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Oestrogen receptor mutations and their influence on breast cancer growth

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2012-03-12
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APPENDICES

Appendix I: Human Ethics Clearance Certificate

Appendix Buffers and Solutions

Buffer/Solut	Working Solution	Concentrated s solution (per litre)
Tri-acetate (TAE)	1 x : 0.04M Tris-acetate 0.001M EDTA	50 x : 242g Tris ba 57.1ml glacia acetic acid 100ml 0.5M E (pH 8.0)
Phosphate Bu Saline (PBS) [0.15M]	8g NaCl 0.2g KCl 1.15g Na_2HPO_4 0.2g KH_2PO_4 Make up to 1 litre w water.	
Sodium aceta (3M) pH 5.2	408.1g sodium ac CH_3CO_2 800ml H_2O Adjust to pH 5.2 wit acetic acid. Adjust litre with H_2O	

Appendix II: Serum preparation and its use in vitro for experiments.

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

Physiological concentrations of steroid hormones are usually in the range of 1 - 10 nM (Eisen, 1973). However, much lower concentrations of steroid have been found in some foetal calf sera (Eisen, 1973). Serum is therefore stripped of endogenous steroid prior to experiments involving response to steroid hormones. Dextran coated charcoal is used to strip endogenous hormones from foetal calf serum. This is a modification of the method of Rae (1968).

Mix 0.02g Dextran (BDH Laboratory Supplies, Poole, England) with 2g activated charcoal (Merck, Darmstadt, Germany) in 50ml FCS (Delta Biologicals, Kempton Park, South Africa).

Centrifuge for 1 hour on a benchtop centrifuge.

Pour off liquid.

Mix pellet with FCS (Delta Biologicals, Kempton Park, South Africa).

Vortex and allow to stand at 56°C for 1 hour with occasional shaking.

Centrifuge and pour off supernatant.

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 Chemical
Poole, England)
100ml methanol (SAARCHEM, Muldersdrift, South Africa
Mix and warm to 50°C
Cool to room temperature, shaking occasiona
Stand for 24 hours and filter through Whatman No. 1 fi
(Clifton, NJ, USA) before use.

C.Preparation of Giemsa Stain.

1g Giemsa powder (BDH Chemicals Ltd)
Add 66ml glycerol (SAARCHEM, Muldersdrift, South A
and heat in a water bath 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 th
1 filter paper (Clifton, NJ, USA) prior to use.

APPENDIX IV: Sequencing electropherograms of ER± exons

A. ER± exon 2

A1. ERa2 (427bp band) (forward primer)

A1. (continued) Exon 2 (427 bp spectrogram of forward primer)

A2. ERa2 (236bp band) electrophoresis (antisense primer)

B. ER± exon 3

B1. ER±3 (322 bp) by touchdown PCR (reverse primer)

C. ER± exon 4

C1a. ER±4 (555bp band) specific forward primer

C1 b E Ra4 (5 5 5 b p b a n d) t r o g r a p h e m e n t a t i o n)

C2a. Edd4 (219bp band) spectrograph (forward primer)

D. ER± exon 5

D1a. ER± exon 5 4-5 bp (band) spectrogram (forward primer)

D1a (control) ± 5 (544bp band) spectrograph (forward primer) c

D1bER±5 (544bp band) spectrograph (complementary primer

D2a. ER±5 (405bp band) spectrograph (forward primer)

D2a (contd). ER±5 (40S) spectrograph (forward component)

D2b. ER±5 (405bp band) spectrograph (complementary prime

D2b (contd). ER±5 (405bp band) spectrograph (complementa
continued

D3. ER±5 (80bp band) (complementary primer)

E. ER± exon 6.

E1a. ER±368 (6p band) spectrogram aprrodepriim

E1a. (cont'd) E3a6 (388bp band) spectrograph (forward primer) co

E1b. ER±6 (388bp band) spectrograph (complementary prime

E1b. (cont'd) ±6 (388bp band) spectrograph (complementary p

continued

F.ER± exon 7.
F1aERǂ (345bp band) spectrograph (forward primer)

F1a. (contd). ER±7 (345bp band) spectrophotometry (followed

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

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

APPENDIX V: Sequencing spectrographs of ER² exo

A. ER² DBD

A1a. ER² DBD (214bp band) spectrograph (forward primer)

A 1 bE Rb D B D (2 1 4 b p b a n d) s p e (c t r o n g r l a p r h e n p t r a i n y e r)

B.ERB LBD

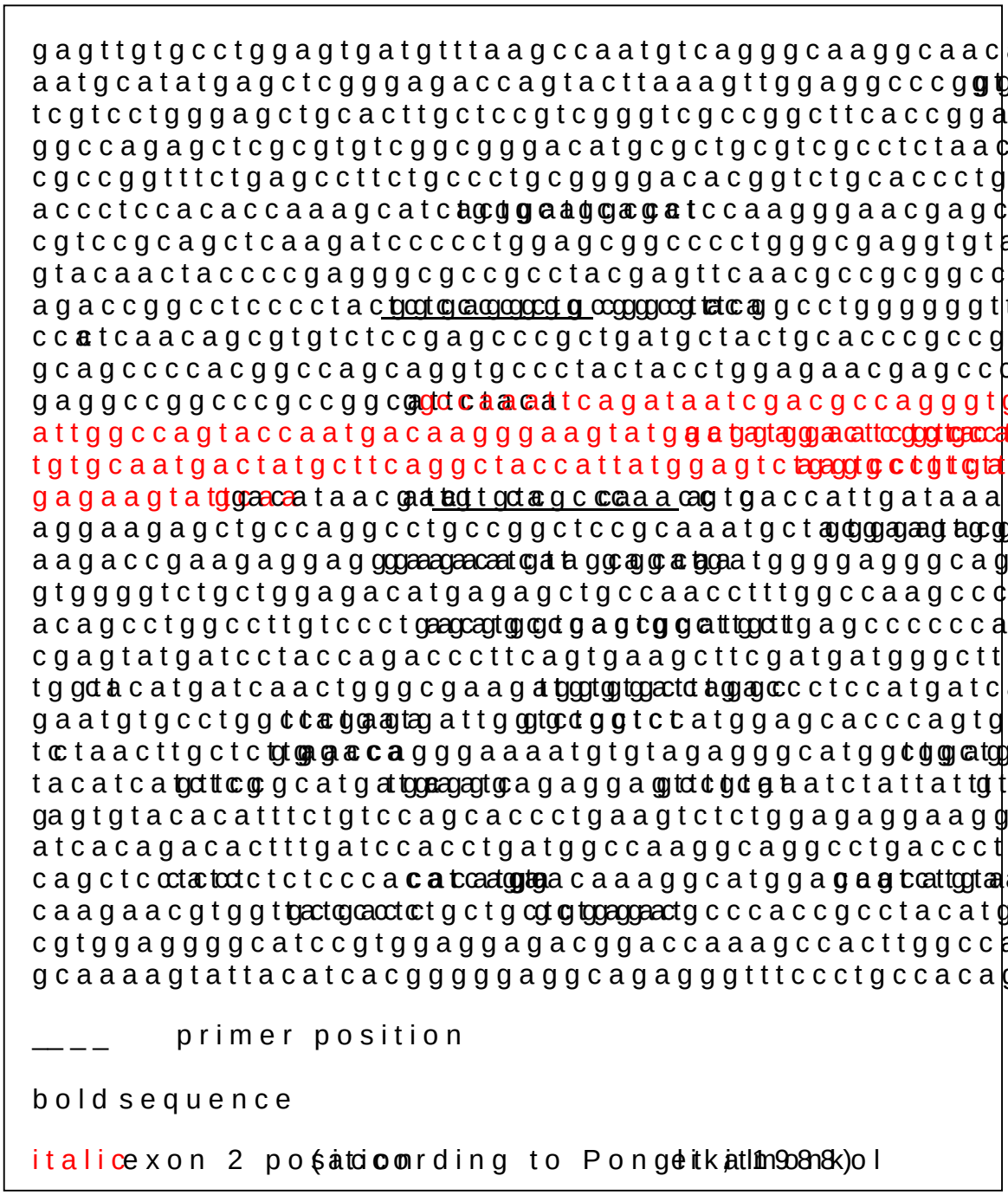
B1aERbLBD(222bp band) spectrophotometer)

