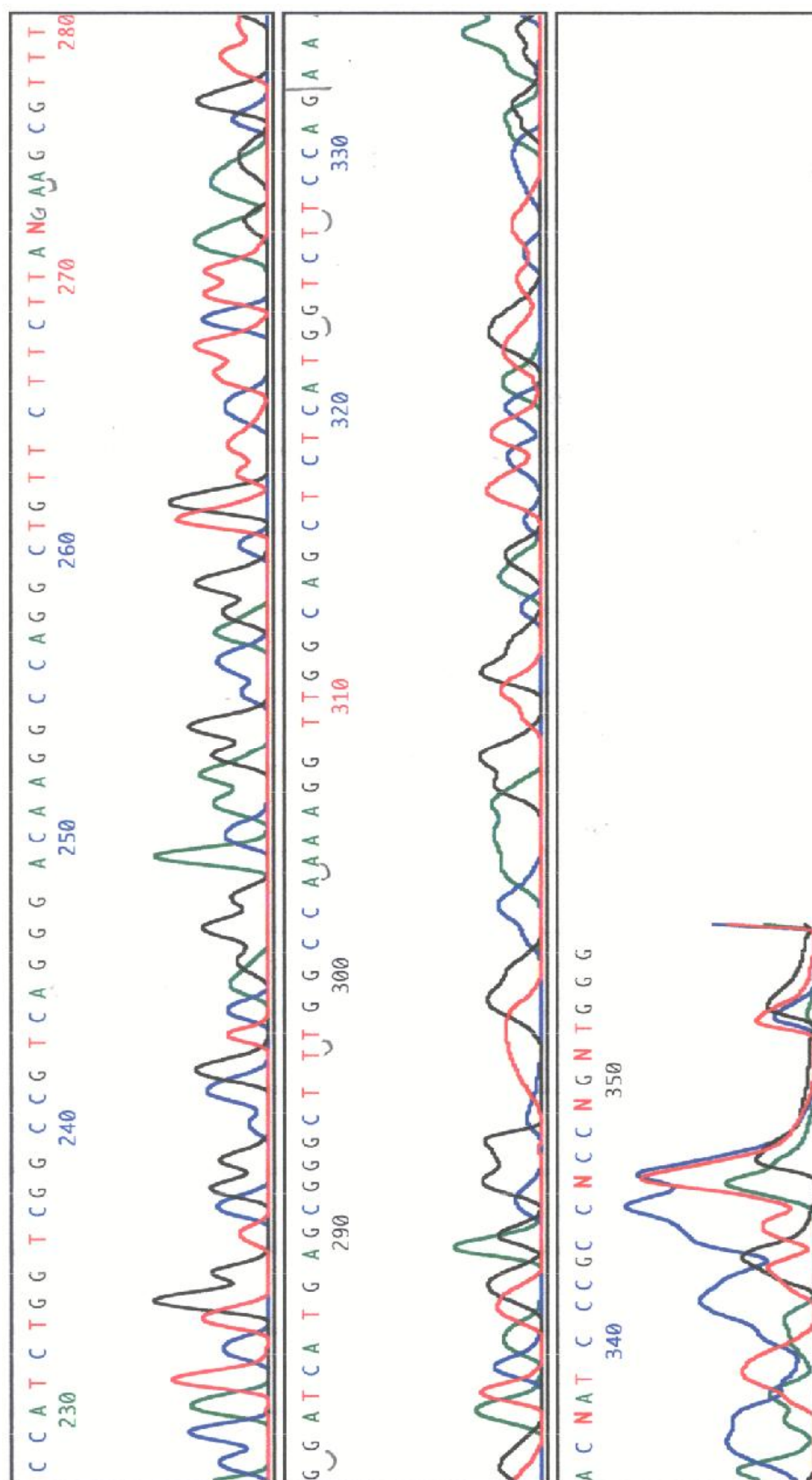
D2a (contd). ERα5 (405bp band) spectrograph (forward primer) continued



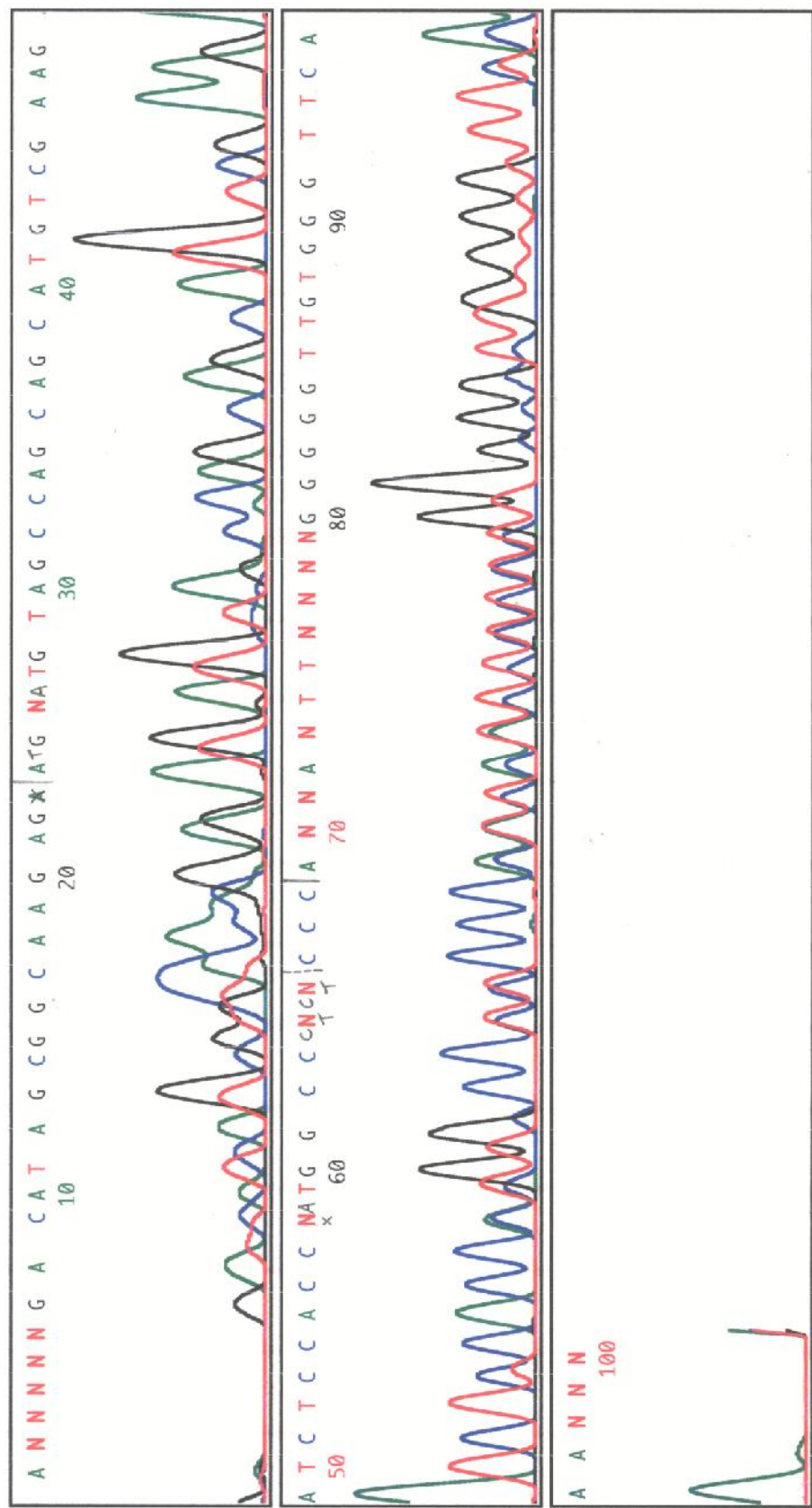
D2b. ERα5 (405bp band) spectrograph (complementary primer)



