The American Journal of Human Genetics

Supplemental Data

TFIIH Subunit Alterations Causing Xeroderma Pigmentosum and Trichothiodystrophy Specifically Disturb Several Steps during Transcription

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Figure S1

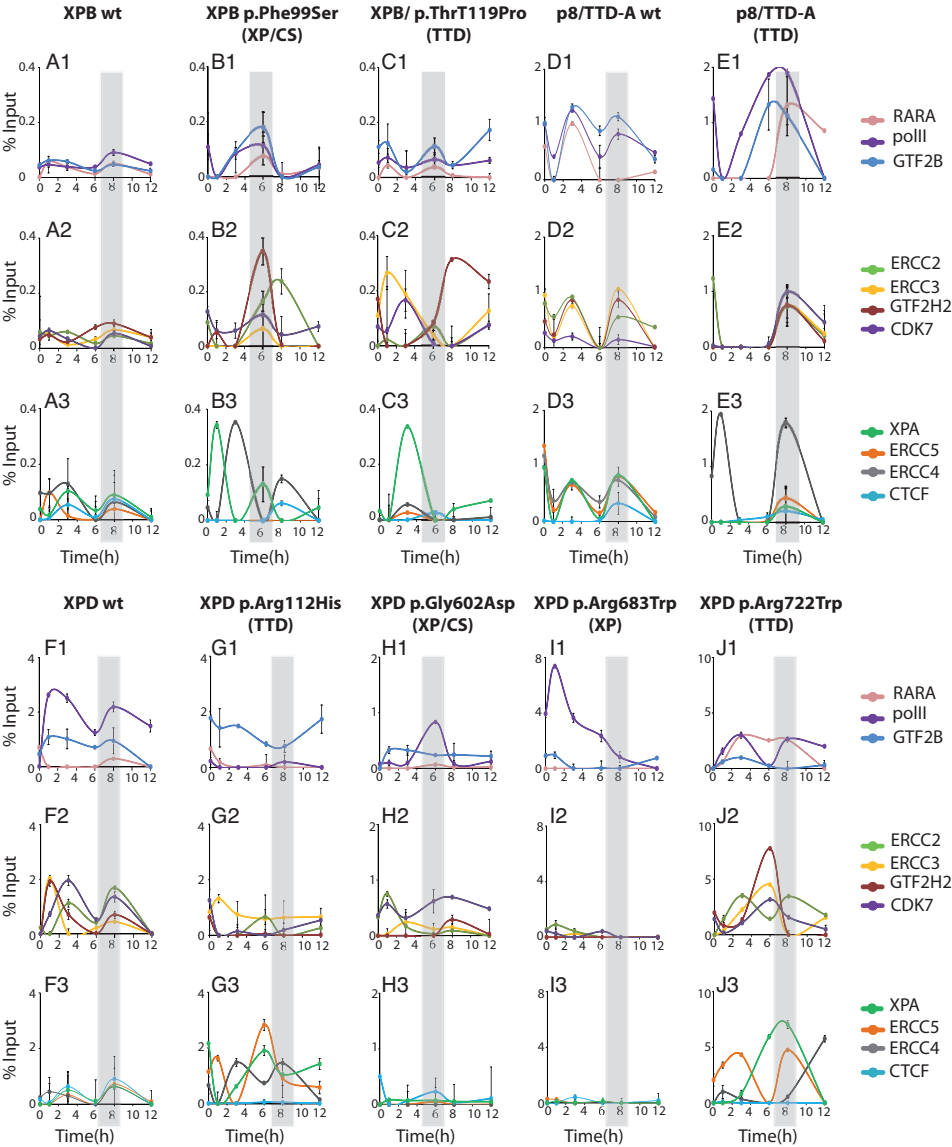

XPB wt
XPB p.Phe99Ser (XP/CS)
XPB/ p.ThrT119Pro (TTD)
p8/TTD-A wt
p8/TTD-A (TTD)
A1
B1
C1
D1
E1
A2
B2
C2
D2
E2
A3
B3
C3
D3
E3
% Input
Time(h)
RARA
polII
GTF2B
ERCC2
ERCC3
GTF2H2
CDK7
XPA
ERCC5
ERCC4
CTCF
XPD wt
XPD p.Arg112His (TTD)
XPD p.Gly602Asp (XP/CS)
XPD p.Arg683Trp (XP)
XPD p.Arg722Trp (TTD)
F1
G1
H1
I1
J1
F2
G2
H2
I2
J2
F3
G3
H3
I3
J3