B 1 bE Rd L B D (2 2 2 b p b a n d) s p e (c t r o n g r l a p r h e m p t r a i m y e r)

APPENDIX V: Positions of primers and PCR products within the ERα gene

A. ERα exon 2

A1. Sequence and position of the 427bp band from nested

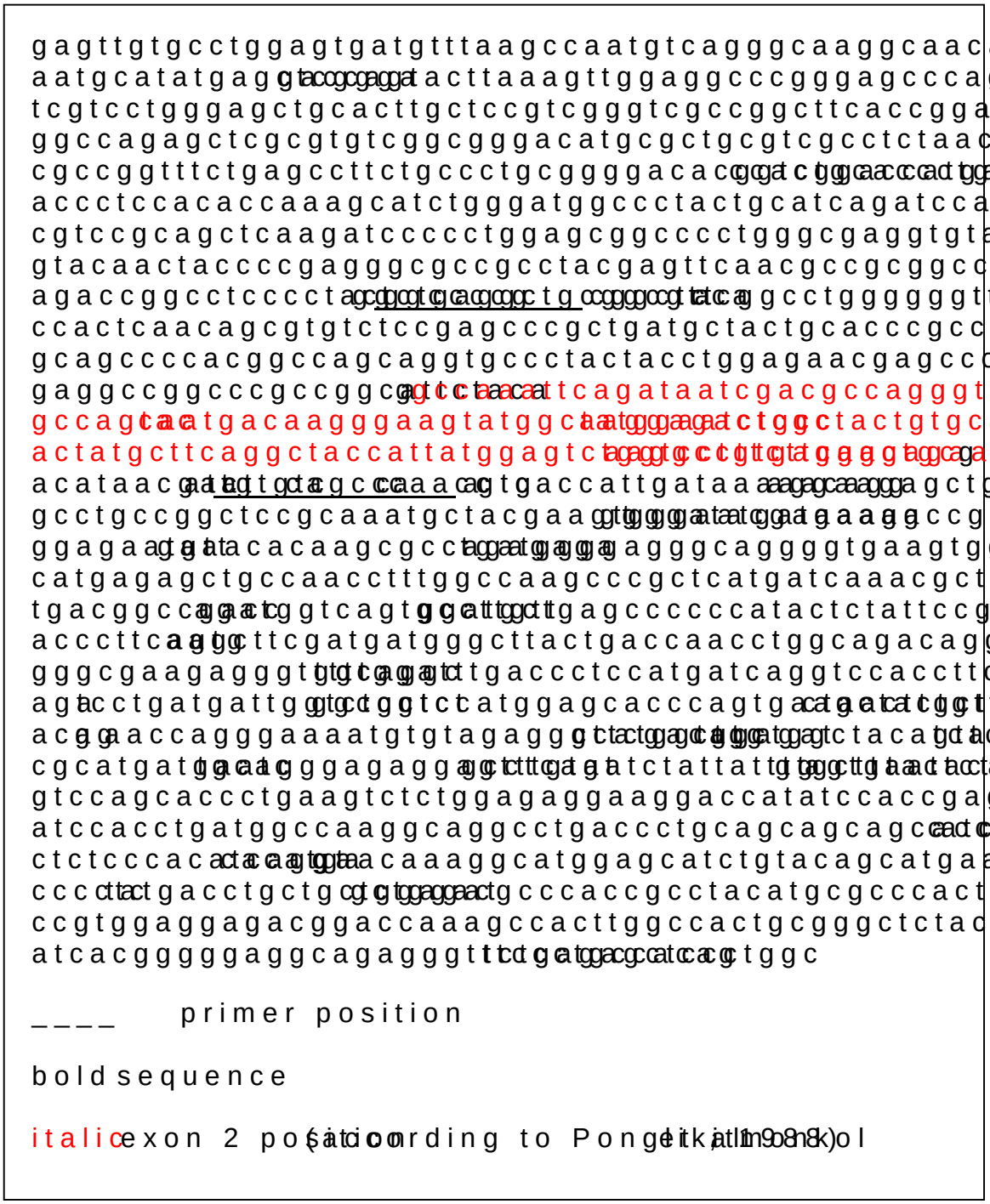


primer position

bold sequence

italic exon 2 position according to Ponglikitmongkol et al. 1988

A2. Sequence and position of the 236bp band from nested



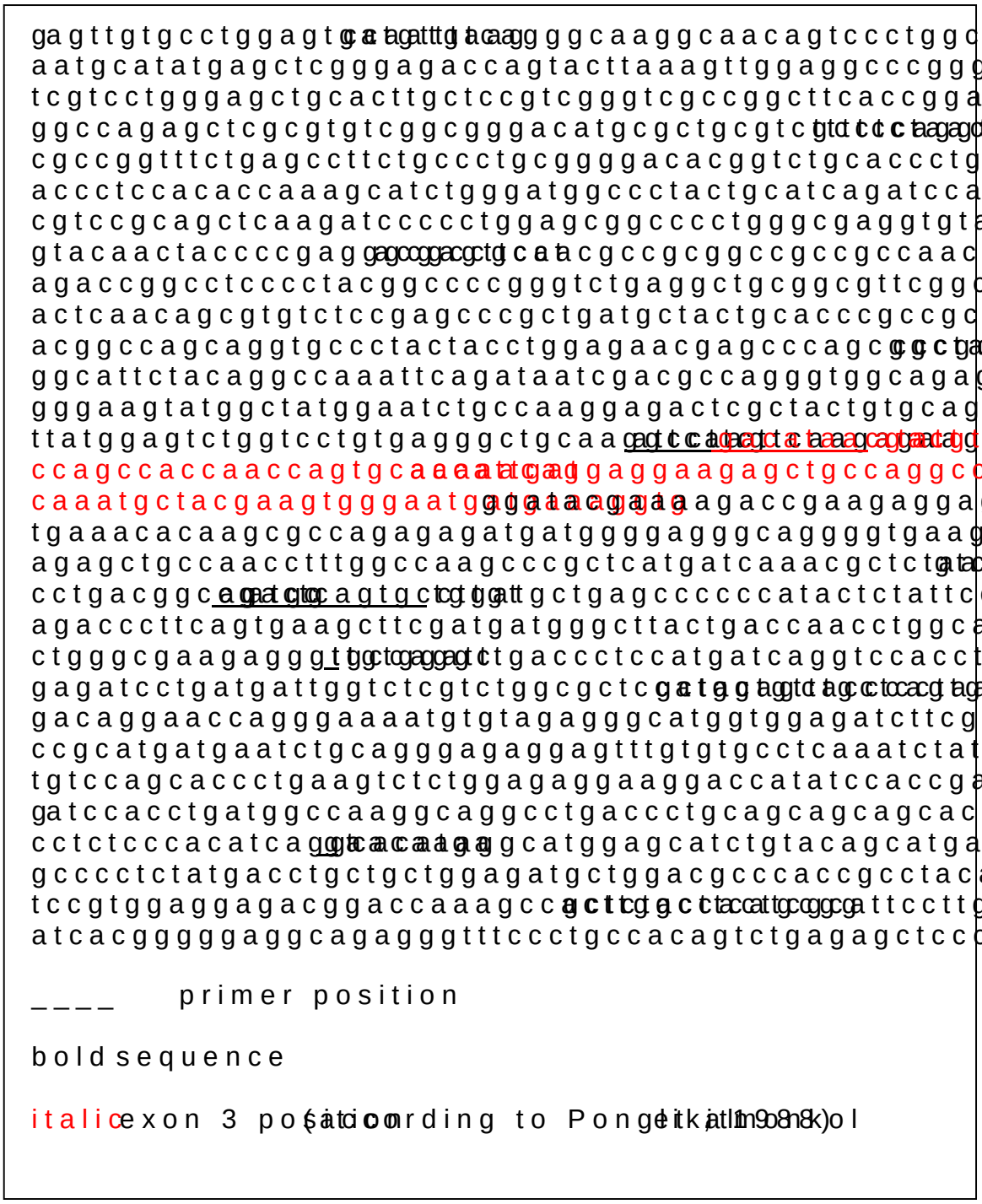
_____ primer position

bold sequence

italic exon 2 position according to Pongetti et al. 1989 (Blood)