D2b (contd). ERα5 (405bp band) spectrograph (complementary primer)
continued

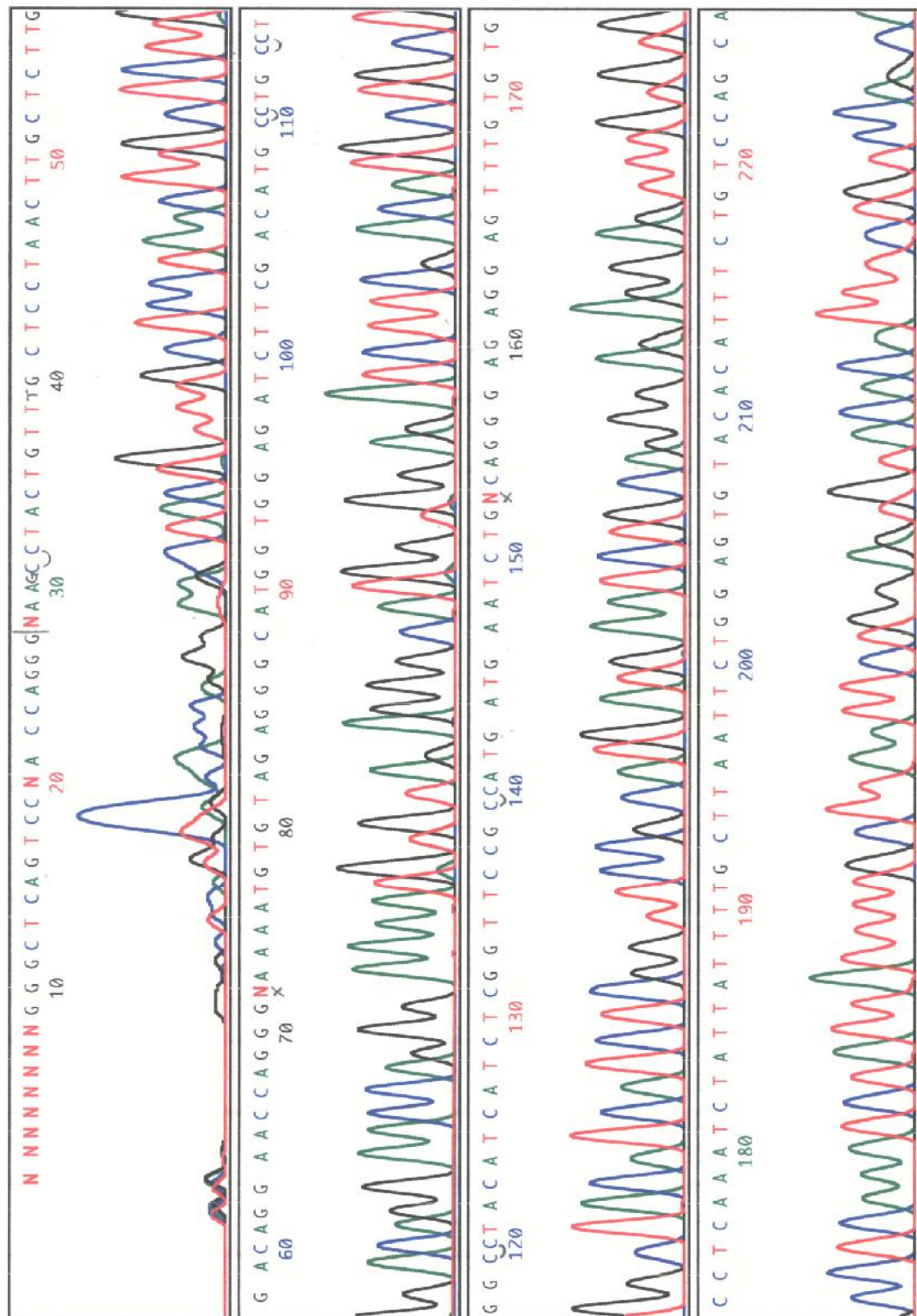


D3. ERα5 (80bp band) spectrograph (complementary primer)

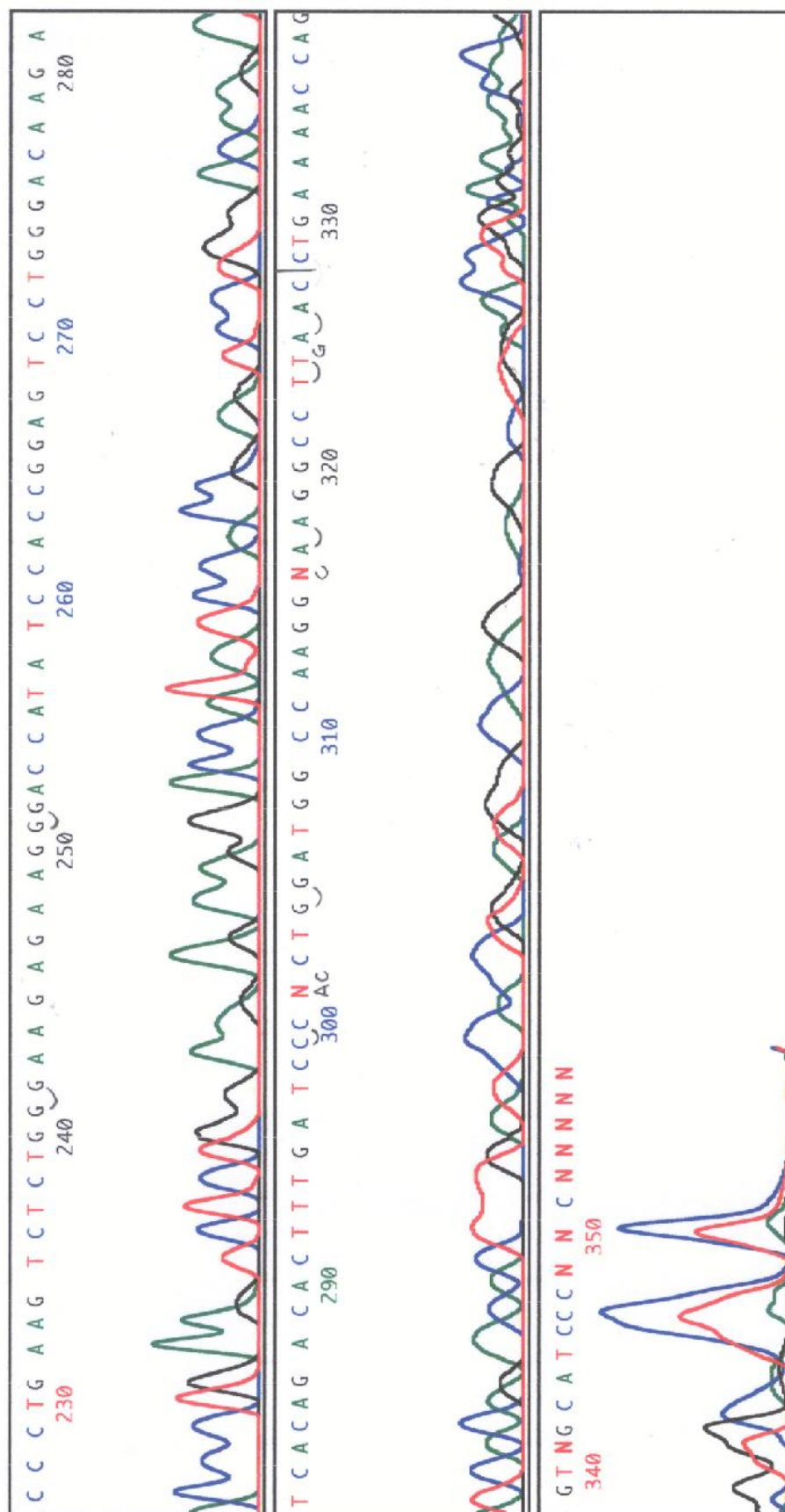


E. ER α exon 6.

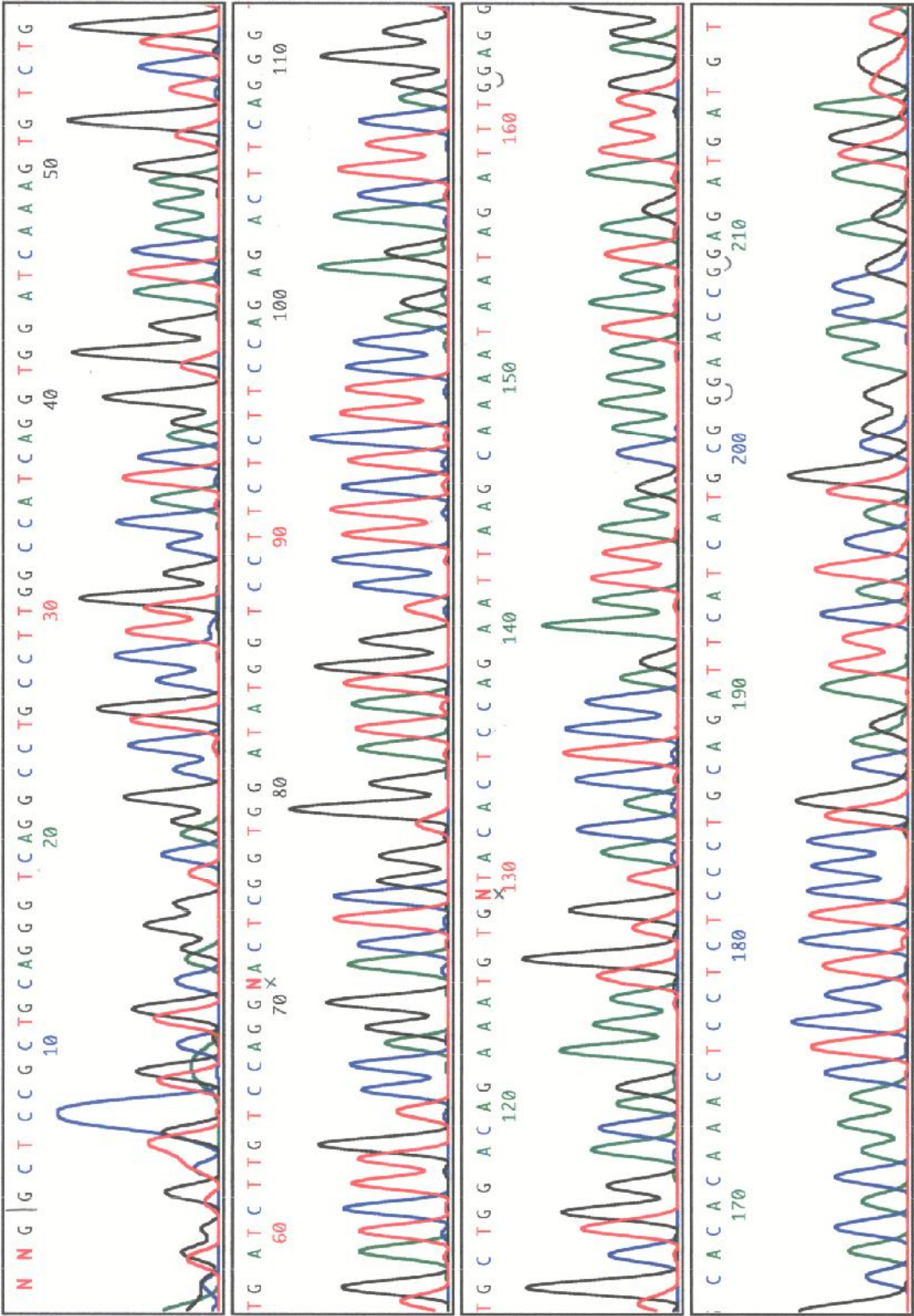
E1a. ER α 6 (388bp band) spectrograph (forward primer)