Figure S2

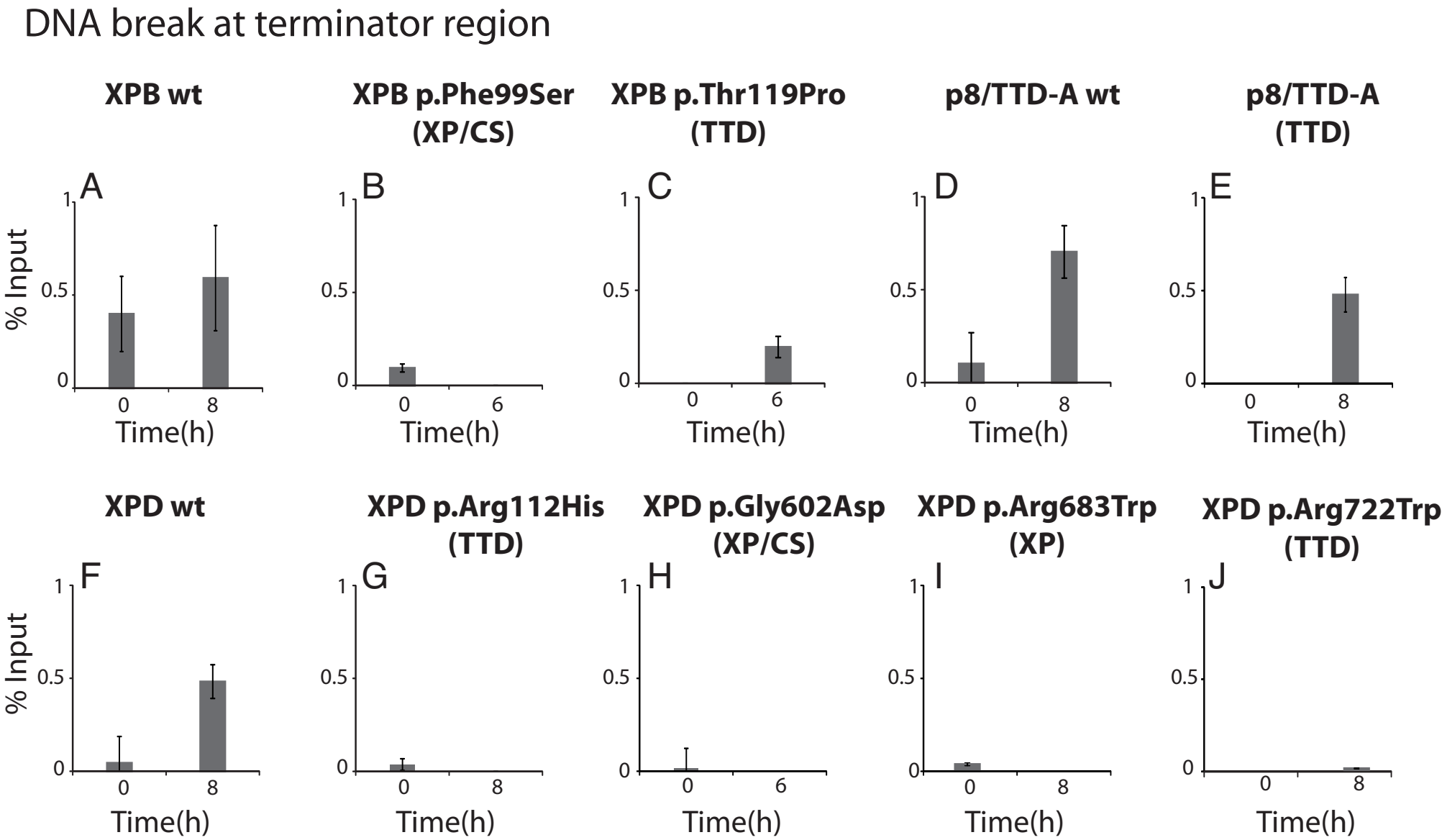