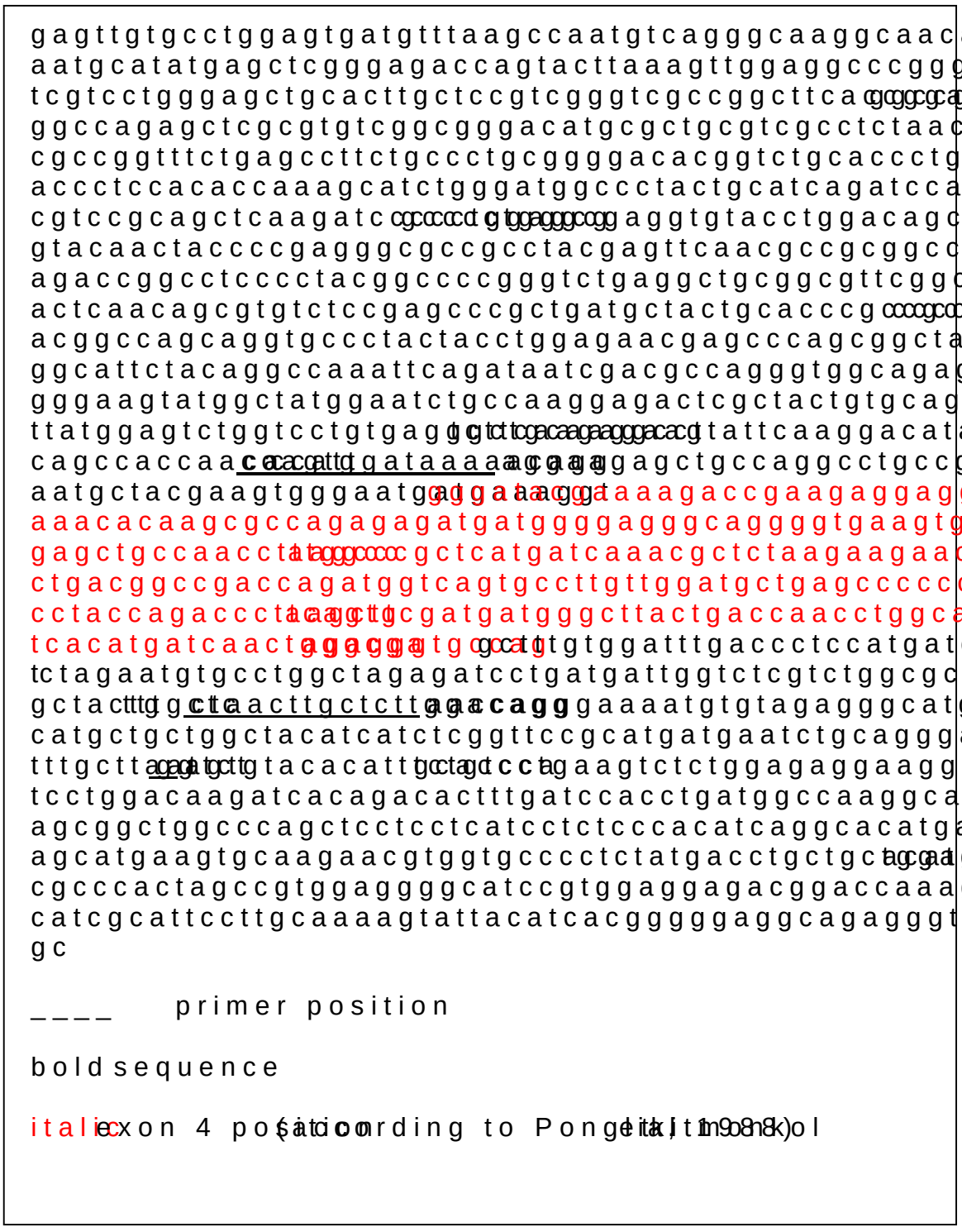
B. ER± exon 3

B1. Sequence and position of the 322bp bER± exon 3 nested

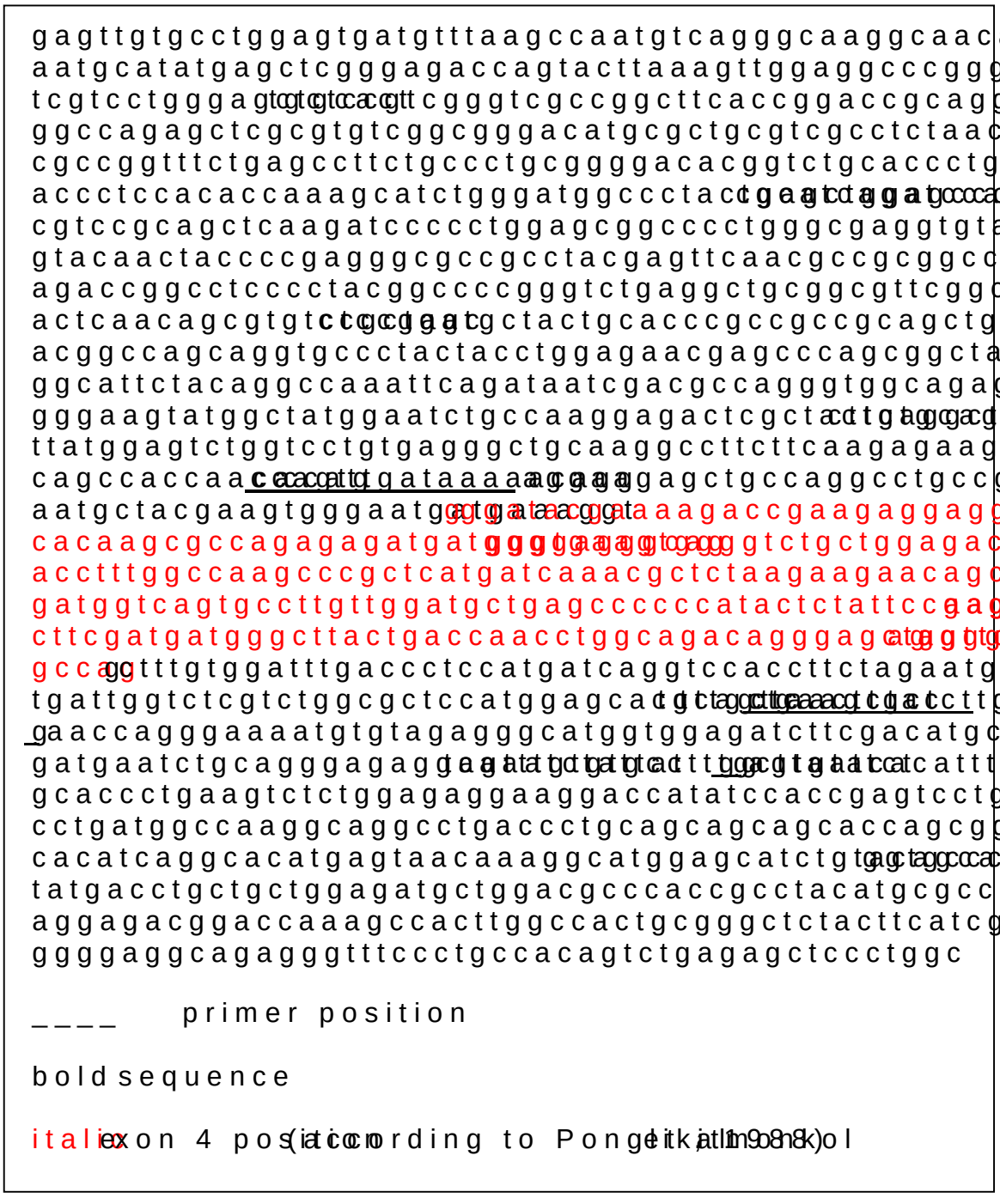


C. ER± exon 4

C1. Sequence and position of the 5' nested ER± PCR product of exon 4

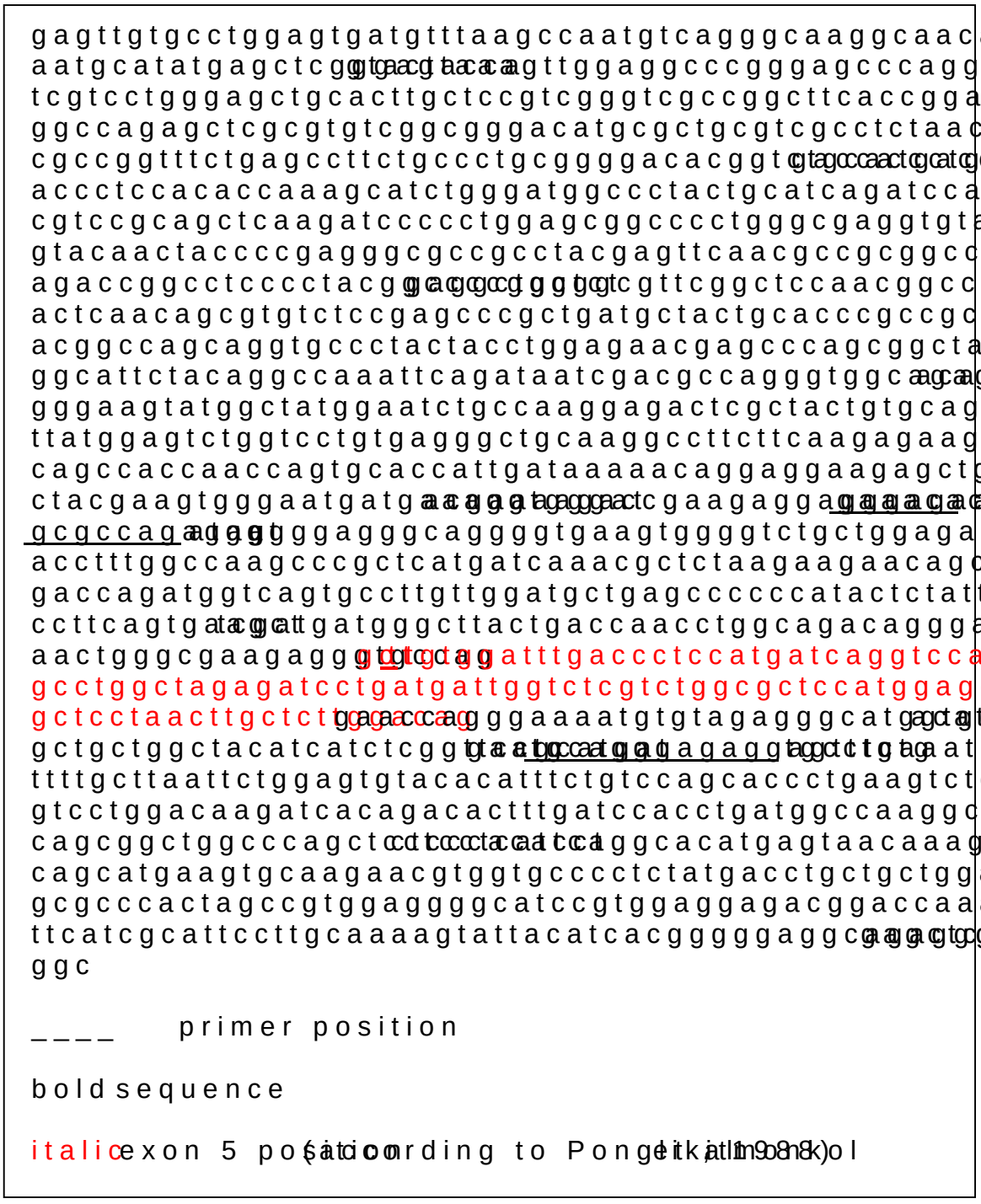