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

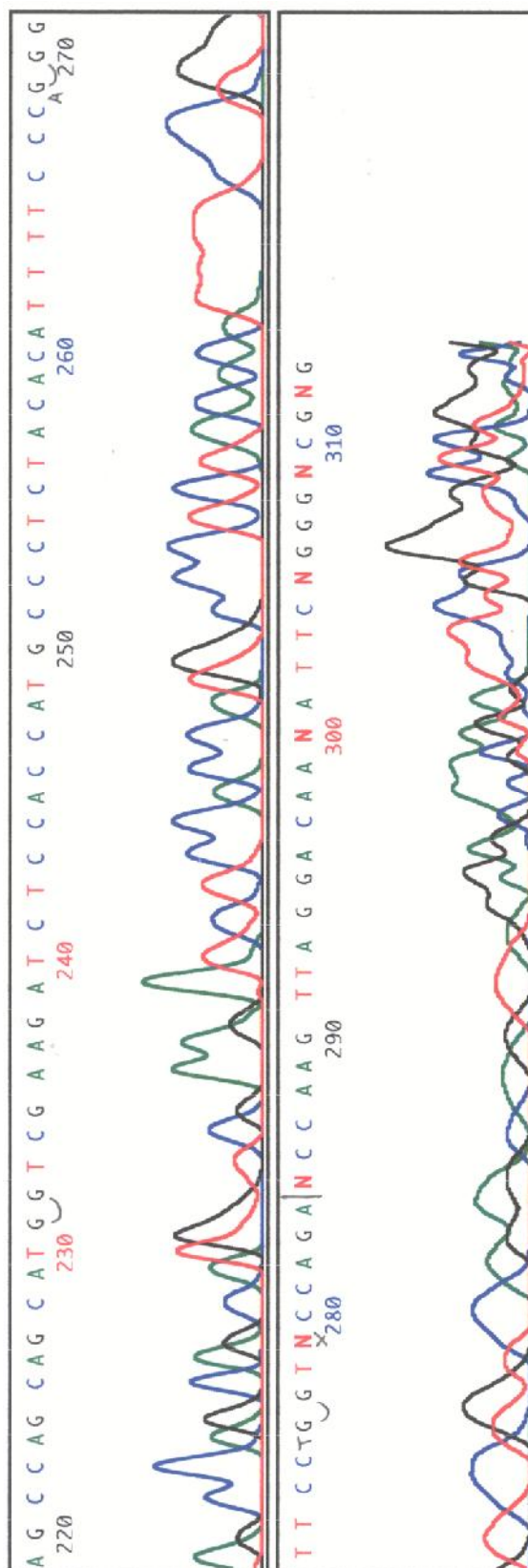


E1b. ERα6 (388bp band) spectrograph (complementary primer)



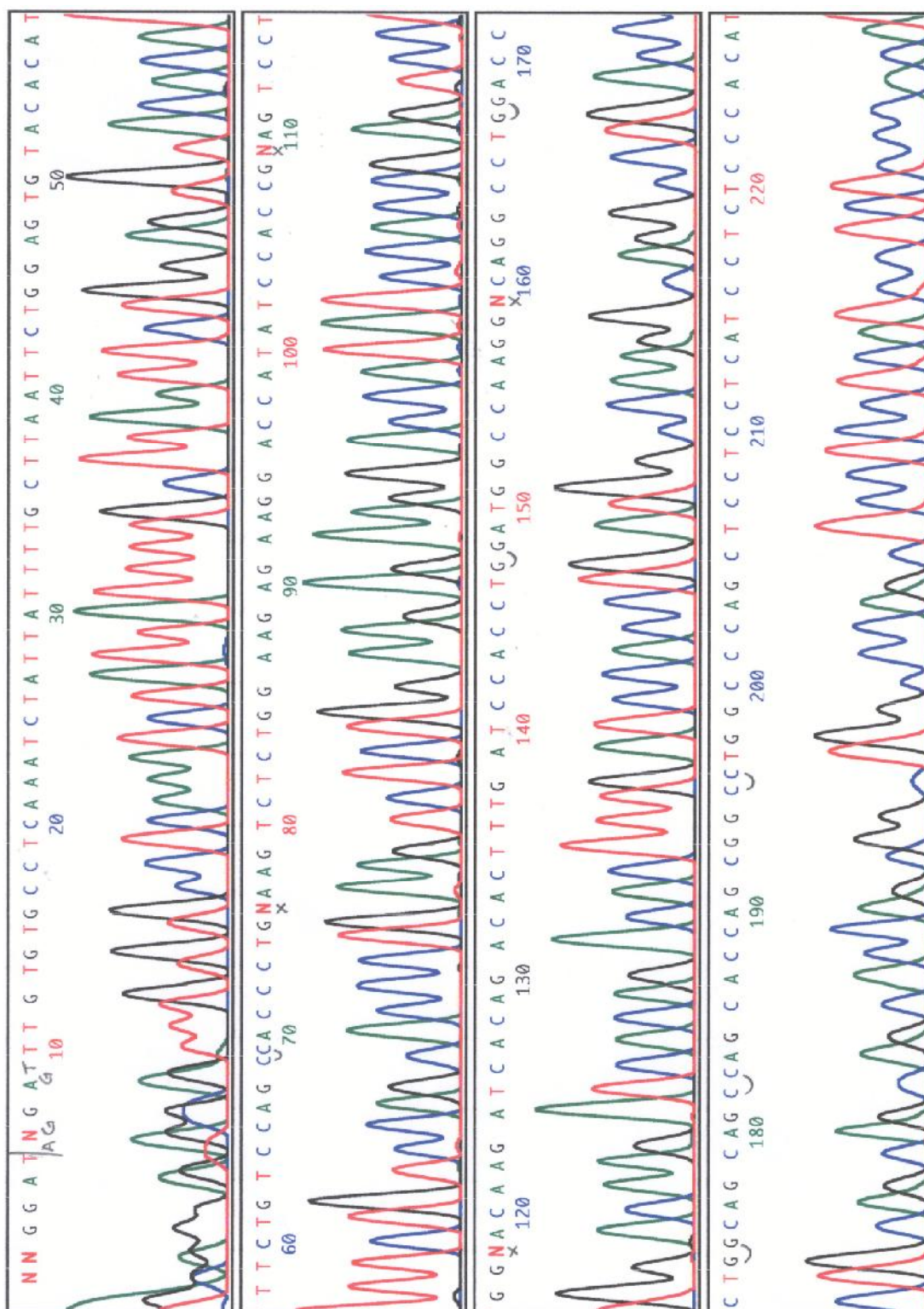
E1b. (contd). ERα6 (388bp band) spectrograph (complementary primer)

