TWO ESSENTIAL SPLICE LARIAT BRANCH POINT SEQUENCES IN ONE INTRON IN HUMAN *XPC* DNA REPAIR GENE

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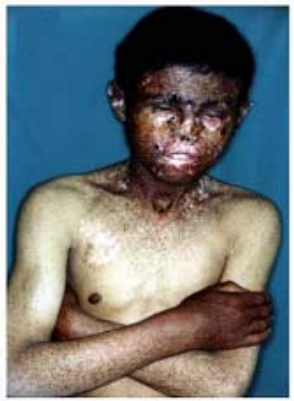
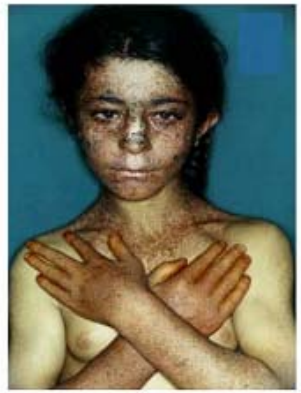
SEVERELY-AFFECTED XP SIBLINGS FROM TURKEY IN FAMILY A

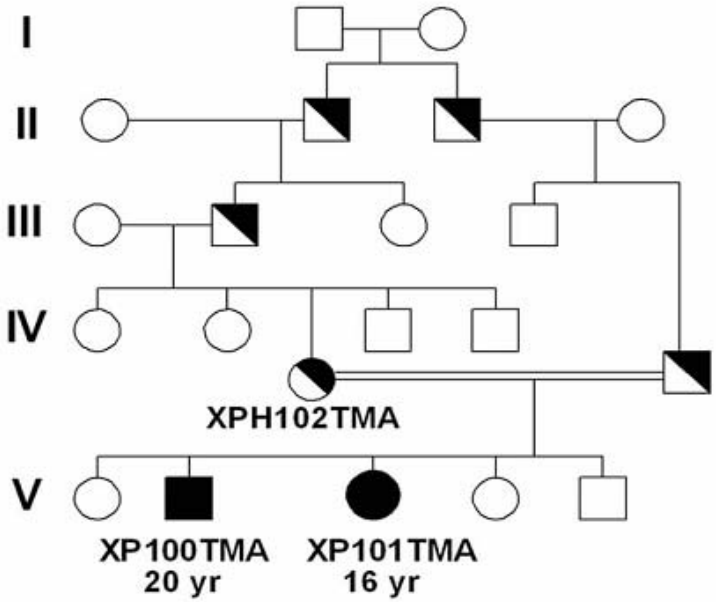


XP100TMA

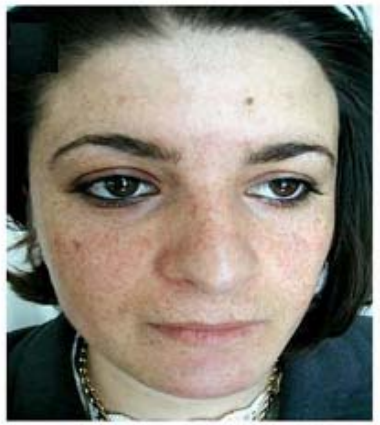


XP101TMA