C2. Sequence and position of the 219bp band from nested



D. ER± exon 5

D1. Sequence and position of the 544bp band from nested

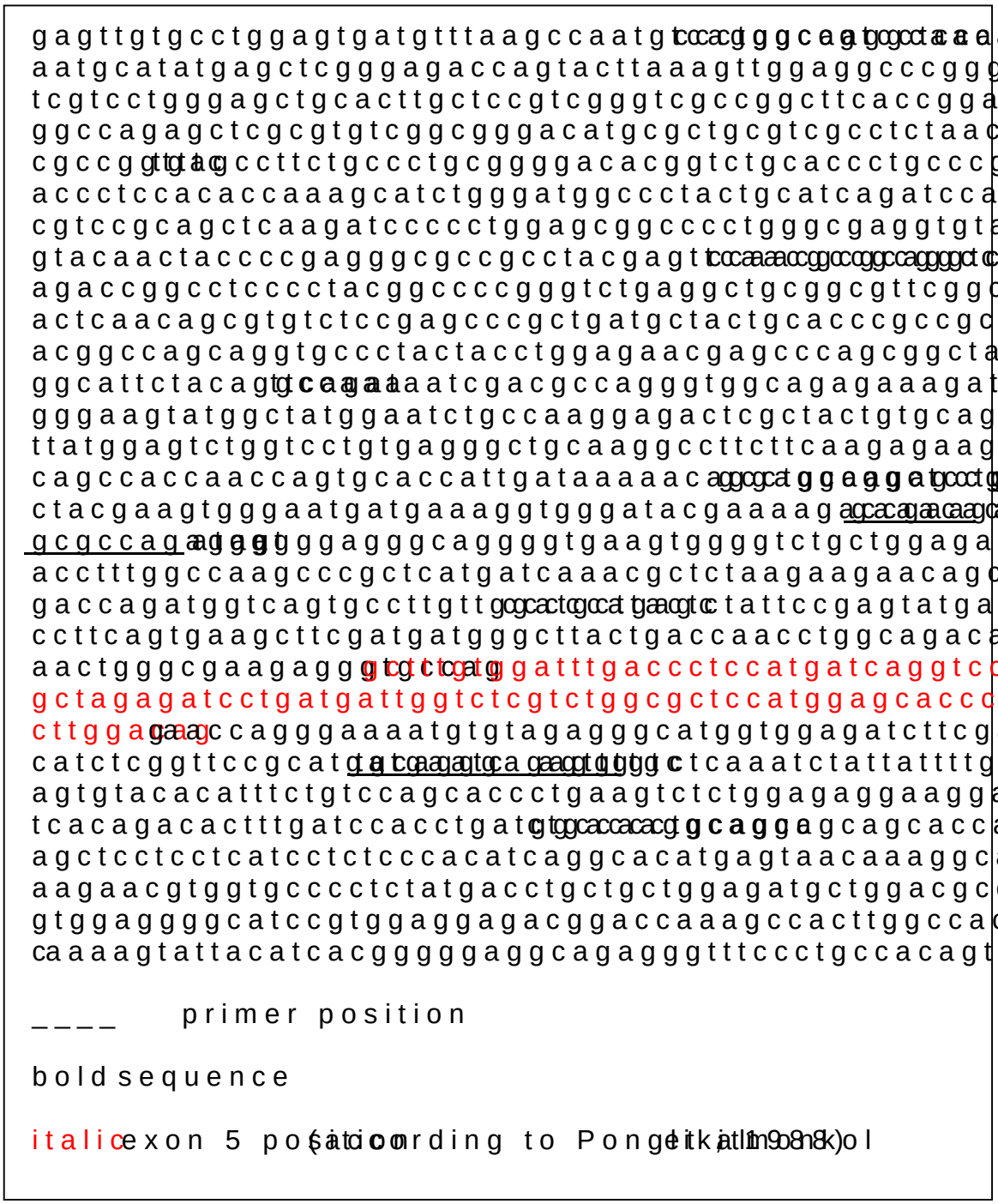


_____ primer position

bold sequence

italic exon 5 position according to Ponglikitmongkol et al 1988