continued

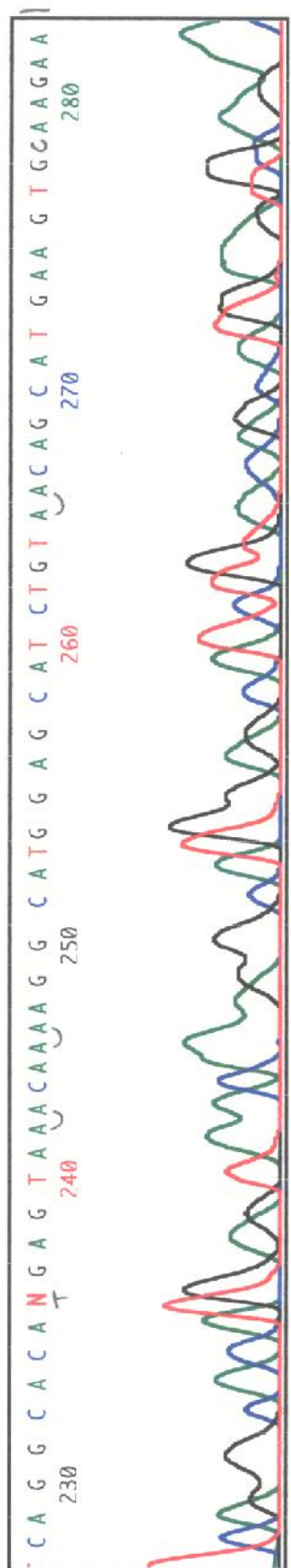


F. ER α exon 7.

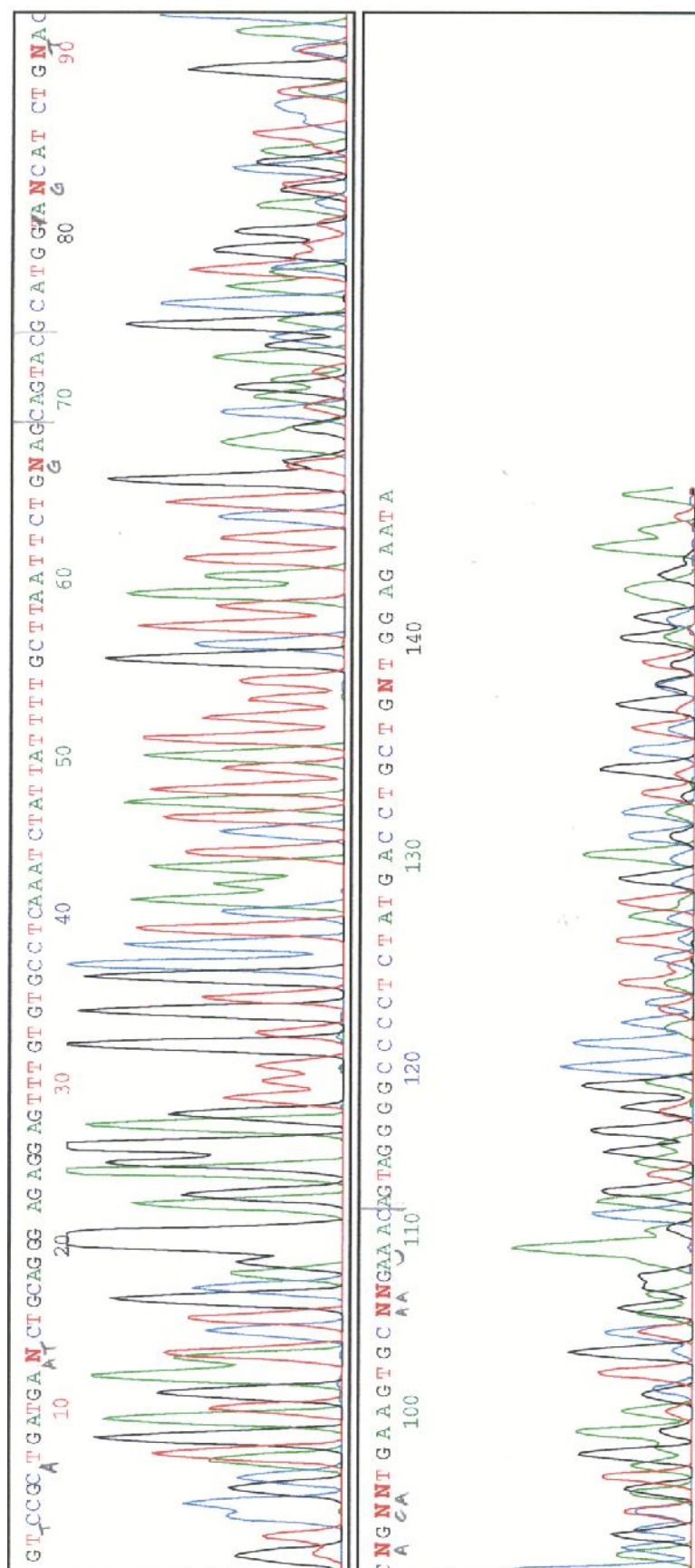
F1a. ER α 7 (345bp band) spectrograph (forward primer)



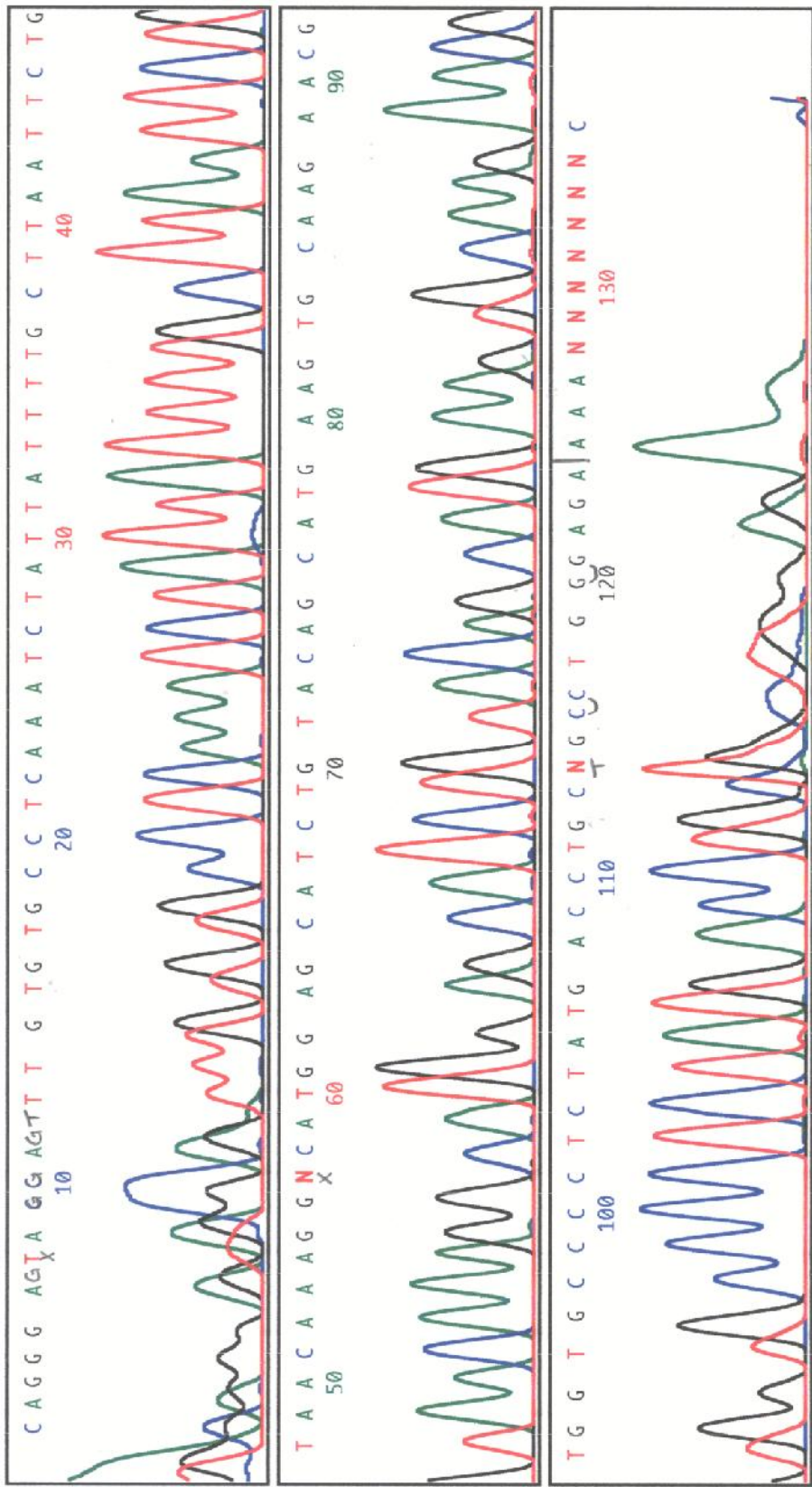
F1a. (contd). ERα7 (345bp band) spectrograph (forward primer) continued



F2a. ERα7 (310bp band) spectrograph (forward primer)



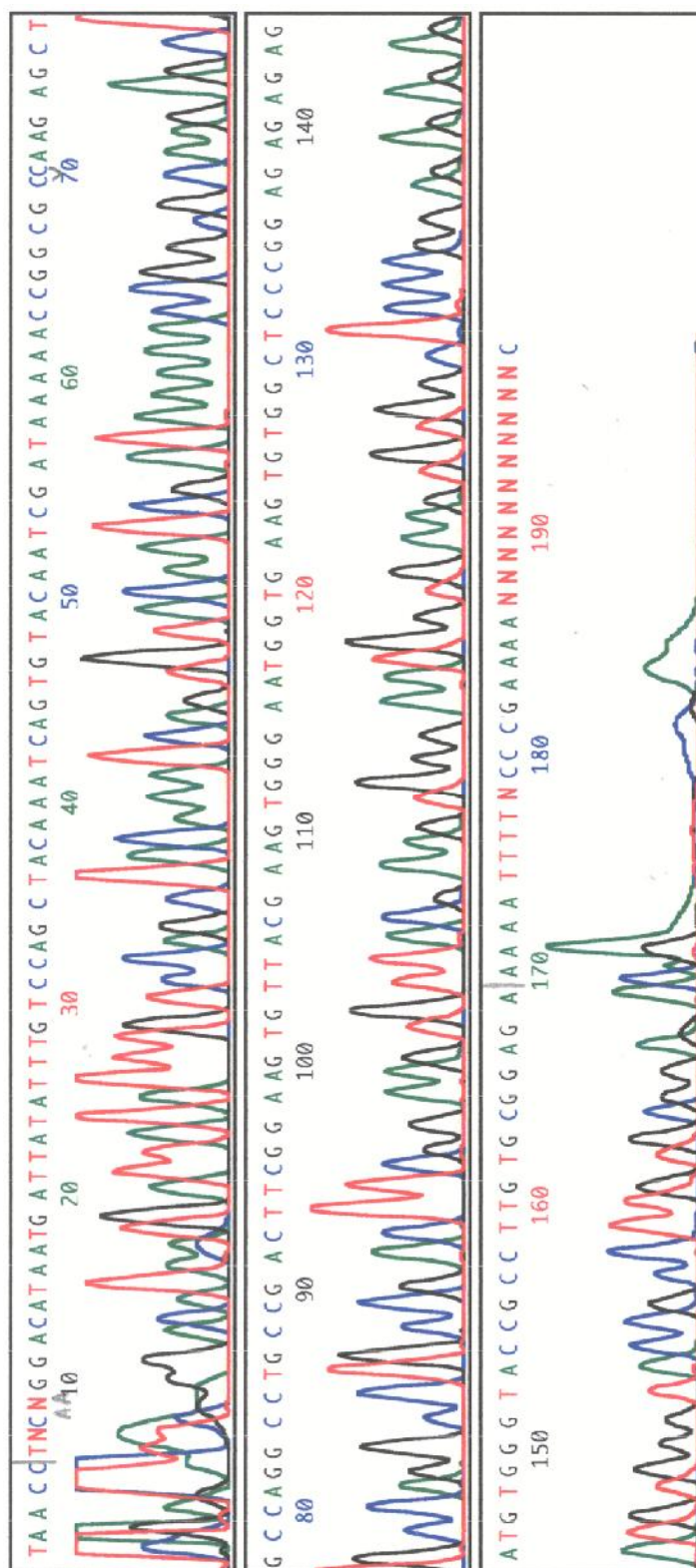
F3a. ERα7 (161bp band) spectrograph (forward primer)