Multiple skin cancers in both patients



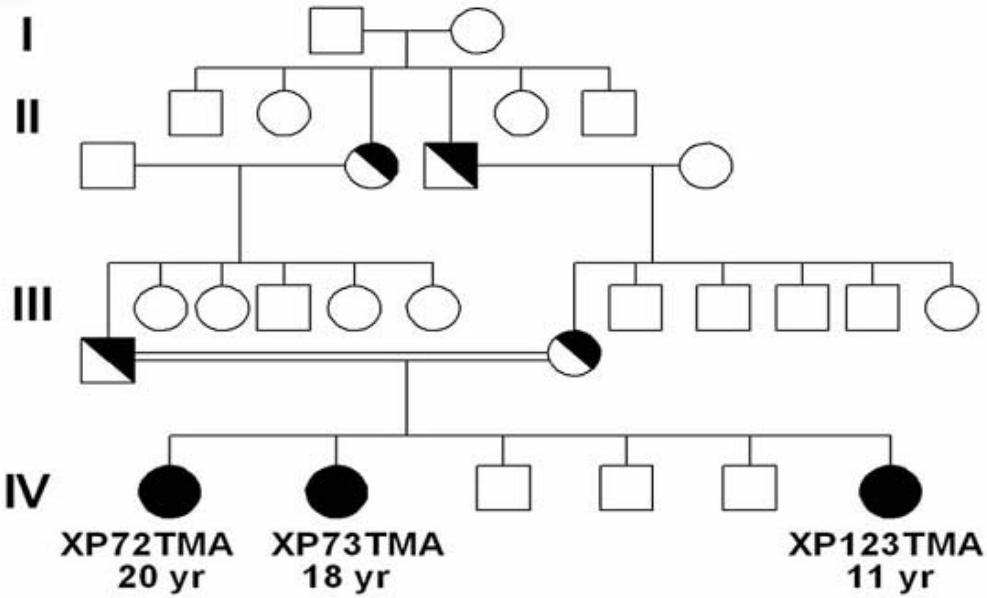
MILDLY-AFFECTED XP SIBLINGS FROM TURKEY IN FAMILY B



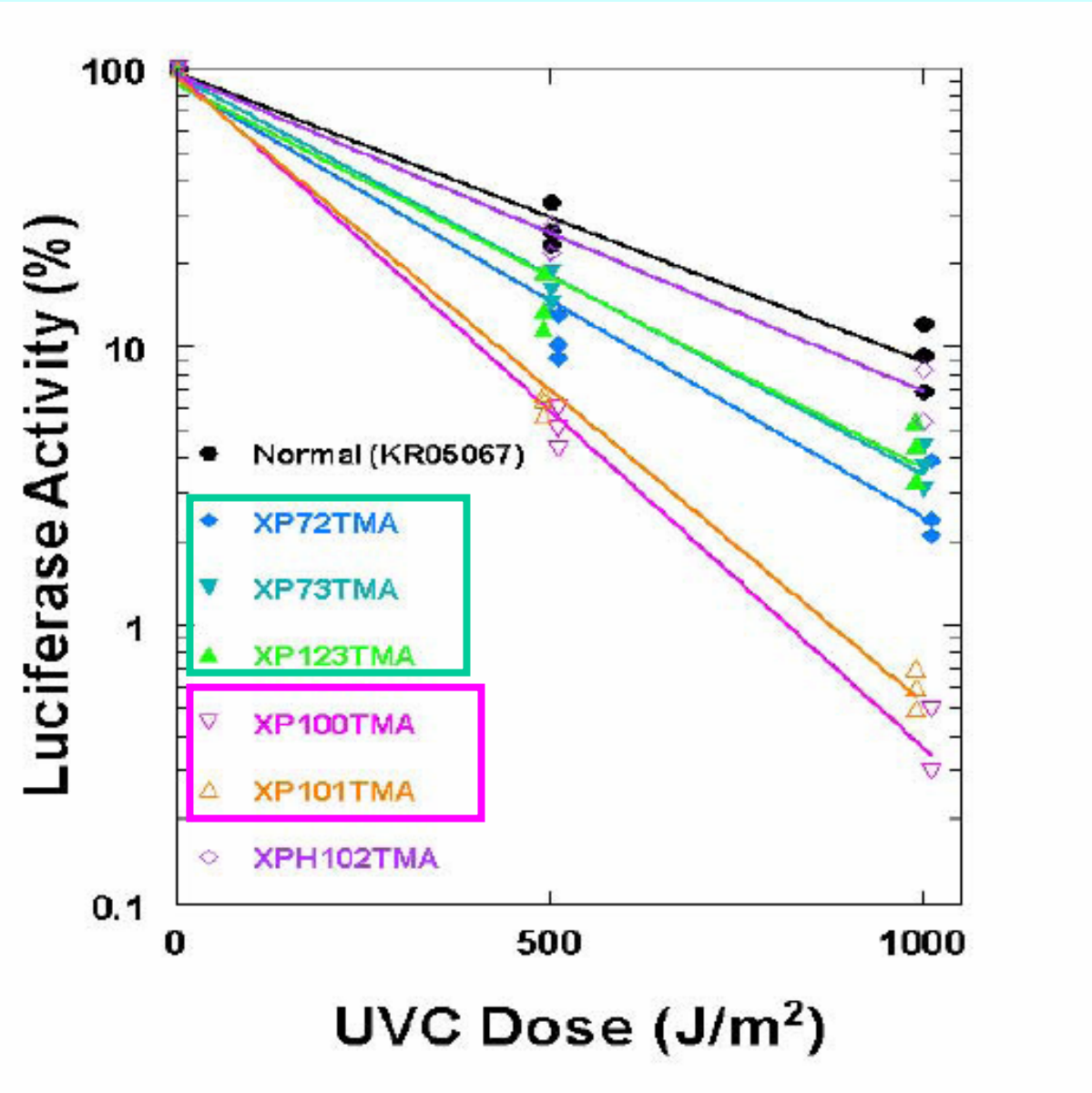
XP72TMA

XP73TMA
Skin cancer