D2. Sequence and position of the 405bp band from nested

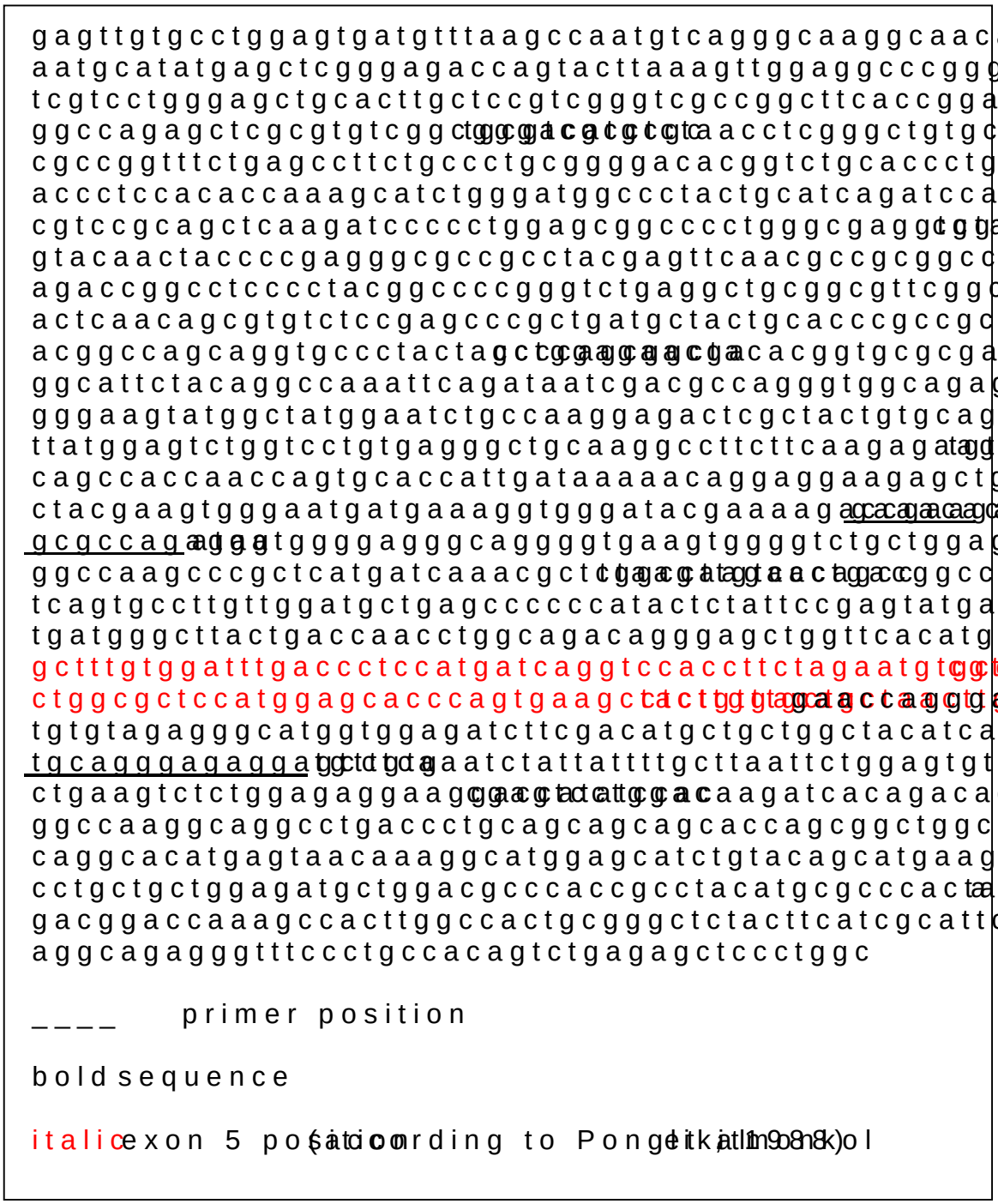


_____ primer position

bold sequence

italic exon 5 position according to Pongetti et al 1989 (ol

D3. Sequence and position of the 80bp band from exon 5 tested



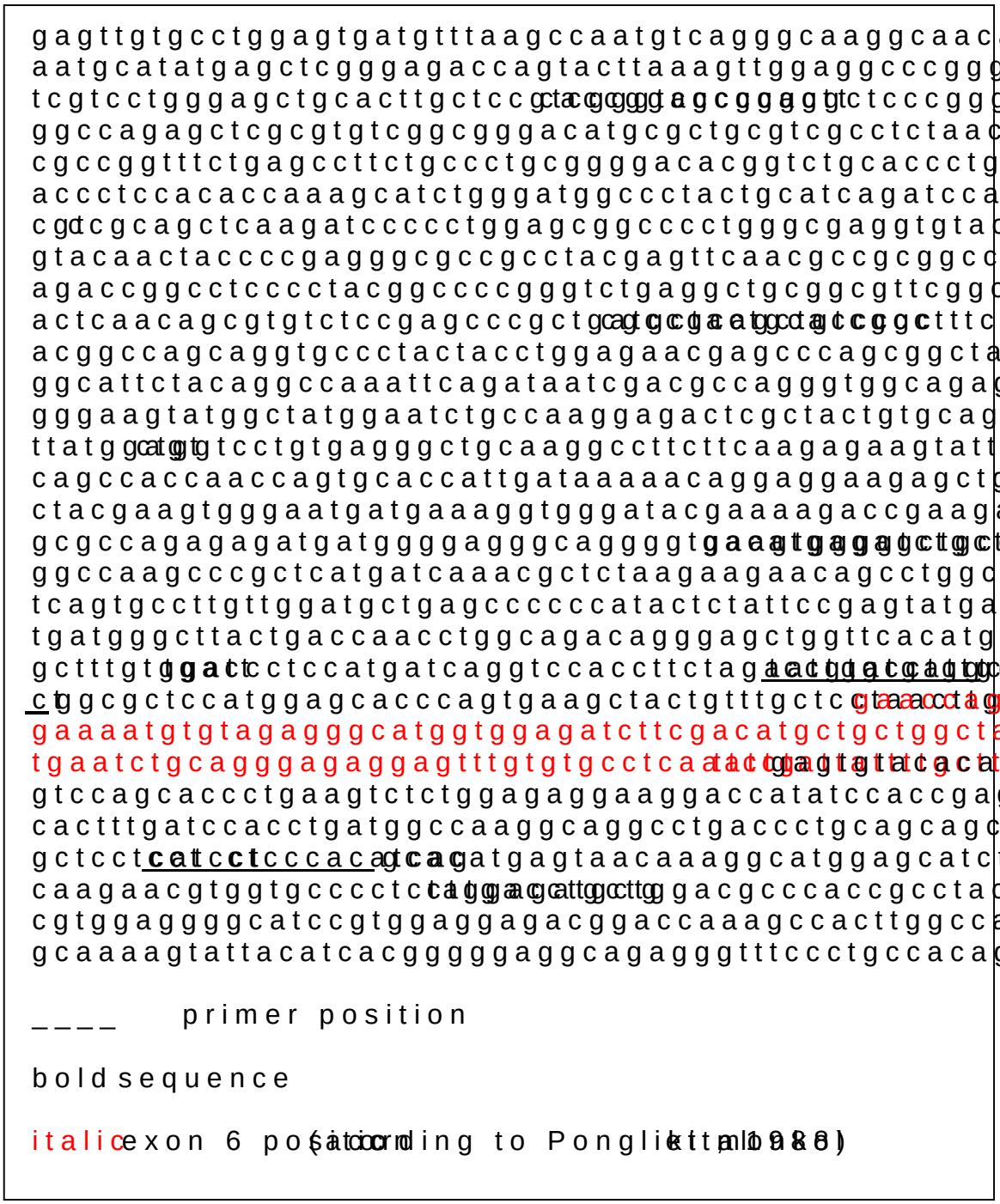
____ primer position

bold sequence

italic exon 5 position according to Pongetti et al 1989 (ol

E. ER± exon 6

E1. Sequence and position of the 388bp band from nested PCR



_____ primer position

bold sequence

italic exon 6 position according to Ponglikitmongkol et al. (1988)