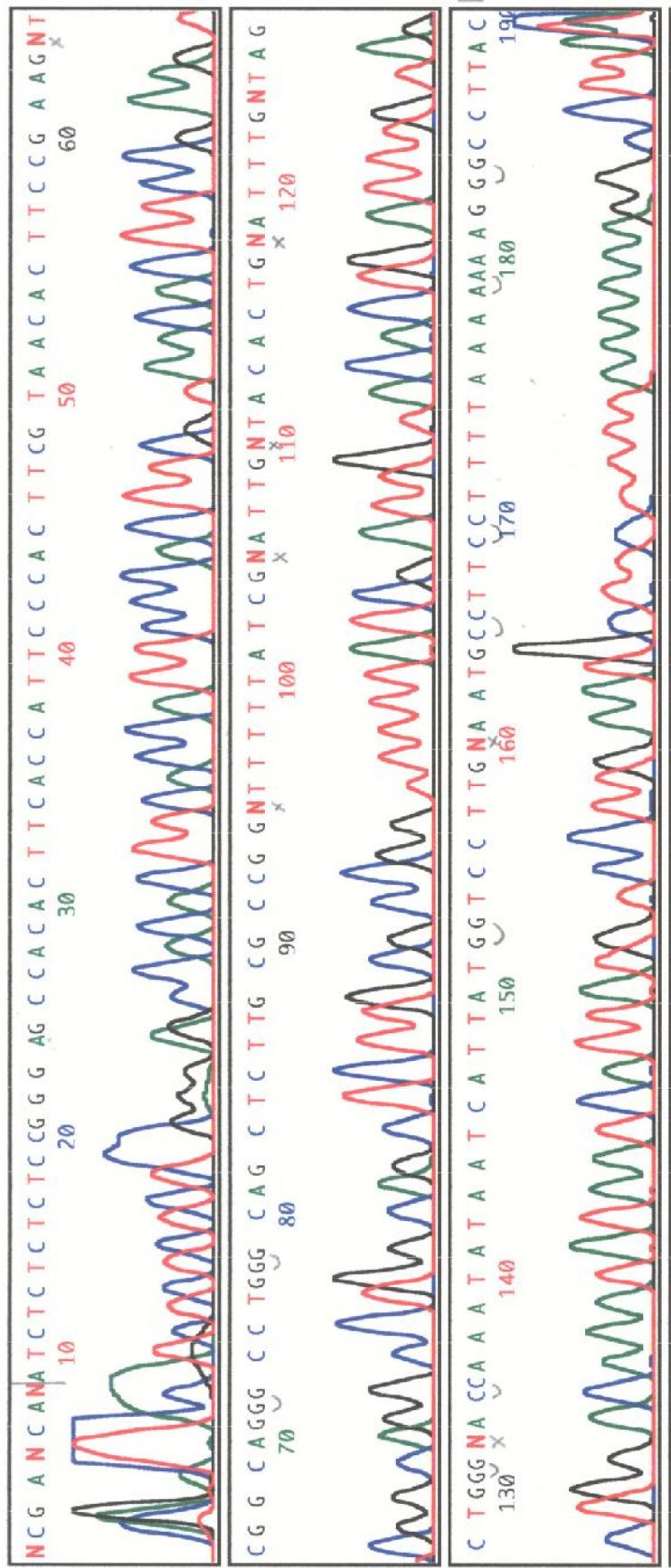
APPENDIX V: Sequencing spectrographs of ER β exons

A. ER β DBD

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

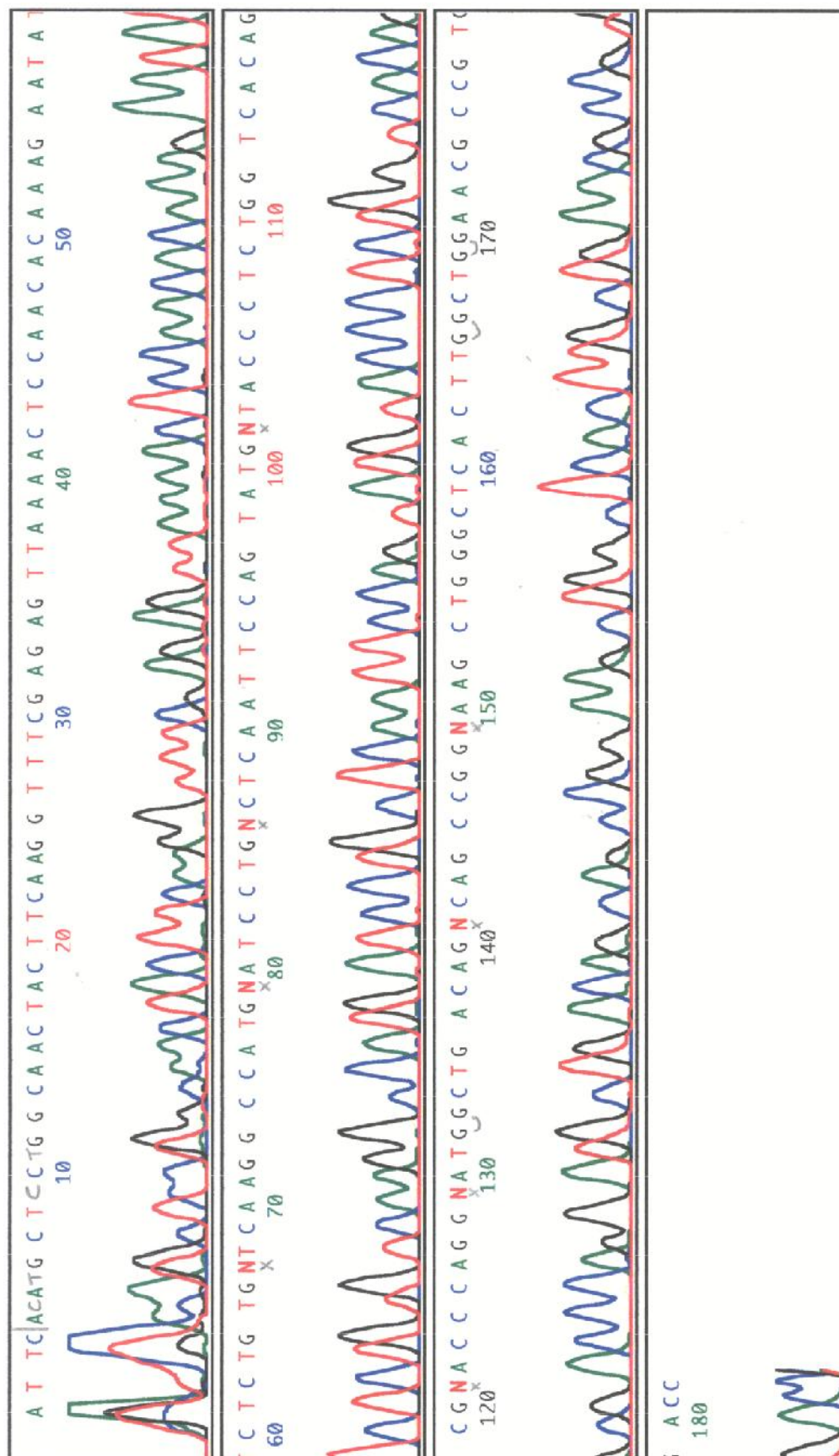


A1b. ERβDBD (214bp band) spectrograph (complementary primer)