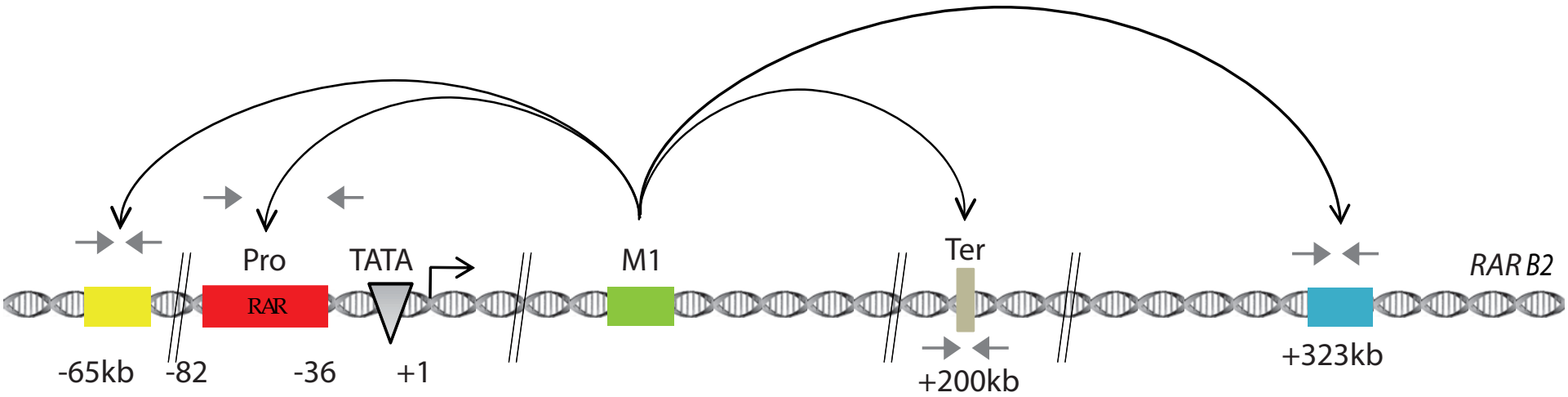