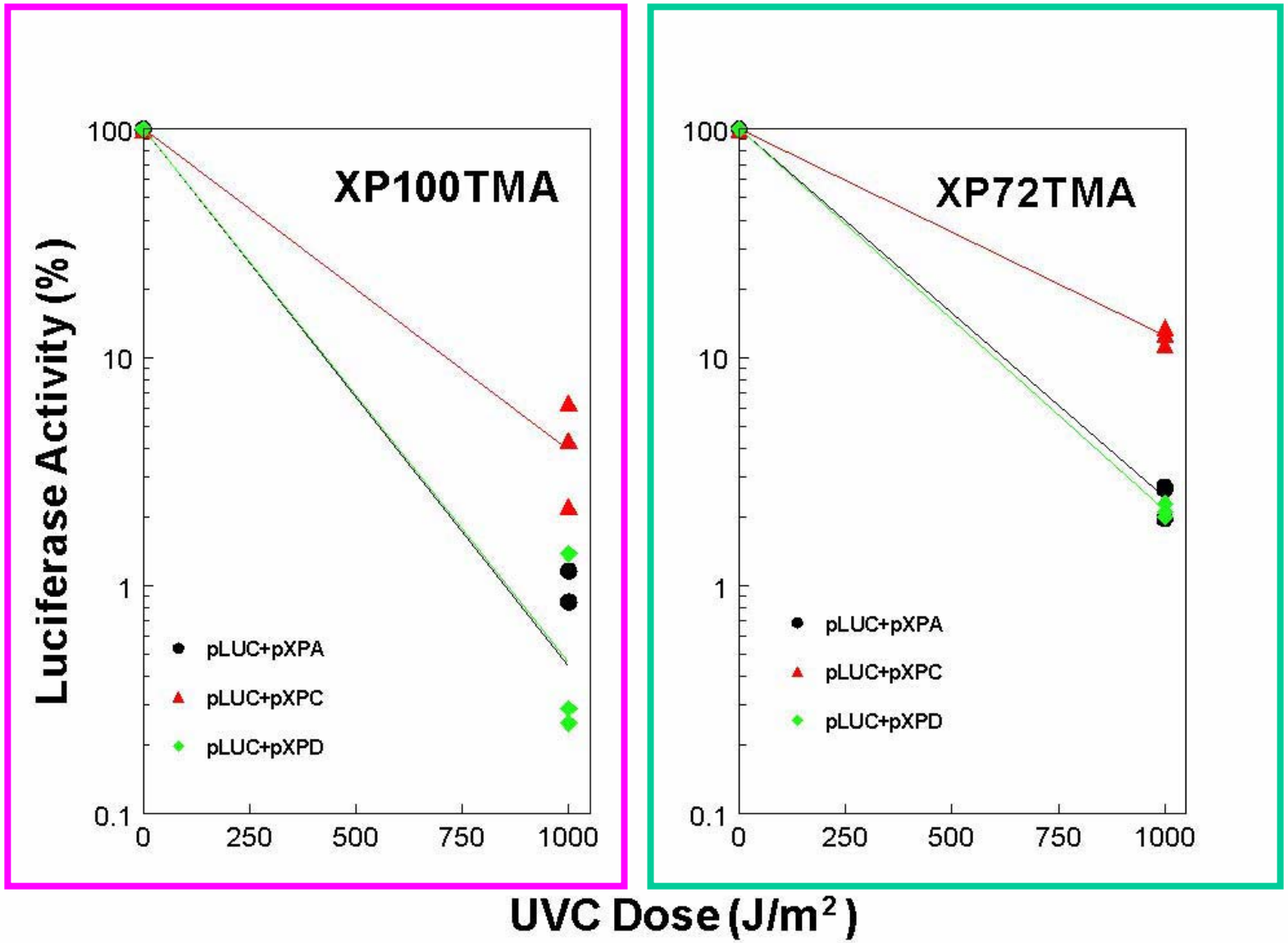
XP123TMA



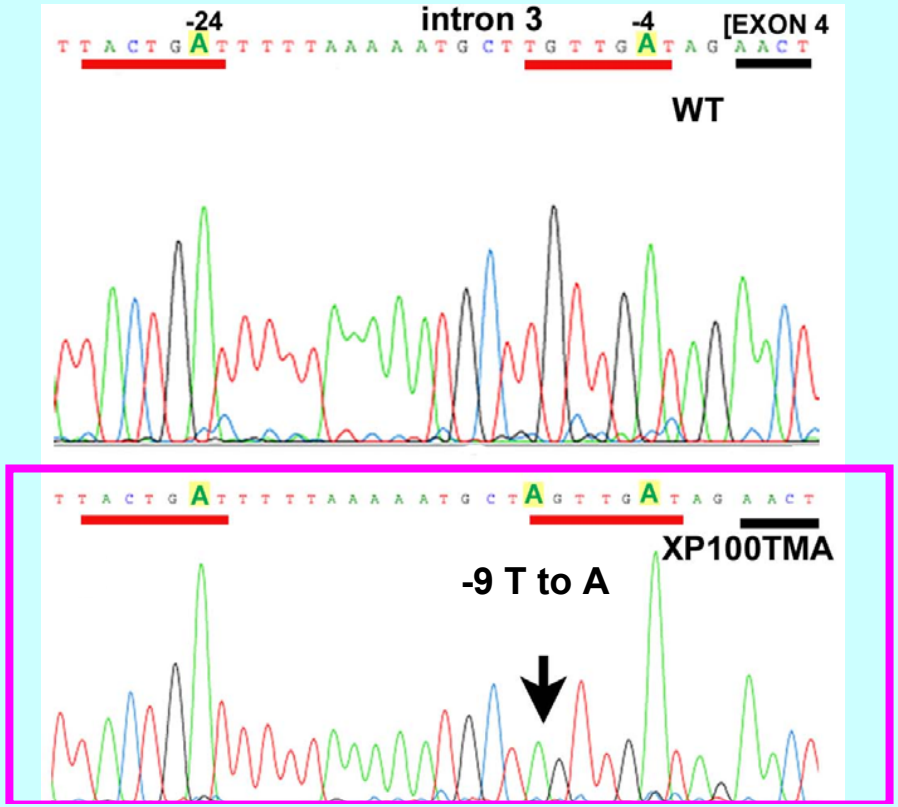
RELATIONSHIP BETWEEN DNA REPAIR STATUS OF THE CELLS AND THE CLINICAL PHENOTYPES IN THE XP PATIENTS