F. ERα exon 7

F1. Sequence and position of the 345bp bERα exon 7 tested

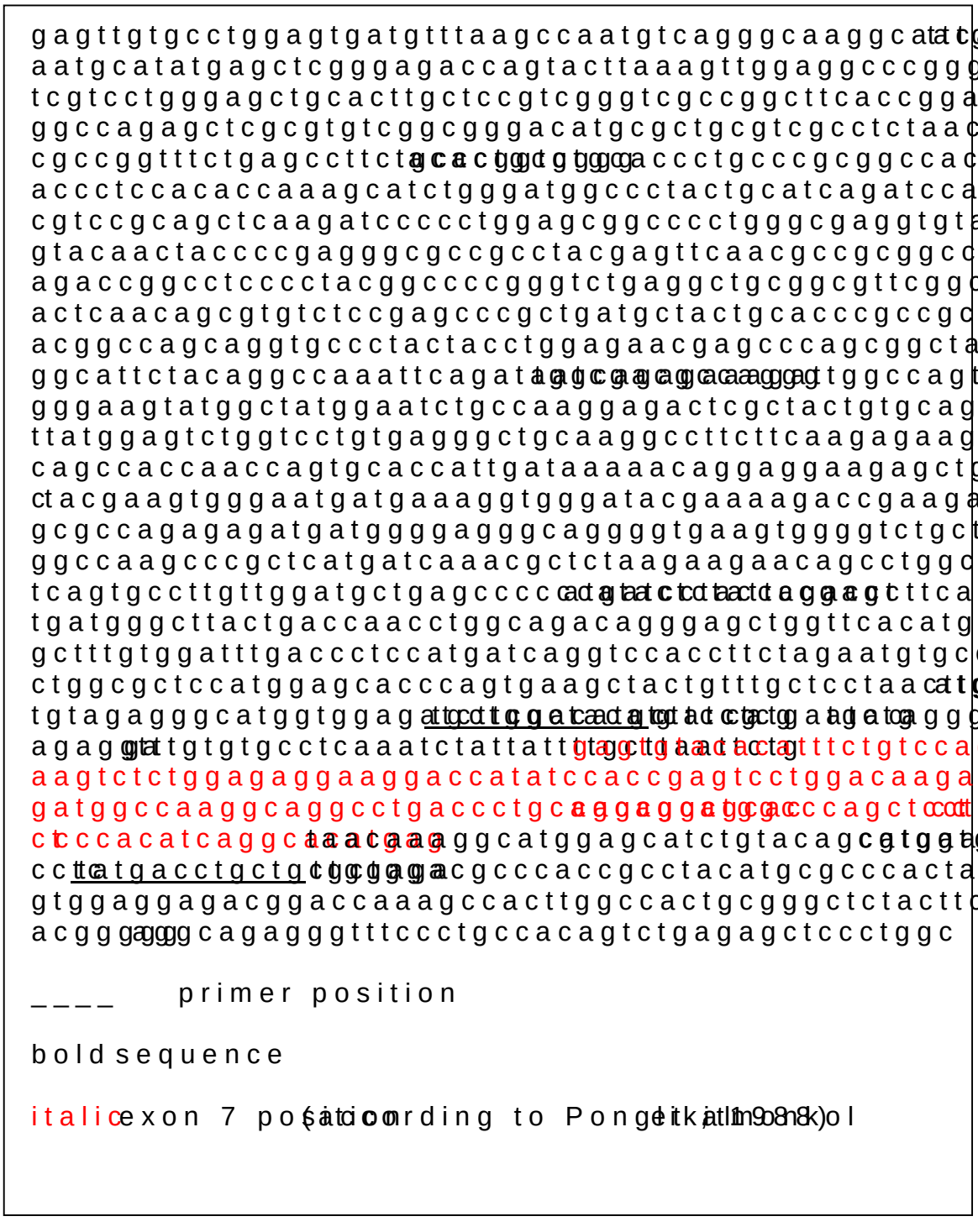


_____primer position

bold sequence

italic exon 7 position according to Ponglikitmongkol et al 1988

F2. Sequence and position of the 310bp band from nested

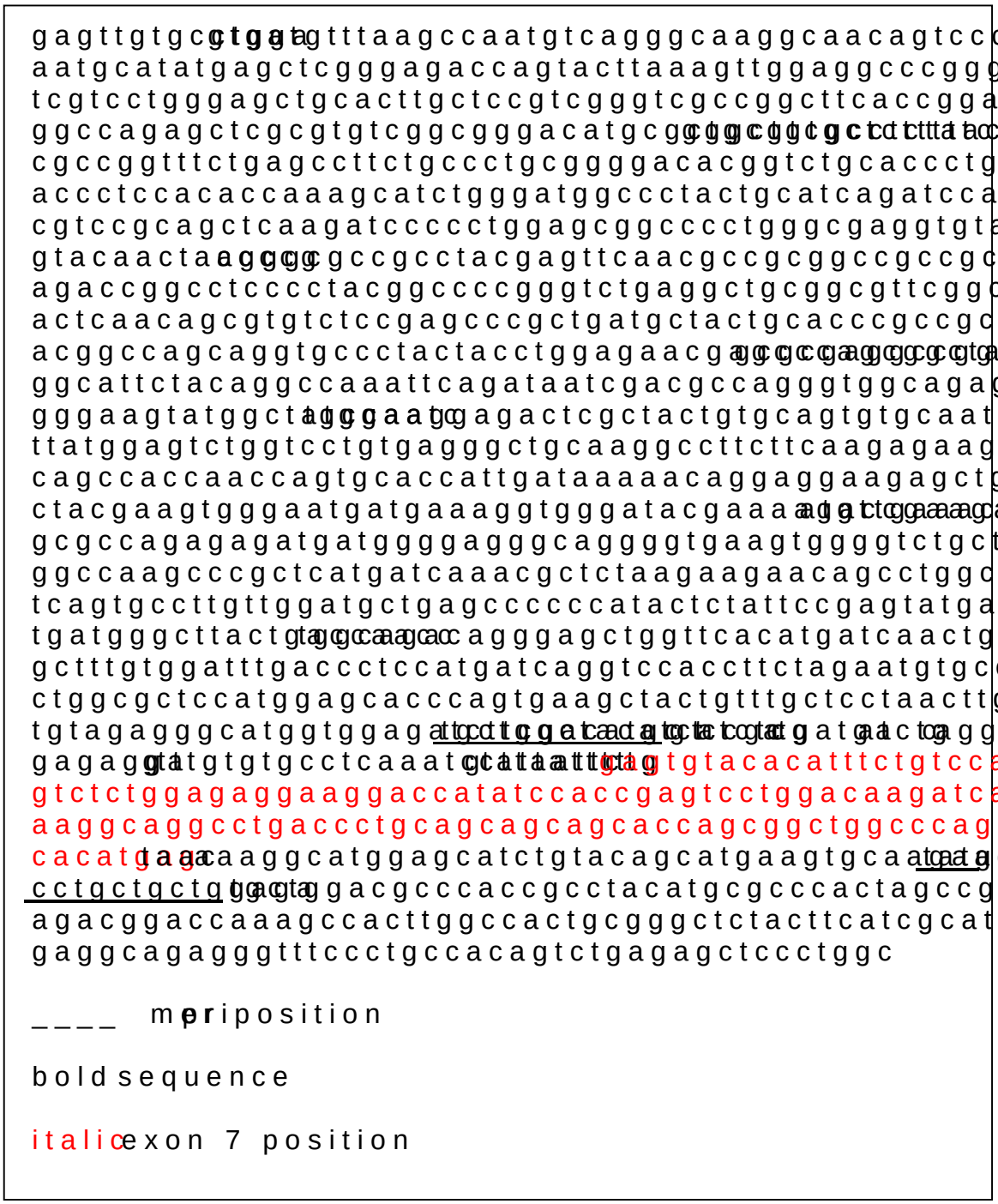


_____ primer position

bold sequence

italic exon 7 position according to Pongetti et al. 1988)