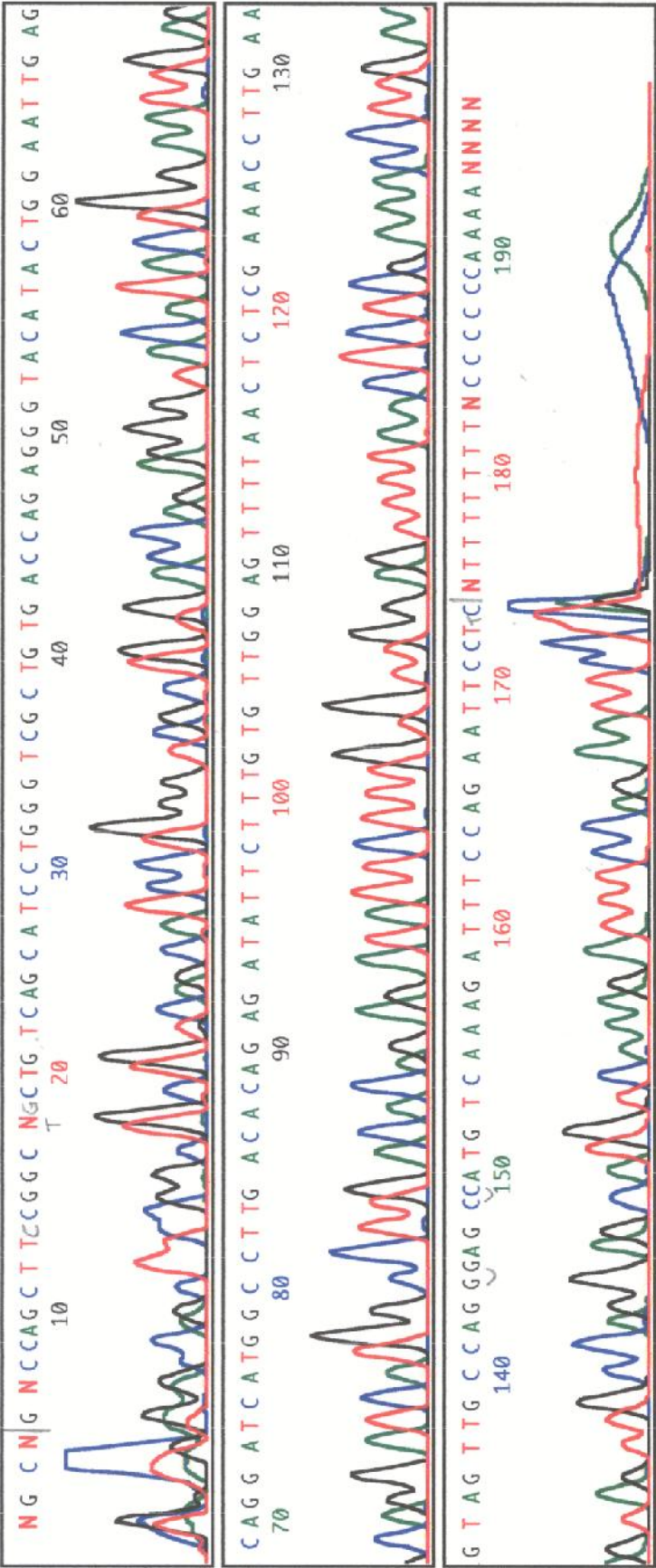


B. ERB LBD

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



B1b. ERβLBD (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

gagttgtgcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgtcctccagcaccttgt
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gcaaaagtattacatcacgggggaggcagagggttccctgccacagtctgagagctccctggc

_____ 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

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgtcctccagcaccttgt
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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

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgtcctccagcaccttgt
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atcacgggggagggcagaggggttccctgccacagtctgagagctccctggc

_____ 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

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgtcctccagcacctttgt
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aaacacaagcgccagagagatgatggggagggcaggggtgaagtggggtctgctggagacatga
gagctgccaacctttggccaagcccgctcatgatcaaacgctctaagaagaacagcctggcctgtcc
ctgacggccgaccagatgggtcagtgccctgttggtgctgagcccccatactctattccgagtatgat
cctaccagacccttcagtgaagcttcgatgatgggcttactgaccaacctggcagacagggagctggt
tcacatgatcaactggggaagaggggtgccaggcctttgtgatttgaccctccatgatcaggtccacct
tctagaatgtgcctggctagagatcctgatgattgggtctcgtctggcgctccatggagcaccagtgaa
gctactgtttgctcctaacttgccttgacaggaaccagggaaaatgtgtagagggcatggtggagatcttga
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tttgcttaattctggagtgtacacatttctgtccagcacctgaagtctctggagaggaaggaccatatccaccgag
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agcggctggccagctcctcctcatcctctcccatcaggcacatgagtaacaaaggcatggagcatctgtac
agcatgaagtgaagaacgtggtgccctctatgacctgctgctggagatgctggacgcccaccgcctacatg
cgccactagccgtggaggggcatccgtggaggagacggaccaaagccactggccactgcgggctctactt
catcgcatctcttgcataaagtattacatcacgggggaggcagaggggttccctgccacagctgagagctccctg
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 ERα exon 4

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgctcctccagcaccttgt
aatgcatatgagctcgggagaccagtaactaaagttggaggcccgaggagctggcggagggcgt
tcgtcctgggagctgcacttgctccgtcgggtcgccggctcaccggaccgcaggctcccgggcagggccgg
ggccagagctcgcgtgctggcgggacatgcgtgcgtgcctctaacctcgggctgtgctctttccagggtggcc
cgccggtttctgagccttctgccctgcggggacacgggtctgcacctgcccgcggccacggaccatgaccatg
accctccacaccaaagcatctgggatggccctactgcatcagatccaagggaacgagctggagcccctgaac
cgccgcagctcaagatccccctggagcggcccctgggcgaggtgtacctggacagcagcaagcccgcctg
gtacaactaccccgagggcgccgcctacgagttaacgcgcggccgcccgaacgcgcagggtctacggctc
agaccggcctccccctacggccccgggtctgagggtgcggcgttcggctccaacggcctggggggtttcccccc
actcaacagcgtgtctccgagcccgtgatgctactgcacccgcgcgcagctgtgcctttctgcagcccc
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gggaagtatggctatggaatctgccaaggagactcgctactgtgcagtgtgcaatgactatgcttcaggctacca
ttatggagtctggtcctgtgagggctgcaaggcctcttcaagagaagtattcaaggacataacgactatatgtgc
cagccaccaaccagtg**caccattgataaaaacaggaggaagagctgccaggcctgccggctccgca**
aatgctacgaagtgggaatgatgaaaggtgggatacgaaaagaccgaagaggaggaggagaatgttgaaa
cacaagcgcagagagatgatggggagggcaggggtgaagtggggctgctggagacatgagagctgcc
acctttggccaagcccgcctcatgatcaaacgctctaagaagaacagcctggcctgtccctgacggccgacca
gatggtcagtgcttgttgatgctgagccccccatactctattccgagtatgatcctaccagacccttcagtgaag
cttcgatgatgggcttactgaccaacctggcagacaggagctggttcacatgatcaactgggcgaagaggggt
gccaggcctttgtggatttgacctccatgatcagggtccaccttctagaatgtgcctggctagagatcctga
tgattgggtctcgtctggcgctccatggagcaccagtggaagctactgttgcctcctaactgctcttgacag
gaaccagggaaaatgtgtagagggcatggtggagatcttcgacatgctgctggctacatcatctcggttccgcat
gatgaatctgcaggagagaggagtttgtgctcaaatctatttttgccttaattctggagtgtacacatttctgtcca
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cctgatggccaaggcaggcctgacctgcagcagcagcaccagcggctggcccagctcctcctcatcctctcc
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tatgacctgctgctggagatgctggacgccaccgcctacatgcgccactagccgtggaggggcatccgtgg
aggagacggaccaaaagccactggccactgcccgtctactcatcgcatccttgcaaaagtattacatcacg
ggggaggcagaggggttccctgccacagtctgagagctccctggc

_____ 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 ERα exon 5

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttggcgtcctccagcaccttgt
aatgcatatgagctcgggagaccagtacttaagttggaggcccgaggagcctggcgaggcggt
tcgtcctgggagctgcacttgctccgtcgggtcgccggcttaccggaccgcaggctcccggggcaggggccgg
ggccagagctcgcgtgctggcgggacatgcgtgcgtgccttaacctcgggtgtgctctttccaggtggcc
cgccggtttctgagccttctgcctgcggggacacgggtcgcacctgcccgcggccacggaccatgaccatg
acctccacaccaaagcatctgggatggcctactgcatcagatccaagggaacgagctggagcccctgaac
cgccgcagctcaagatccccctggagcggccccctgggaggtgtacctggacagcagcaagcccgcctg
gtacaactaccccgagggcgccgctacgagttcaacgcgcggccgccaacgcgcaggctacggtc
agaccggcctccctacggccccgggtctgaggtcgcgggttcggctccaacggcctggggggttcccccc
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gggaagtatggctatggaatctgccaaaggagactcgctactgtgcagtgtgcaatgactatgcttcagggtacca
ttatggagtctggtcctgtgaggggtgcaaggccttctcaagagaagtattcaaggacataacgactatatgtgc
cagccaccaaccagtgcaccattgataaaaacaggaggaagagctgccaggcctgcgggtccgcaaattg
ctacgaagtgggaatgatgaaagggtgggatacgaagaccgaagaggaggaggaatgttgaaacacaa
gcgccagagagatgatggggagggcaggggtgaagtggggtctgctggagacatgagagctgcca
accttggccaagcccgcctcatgatcaaacgctctaagaagaacagcctggccttgcctgacggcc
gaccagatggctcagtgccctgttgatgctgagcccccatactctattccgagtatgatcctaccagac
ccttcagtgaagcttcgatgatgggcttactgaccaacctggcagacagggagctggttcacatgatc
aactgggcgaagaggggtgccag***gctttgtggaattgaccctccatgatcaggtccaccttctagaatgt***
gcctggctagagatcctgatgattgggtctcgtctggcgctccatggagcaccagtgaaagctactgttt
gctcctaacttgctcttgacaggaaccagggaaaatgtgtagagggcatggtggagatcttcgacat
gctgctggctacatcatctcggtccgcatgatgaatctgcagggagaggagttgtgtgcctcaaacttatta
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cagcgggtggcccagctcctcctcatcctctccacatcaggcacatgagtaacaaaggcatggagcatctgta
cagcatgaagtgaagaacgtggtgccctctatgacctgctgctggagatgctggacgccaccgcctacat
gcgccactagccgtggaggggcatccgtggaggagacggaccaaagccacttggccactgcgggtctac
ttcatcgcatccttgcaaaagtattacatcacgggggagggcagaggggttccctgccacagtctgagagctccct
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 ERα exon 5