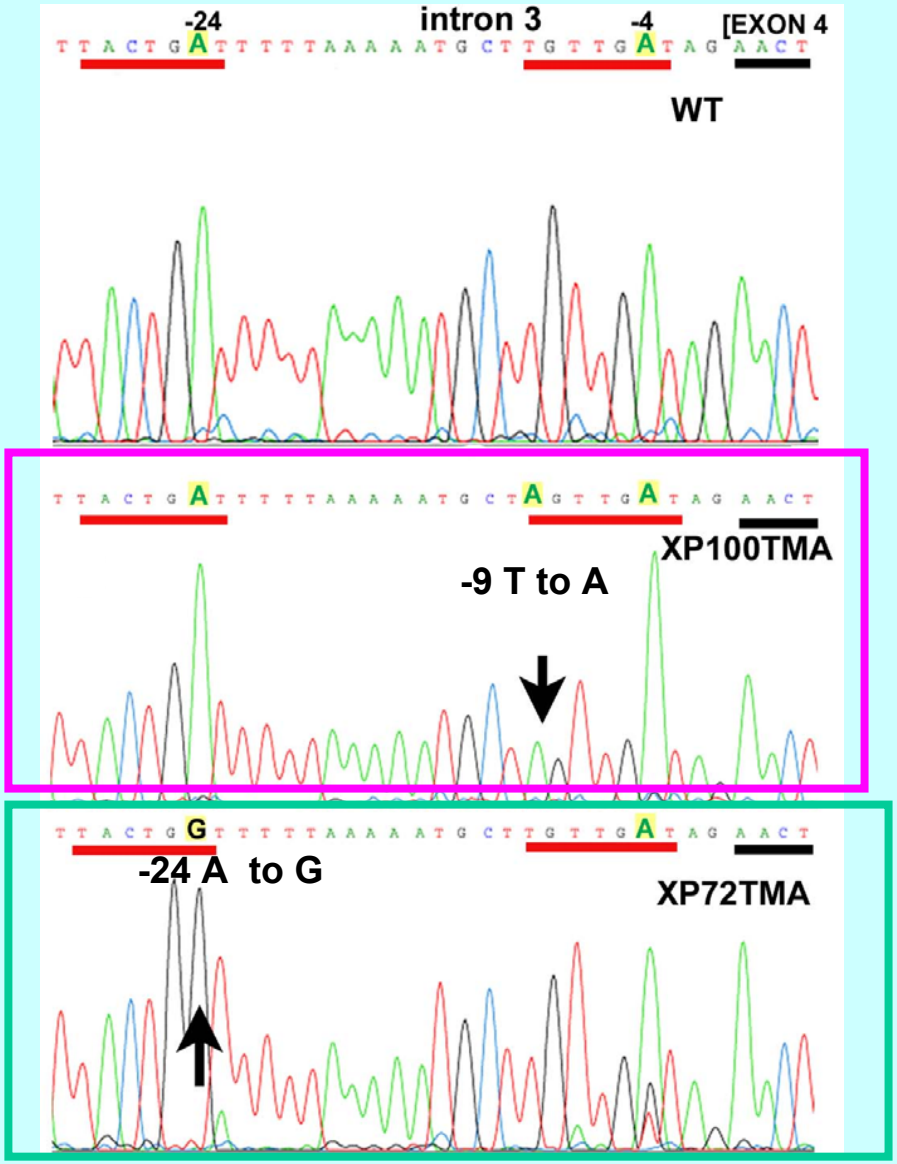
ASSIGNMENT OF CELLS FROM PATIENTS TO XP-C BY HCR ASSAY



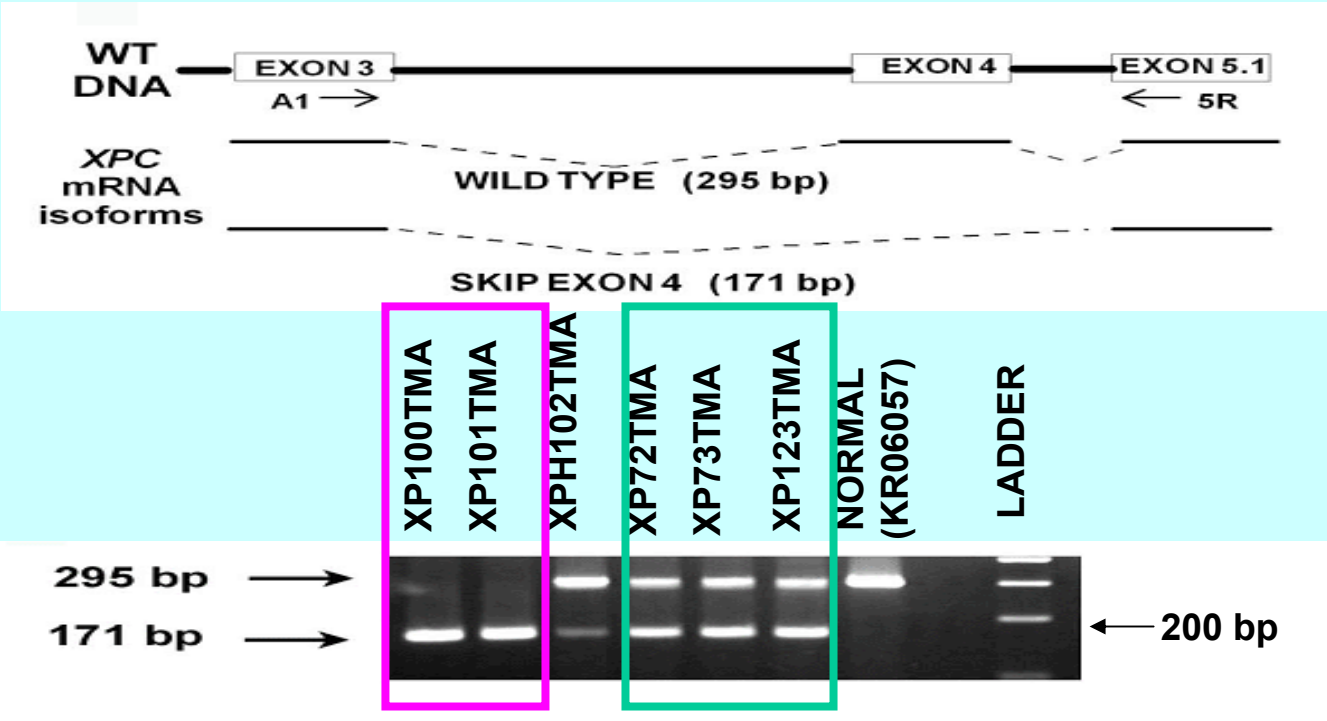
Mutational analysis of the *XPC* gene in cells from proband of each XP-C family from Turkey