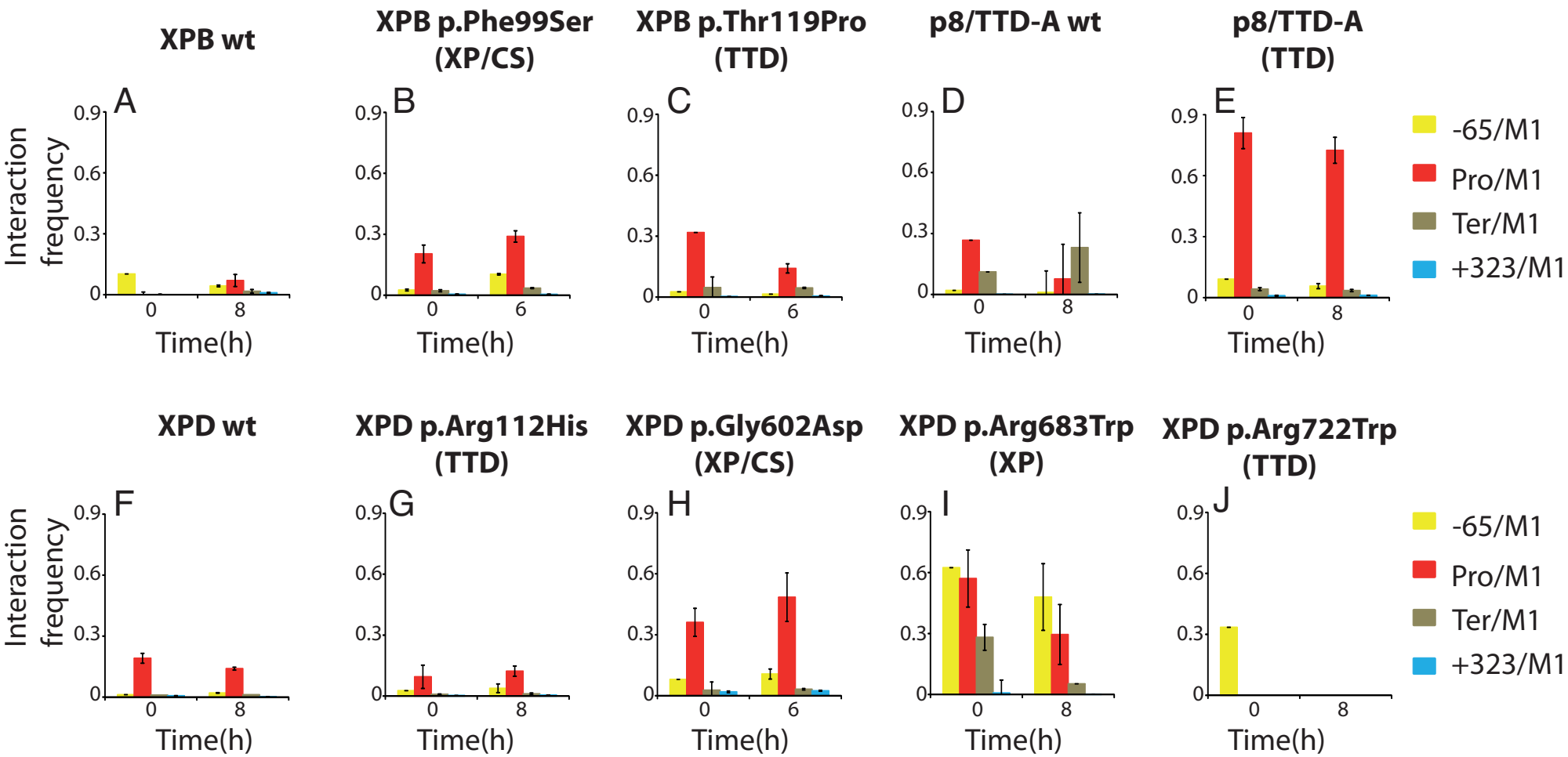