F3. Sequence and position of the 161bp band from exon 7 tested



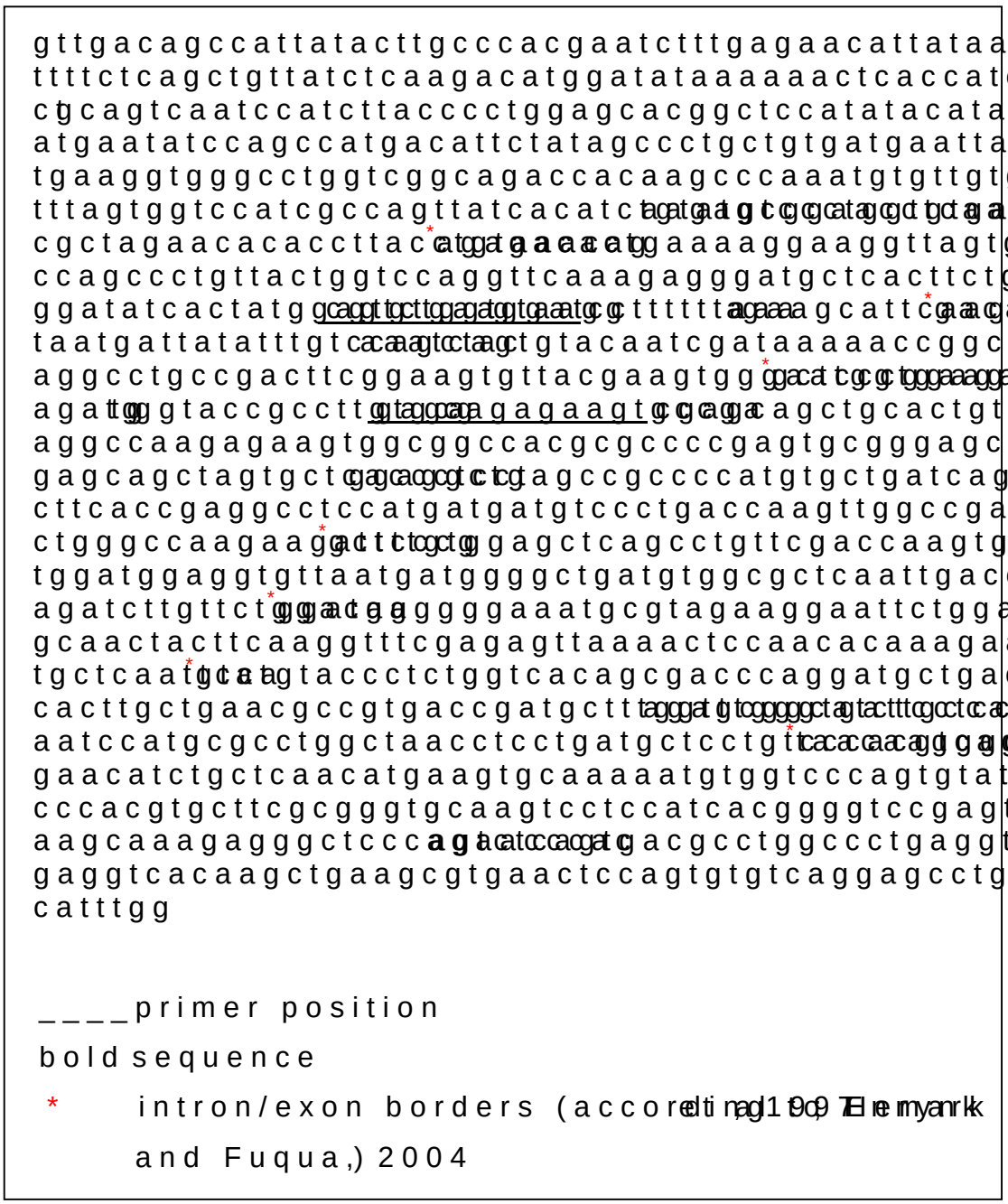
APPENDIX VII: Positions of primers and PCR prod

within the ER² gene.

A. ER² DBD

A1. Sequence and position of the 214bp band from neste

ER²DBD



B. ER² LBD

B1 Sequence and position of the 222bp band from nested P



____ primer position

bold sequence

* intron/exon borders (according to Ensembl 19.1, Fuqua, 2004)