MUTATIONAL ANALYSIS OF THE *XPC* GENE IN CELLS FROM PROBAND OF EACH XP-C FAMILY FROM TURKEY



ANALYSIS OF *XPC* cDNA EXON 4 SKIPPING



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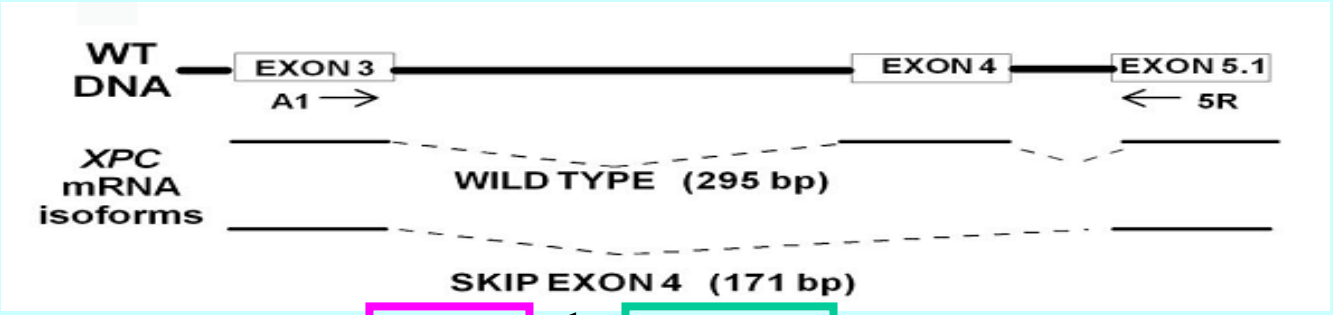