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgtcctccagcaccttgt
aatgcatatgagctcgggagaccagtagctaaagttggaggcccgaggagctggcggagggcgt
tcgtcctgggagctgcacttgctccgtcgggtcgccggctcaccggaccgcaggctcccgggcagggccgg
ggccagagctcgcgtgctggcggaacatgcgtgcgtgcctctaacctcgggtgtgctctttccaggtggcc
cgccggtttctgagccttctgccctgcggggacacgggtctgcacctgcccgcggccacggaccatgaccatg
accctccacaccaaagcatctgggatggccctactgcatcagatccaagggaacgagctggagcccctgaac
cgccgcagctcaagatccccctggagcggcccctgggcgaggtgtacctggacagcagcaagcccgcgt
gtacaactaccccgagggcgccgcctacgagttcaacgcgcggccgcccgaacgcgcagggttacggtc
agaccggcctcccctacggccccgggtctgagggtgcggcgttcgggtccaacggcctggggggttcccccc
actcaacagcgtgtctccgagcccgtgatgctactgcacccgcgcgcagctgtgccttctcagcccc
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ggcattctacaggccaaatcagataatcgacgccagggtggcagagaaagattggccagtagcaatgacaa
gggaagtatggctatggaatctgccaaggagactcgctactgtgcagtgtgcaatgactatgcttcagggtacca
ttatggagtctggtcctgtgagggctgcaaggccttctcaagagaagtattcaaggacataacgactatatgtgtc
cagccaccaaccagtgaccattgataaaaacaggaggaagagctgccaggcctgccggctccgcaaatg
ctacgaagtgggaatgatgaaagggtgggatacgaaaagaccgaagaggaggagaatgtgaaacacaa
gcgccagagagatgatggggagggcaggggtgaagtgggggtctgctggagacatgagagctgcca
acctttggccaagcccgtcatgatcaaacgctctaagaagaacagcctggccttgcctgacggcc
gaccagatggtcagtgccctgttgatgctgagcccccatactctattccgagtagtgcctaccagac
ccttcagtgaagcttcgatgatgggcttactgaccaacctggcagacagggagctggttcacatgatc
aactgggcgaagagggtgccag*gcttgtggattgaccctccatgatcagggtccaccttctagaatgtgcctg*
gctagagatcctgatgattggtctcgtctggcgctccatggagcaccagtgaaagctactgttggctcctaacttgc
cttggacaggaaccagggaaaatgtgtagagggcatggtggagatcttcgacatgctgctggctacat
catctcggttccgcatgatgaatctgcagggagaggagttgtgtgcctcaaattctattttgcttaattctgg
agtgtacacatttctgtccagcacctgaagtctctggagaggaaggaccatatccaccgagtcctggacaaga
tcacagacactttgatccacctgatggccaaggcaggcctgacctgcagcagcagcaccagcggctggccc
agctcctctcatcctctcccacatcaggcacatgagtaacaaaggcatggagcatctgtacagcatgaagtgc
aagaacgtggtgccctctatgacctgctgctggagatgctggacgcccaccgcctacatgcgccactagcc
gtggaggggcatccgtggaggagacggaccaaagccactggccactgcgggctctactcatgcattccttg
caaaagtattacatcacgggggaggcagaggggttcctgccacagtctgagagctccctggc

_____ 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 ERα exon 5

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgctcctccagcaccttgt
aatgcatatgagctcgggagaccagtagtactaaagttggaggcccgaggagccagagctggcggagggcgt
tcgtcctgggagctgcacttgctccgtcgggtcgccggctcaccggaccgcaggctcccgggcagggccgg
ggccagagctcgcgtgctggcgggacatgcgtgcgtgcctctaacctcggtgtgctctttccagggtggcc
cgccggtttctgagccttctgccctgcggggacacgggtctgcacctgcccgcggccacggaccatgaccatg
accctccacaccaaagcatctgggatggccctactgcatcagatccaagggaacgagctggagcccctgaac
cgccgcagctcaagatccccctggagcggcccctgggcgaggtgtacctggacagcagcaagcccgcgt
gtacaactaccccgagggcgccgctacgagttaacgcgcggccgcccgaacgcgcagggtctacggtc
agaccggcctcccctacggccccgggtctgagggtgcggcgttcgggtccaacggcctggggggttcccccc
actcaacagcgtgtctccgagcccgtgatgctactgcacccgcgcgcagctgtgccttctctgcagcccc
acggccagcagggtgccctactacctggagaacgagcccagcggctacacgggtgcgcgaggccggcccgc
ggcattctacaggccaaattcagataatcgacgccagggtggcagagaaagattggccagtagcaatgacaa
gggaagtatggctatggaatctgccaaggagactcgctactgtgcagtgtgcaatgactatgcttcagggtacca
ttatggagtctggtcctgtgagggctgcaaggccttctcaagagaagtattcaaggacataacgactatatgtgtc
cagccaccaaccagtgaccattgataaaaaacaggaggaagagctgccaggcctgccggctccgcaaatg
ctacgaagtgggaatgatgaaagggtgggatacgaaaagaccgaagaggaggagaatgtgaaacacaa
gcgccagagagatgatggggagggcaggggtgaagtgggtctgctggagacatgagagctgccaacctt
ggccaagcccgtcatgatcaaacgctctaagaagaacagcctggccttgcctgacggccgaccagatgg
tcagtgcctgttgatgctgagccccatactctattccgagtatgatcctaccagacccttcagtgaagctcga
tgatgggtactgaccaacctggcagacaggagctggttcacatgatcaactgggcgaagagggtgccag
gcttgtgagattgaccctccatgatcaggtccacctttagaatgtgcctggctagagatcctgatgattggtctcgt
ctggcgtccatggagcaccagtgaaagctactgttgccttaacttgctcttggaacaggaaccaggga
aaatgtgtagagggcatgggtggagatcttcgacatgctgctggctacatcatctcggttccgcatgatgaatc
tgcagggagaggagttgtgtgcctcaaataattttgcttaattctggagtgtacacatttctgcccagcacc
ctgaagtctctggagaggaaggaccatatccaccgagtcctggacaagatcacagacactttgatccacctgat
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caggcacatgagtaaaaaggcatggagcatctgtacagcatgaagtgaagaacgtggtgcccctctatga
cctgctgctggagatgctggacgccaccgcctacatgcgccactagccgtggaggggcatccgtggagga
gacggaccaaagccactggccactgcgggtctactcatgcattccttgcaaaagtattacatcacggggg
aggcagaggggttccctgccacagtctgagagctcctggc

_____ 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 ERα exon 6

gagttgtgcctggagtgatgtttaagccaatgtcagggcaaggcaacagtcacctggcgtcctccagcaccttgt
aatgcatatgagctcgggagaccagtacttaagttggaggcccgaggagctggcgaggggcgt
tcgtcctgggagctgcacttgcctcggtcgccggttcaccggaccgcaggctcccggggcaggggccgg
ggccagagctcgcgtgctggcgggacatgcgtgcgtgcctctaacctcgggtgtgctctttccagggtggcc
cgccggtttctgagccttctgcctgcggggacacgggtctgcacctgcccgcggccacggaccatgaccatg
acctccacaccaaagcatctgggatggcctactgcatcagatccaagggaacgagctggagcccctgaac
cgccgcagctcaagatccccctggagcggccccctgggcgaggtgtacctggacagcagcaagcccgcct
gtacaactaccccgagggcgccgcctacgagttcaacgccgcggccgccaacgcgcaggctacggtc
agaccggcctcccctacggccccgggtctgaggtcgggcgttcggctccaacggcctggggggttcccccc
actcaacagcgtgtctccgagcccgtgatgctactgcacccgcggccgcagctgtcgcttctgcagcccc
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ggcattctacaggccaaattcagataatcgacgccaggggtggcagagaaagattggccagtaccaatgacaa
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ttatggagtctggtcctgtgaggggtgcaaggccttctcaagagaagtattcaaggacataacgactatatgtgc
cagccaccaaccagtgcaccattgataaaaacaggaggaagagctgccaggcctgcgggtccgcaaattg
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gcgccagagagatgatggggagggcaggggtgaagtgggtctgctggagacatgagagctgccaaccttt
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tcagtgccttgttgatgctgagcccccatactctattccgagtatgatcctaccagacccttcagtgaagcttga
tgatgggcttactgaccaacctggcagacagggagctggttcacatgatcaactgggccaagaggggtgccag
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gaaaatgtgtagagggcatggtggagatcttcgacatgctgctggctacatcatctcggttccgcatga
tgaatctgcaggagaggagttgtgtgcctcaaatctatttttgcttaattctggagtgtacacattct
gtccagcaccctgaagtctctggagaggaaggaccatatccaccgagtcctggacaagatcacaga
cactttgatccacctgatggccaaggcaggcctgacctgcagcagcagcaccagcggctggccca
gctcctcctcatcctctccacatcaggcacatgagtaacaaaggcatggagcatctgtacagcatgaagt
caagaacgtggtgcccctctatgacctgctgctggagatgctggacgccaccgcctacatgcgccactagc
cgtggaggggcatccgtggaggagacggaccaaaagccacttgccactgcgggctctactcatgcattcct
gcaaaagtattacatcacgggggagggcagagggttccctgccacagtctgagagctccctggc