DNA break at terminator region
XPB wt
XPB p.Phe99Ser (XP/CS)
XPB p.Thr119Pro (TTD)
p8/TTD-A wt
p8/TTD-A (TTD)
XPD wt
XPD p.Arg112His (TTD)
XPD p.Gly602Asp (XP/CS)
XPD p.Arg683Trp (XP)
XPD p.Arg722Trp (TTD)
A
B
C
D
E
F
G
H
I
J
% Input
Time(h)
0
0.5
1
6
8

Figure S3


Pro
TATA
M1
Ter
RAR B2
RAR
-65kb
-82
-36
+1
+200kb
+323kb


XPB wt
XPB p.Phe99Ser (XP/CS)
XPB p.Thr119Pro (TTD)
p8/TTD-A wt
p8/TTD-A (TTD)
XPD wt
XPD p.Arg112His (TTD)
XPD p.Gly602Asp (XP/CS)
XPD p.Arg683Trp (XP)
XPD p.Arg722Trp (TTD)
A
B
C
D
E
F
G
H
I
J
Interaction frequency
Time(h)
-65/M1
Pro/M1
Ter/M1
+323/M1

Supplemental Figure Legends

Figure S1. Transcriptional machinery and NER factors recruitment on the *RARB2* terminator are disturbed in cells bearing mutations in genes encoding TFIIH subunits

ChIP monitoring the t-RA-dependent recruitment of RARA, pol II, GTF2B (panels A1-J1), ERCC3, ERCC2, GTF2H2, CDK7 subunits of TFIIH (panels A2-J2) and XPA, ERCC5, ERCC4, CTCF (panels A3-J3) on the *RARB2* terminator; Each series of ChIP is representative of at least two independent experiments. Values are expressed as percentage of the input. Error bars represent standard deviation.

Figure S2. Mutations in genes encoding TFIIH subunits dysregulated DNA breaks on the *RARB2* terminator

(A-J) Detection of DNA breaks at *RARB2* terminator at 0 and either at 6 or 8 hours post t-RA treatment depending on the formation of the transcriptional machinery corresponding to RNA expression peak (see Figure 2 shadowed areas). Each series of BioChIP is representative of three independent experiments and values are expressed as percentage of the input. Error bars represent standard deviation.

Figure S3. 3C Controls for TFIIH mutated cells

(Upper panel) Schematic representation of the quantitative chromatin conformation capture (q3C). One probe was designed at the *RARB2* intronic region (M1) to investigate the associations between the different elements including upstream (-65 kb), promoter (Pro), terminator (Ter), and downstream (+323 kb) regions as indicated by the black arrows.

(Lower panels, A-J) q3C assays were performed using crosslinked and HindIII-digested chromatin from all the cells as indicated at 0 and either at 6 or 8 hours post t-RA treatment depending on the formation of the transcriptional machinery corresponding to RNA expression peak (see Figure 2 shadowed areas). The bar chart (y axis) shows the enrichment of PCR product (%) normalized to the enrichment within human *ERCC3* (=100%). Each PCR was performed at least three times. Signals were normalized to the total amount of DNA used, estimated with an amplicon located within a HindIII fragment in *RARB2*. Error bars represent standard deviation.

Table S1. List of the primers used in the study.

Primers	Forward	Reverse
mRNA		
GAPDH	AGCTCACTGGCATGGCCTTC	ACGCCTGCTTCACCACCTTC
RARβ2	CCAGCAAGCCTCCATGTTC	TACACGCTCTGCACCTTTAGC
ChIP		
RARβ2 Promoter	TGGTGATGTCAGACTAGTTGGGTC	GCTCACTTCCTACTACTTCTGTCAC
RARβ2 Terminator	TGTTTGTGCTCTTTGGGCACT	CGGTCGGGCTAGGAAACAAGTAAA
3C primers		
-65	CCTGGCAATTGAAACATGAAAGT	
Pro	TCCAAAGATGCCTATTAAGTTGTAAGAG	
M1	AGCAGCAAAATGCAGGCTTTA	TGACACCAGTGAAAAGGAAGCA
Ter	AAGATGCAGTTTGAGAGCATC	CTGGGCAACATGAAATAAAAGATG
323	CCAAACAATTTTCTTCATGGTCATT	
RARβ2 promoter	CAGACTAGTTGGGTCATTTGAAGGT	TTGAATTGCCTAATATATGCGAGTGA
XPB	CGGTGAGGTGAGTTTGTGGAAT	AGGATCTCTGTTTAATGGAAAAGCTT
3C Probes		
Ter probe	6[FAM]TTGCTCTTTCTGATGCTCTCAAA[TAM]	
M1 probe	6[FAM]CAGTACAGTCAAGGTGGCCCGTCT[TAM]	
RARβ2 probe	6[FAM]AGCCCGGGTAGGGTTCACCGAAAG[TAM]	
XPB probe	6[FAM]AAGGATGAAGGCGTGATCCGACTCTG[TAM]	