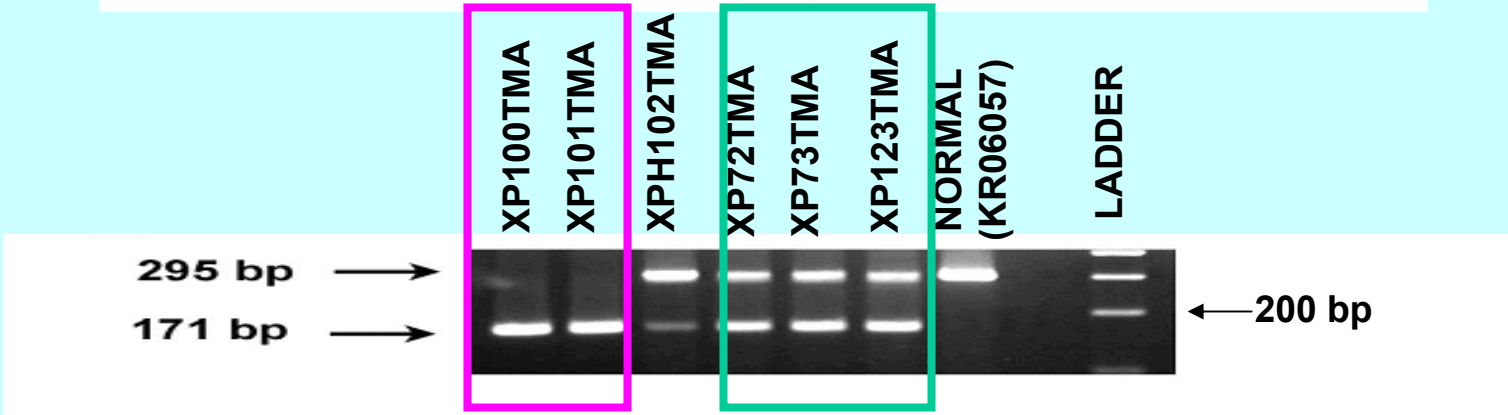

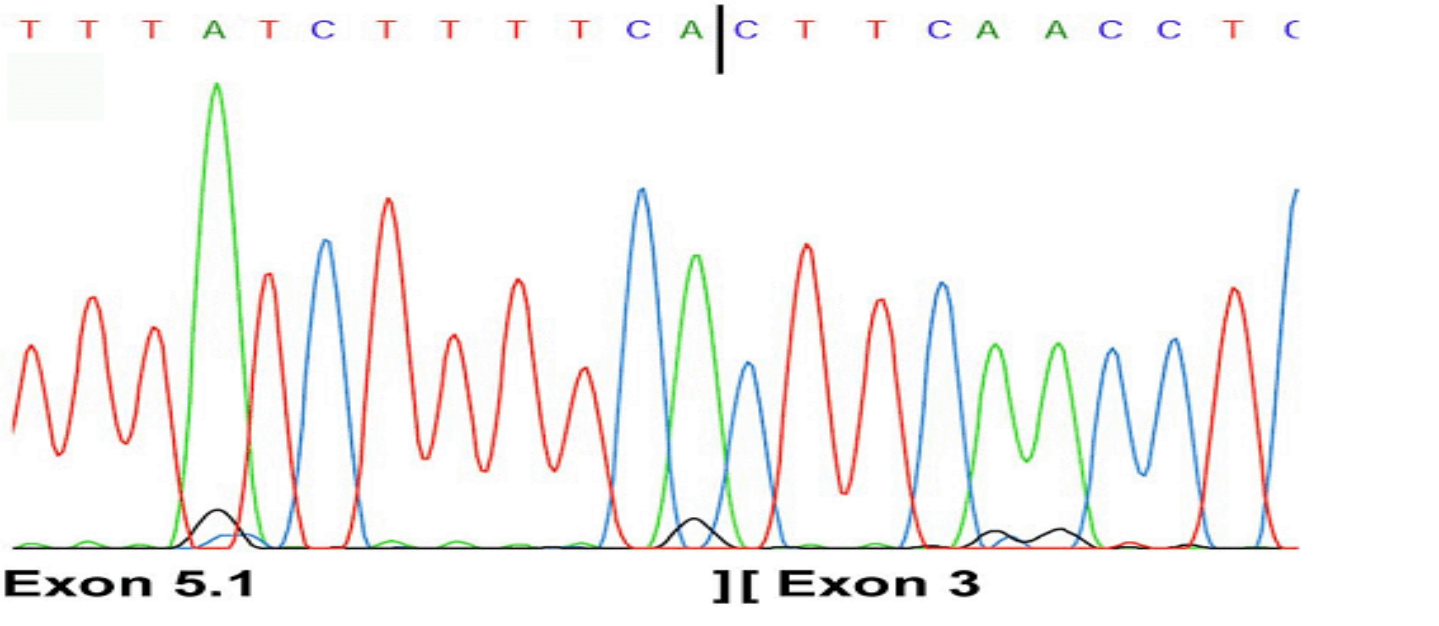

FULL-LENGTH AND ALTERNATIVELY SPLICED *XPC* mRNA ISOFORMS IN CELLS FROM XP-C PATIENTS

CELL LINE	XPC EXON 4 INCLUSION Average fg[1]	XPC EXON 4 SKIPPING Average fg	XPC EXON 7 INCLUSION Average fg	XPC EXON 12 INCLUSION Average fg