_____ 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 ERα exon 7

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttggcgtcctccagcaccttgt
aatgcatatgagctcgggagaccagtacttaaagttggaggcccgaggagctggcgaggggcgt
tcgtcctgggagctgcacttgctccgtcgggtcgccggcttcaccggaccgcaggctcccggggcaggggccgg
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acctccacaccaaagcatctgggatggcctactgcatcagatccaagggaacgagctggagcccctgaac
cgccgcagctcaagatccccctggagcggccccctgggagaggtgtacctggacagcagcaagcccgcct
gtacaactaccccgagggcgccgctacgagttcaacgcgcggccgccaacgcgcaggcttacggtc
agaccggcctcccctacggccccgggtctgaggtcggcggttcggctccaacggcctggggggttcccccc
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ggcattctacaggccaaattcagataatcgacgccagggtggcagagaaagattggccagtaccaatgacaa
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ttatggagctgtgtcctgtgaggggtgcaaggccttctcaagagaagtattcaaggacataacgactatatgtgc
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gcgccagagagatgatggggagggcaggggtgaagtgggtctgctggagacatgagagctgccaaccttt
ggccaagcccgtcatgatcaaacgctctaagaagaacagcctggcctgtccctgacggccgaccagatgg
tcagtgccttgttgatgctgagcccccatactctattccgagtatgatcctaccagacccttcagtgaagcttga
tgatgggcttactgaccaacctggcagacagggagctggttcacatgatcaactgggccaagaggggtgccag
gctttgtgattgacctccatgatcaggtccaccttctagaatgtgcctggctagagatcctgatgattggtctgt
ctggcgtccatggagcaccagtgaaagctactgtttgctcctaactgtcttggacaggaaccagggaataatg
tgtagaggcatggtggagatcttcgacatgctgctggctacatcatctcggttccgcatgatgaatctgcagg
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gaagtctctggagaggaaggaccatatccaccgagtcctggacaagatcacagacactttgatccac
ctgatggccaaggcaggcctgaccctgcagcagcagcaccagcggctggccagctcctcctcatc
ctctccacatcaggcacatgag***taacaaaggcatggagcatctgtacagcatgaagtgaagaacg***
tggtgccctctatgacctgctgctggagatgctggacgccaccgcctacatgcgccactagccgtggaggg
gcatccgtggaggagacggaccaaagccactggccactgcgggtctacttcacgcattccttgc aaaagta
ttacatcacgggggaggcagaggggttccctgccacagtctgagagctccctggc

_____ 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

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgtcctccagcaccttgt
aatgcatatgagctcgggagaccagtagtactaaagttggaggcccgaggagctggcggagggcgt
tcgtcctgggagctgcacttgctccgtcgggtcgccggctcaccggaccgcaggctcccggggcagggccgg
ggccagagctcgcgtgctggcgggacatgcgtgcgtgcctctaacctcggtgtgctctttccagggtggcc
cgccggtttctgagccttctgccctgcggggacacgggtctgcacctgcccgcggccacggaccatgaccatg
accctccacaccaaagcatctgggatggccctactgcatcagatccaagggaacgagctggagcccctgaac
cgtccgcagctcaagatccccctggagcggcccctgggcgaggtgtacctggacagcagcaagcccgcgt
gtacaactaccccgagggcgccgcctacgagttaacgcgcggccgcccgaacgcgcagggttacggtc
agaccggcctcccctacggccccgggtctgagggtgcggcgttcgggtccaacggcctggggggttcccccc
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acggccagcagggtgccctactacctggagaacgagcccagcggctacacgggtgcgcgaggccggcccgc
ggcattctacaggccaaattcagataatcgacgccagggtggcagagaaagattggccagtagcaatgacaa
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cagccaccaaccagtgaccattgataaaaacaggaggaagagctgccaggcctgccggctccgcaaatg
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gcgccagagagatgatggggagggcaggggtgaagtgggtctgctggagacatgagagctgccaacctt
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aagtctctggagaggaaggaccatatccaccgagtcctggacaagatcacagacactttgatccacct
gatggccaaggcaggcctgaccctgcagcagcagcaccagcggctggcccagctcctcctcatcct
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cctctatgacctgctgctggagatgctggacgcccaccgcctacatgcgccactagccgtggaggggcatcc
gtggaggagacggaccaaaagccactggccactgcgggtctactcatgcattccttgcaaaagtattacatc
acgggggagggcagaggggttcctgccacagtctgagagctccctggc

_____ 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

gagttgtcctggagtgatgtttaagccaatgtcagggcaaggcaacagtccttgccgctcctccagcaccttgt
aatgcatatgagctcgggagaccagtagtactaaagttggaggcccgaggagctggcggagggcgt
tcgtcctgggagctgcacttgctccgtcgggtcgccggctcaccggaccgcaggctcccggggcagggccgg
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cagccaccaaccagtgacaccattgataaaaacaggaggaagagctgccaggcctgccggctccgcaaatg
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gcttctgtgattgaccctccatgatcagggtccaccttctagaatgtgcctggctagagatcctgatgattggtcgt
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*cacatgagta***acaaaggcatggagcatctgtacagcatgaagtgaagaacgtggtgcccctctatga**
cctgctgctggagatgctggacgcccaccgcctacatgcgcccactagccgtggaggggcatccgtggagg
agacggaccaaaagccacttgccactgcgggctacttcatcgcattccttgcaaaagtattacatcacgggg
gaggcagaggggttccctgccacagtctgagagctccctggc

_____ 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

gttgacagccattatacttgcccacgaatctttgagaacattataatgacctttgtgcctcttcttgaagggtg
ttttctcagctgttatctcaagacatggatataaaaaactcaccatctagccttaattctccttctctacaa
ctgcagtcaatccatcttaccctggagcacggctccatatacataccttctcctatgtagacagccacc
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tgaagggtggcctggcggcagaccacaagcccaaatgtgttggtggccaacacctgggcacctttctcc
tttagtggtccatcgccagttatcacatctgtatgcggaacctcaaaagagtccttggtgtgaagcaagat
cgctagaacacaccttacctgtaaacag agagacactgaaaaggaagggttagtgggaaccgttgcg
ccagccctgttactggccaggtcaaagagggtatgctcacttctgcgtgtctgcagcgattacgcatcg
ggatatcactatggagctcgtgctgtgaaggatgtaaggccttttttaaagaagcattcaag gaca
taatgattatatttgtccagctacaaatcagtgtaaatcgataaaaaccggcgcaagagctgcc
aggcctgccgacttcggaagtgttacgaagtgggaatgggtgaagtgtg gctcccgagagagag
agatgtgggtaccgccttgtgcggagacagagaagtgcggacgagcagctgcactgtgccggca
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aagcaaagagggctcccagaaccacagctctcagtgacgcctggccctgaggtgaactggcccaca
gaggtcacaagctgaagcgtgaactccagtggtcaggagcctgggcttcatcttctgctgtgtggccct
catttgg

_____ primer position

bold sequence

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

B. ERβ LBD

B1. Sequence and position of the 222bp band from nested PCR of ERβLBD

gttgacagccattataacttgcccacgaatctttgagaacattataatgacctttgtgcctcttcttgaaggtgtttc
tcagctgttatctcaagacatggatataaaaaactcaccatctagccttaattctcctcctacaactgcagt
caatccatcttaccttgaggacggctccatatacatccttctcctatgtagacagccaccatgaatatcc
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tgaaggatgaaggccttttttaaagaagcattcaag* gacataatgattatattgtccagctacaaatcagt
gtacaatcgataaaaaccggcgcaagagctgccaggcctgccgacttcggaagtgttacgaagtgggaat
ggtgaagtgtg* gctccggagagagagatgtgggtaccgccttgcggagacagagaagtgccgacga
gcagctgcactgtgccggcaaggccaagagaagtggcggccacgcgccccgagtgccggagctgctgct
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gccccagtgccgcttcaccgaggcctccatgatgatgtccctgaccaagttggccgacaaggagttggtac
acatgatcagctgggccaagaagattccc* gcttgtggagctcagcctgttcgaccaagtgcggctcttg
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gtccagatcttcttgacag* **ggatgaggggaaatgcctagaaggaattctggaaatctttgacatg**
ctcctggcaactacttcaagggttcgagaggttaaaactccaacacaaagaatatctctgtgtcaaggc
catgatcctgctcaattcca* **gtatgtaccctctggtcacagcgaccaggatgctgacagcagccg**
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ggtcacaagctgaagcgtgaactccagtggtcaggagcctgggctcatcttctgctgtgtggtccctcattg
g

_____ primer position

bold sequence

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