XP100TMA	<0.1 (<0.1%)	49.7 (12x)	23.5 (13%)	46.1 (19%)
XP101TMA	<0.1 (<0.1%)	47.9 (11x)	21.3 (12%)	45.2 (19%)
XPH102TMA	38.0 (22%)	24.8 (6x)	48.7 (28%)	72.9 (30%)
KR06057 (NL)	171.0 (100%)	4.3 (1x)	177.0 (100%)	240.0 (100%)

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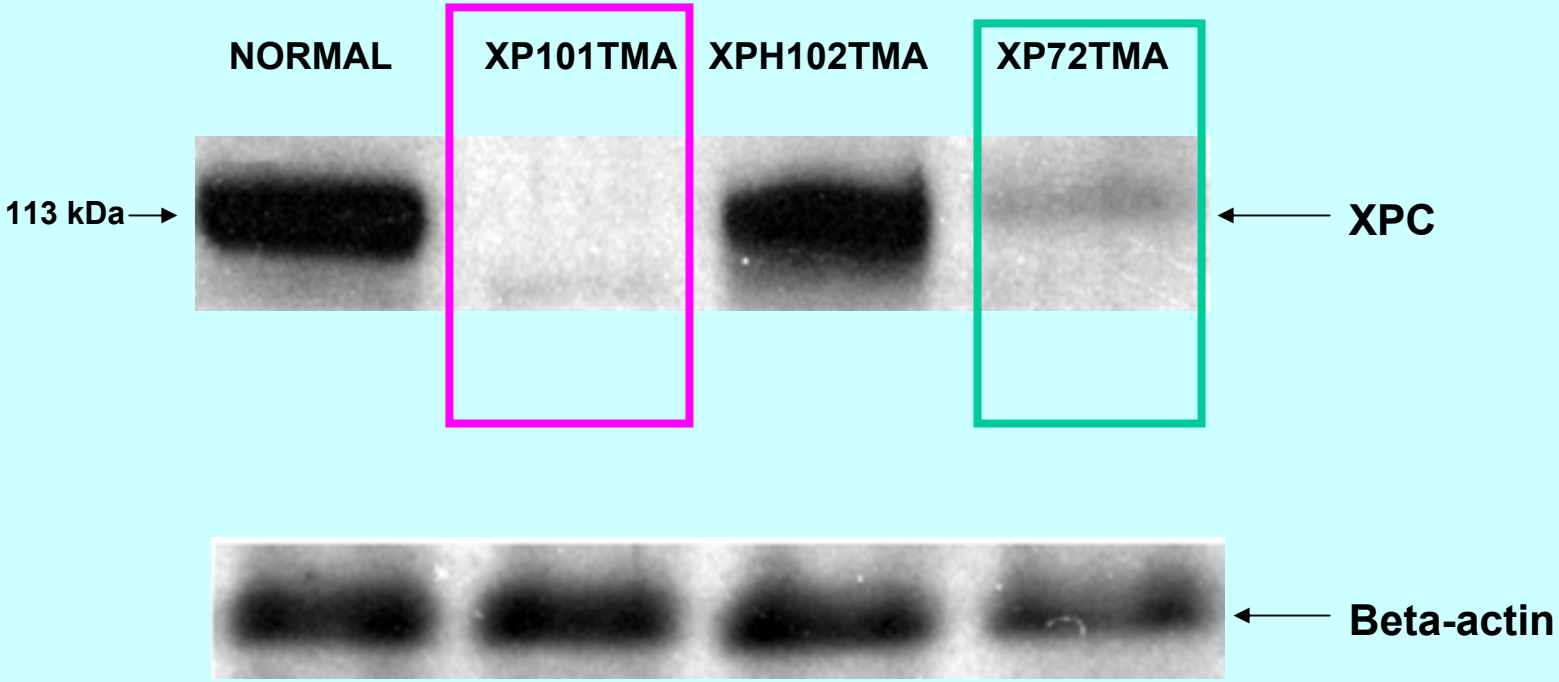
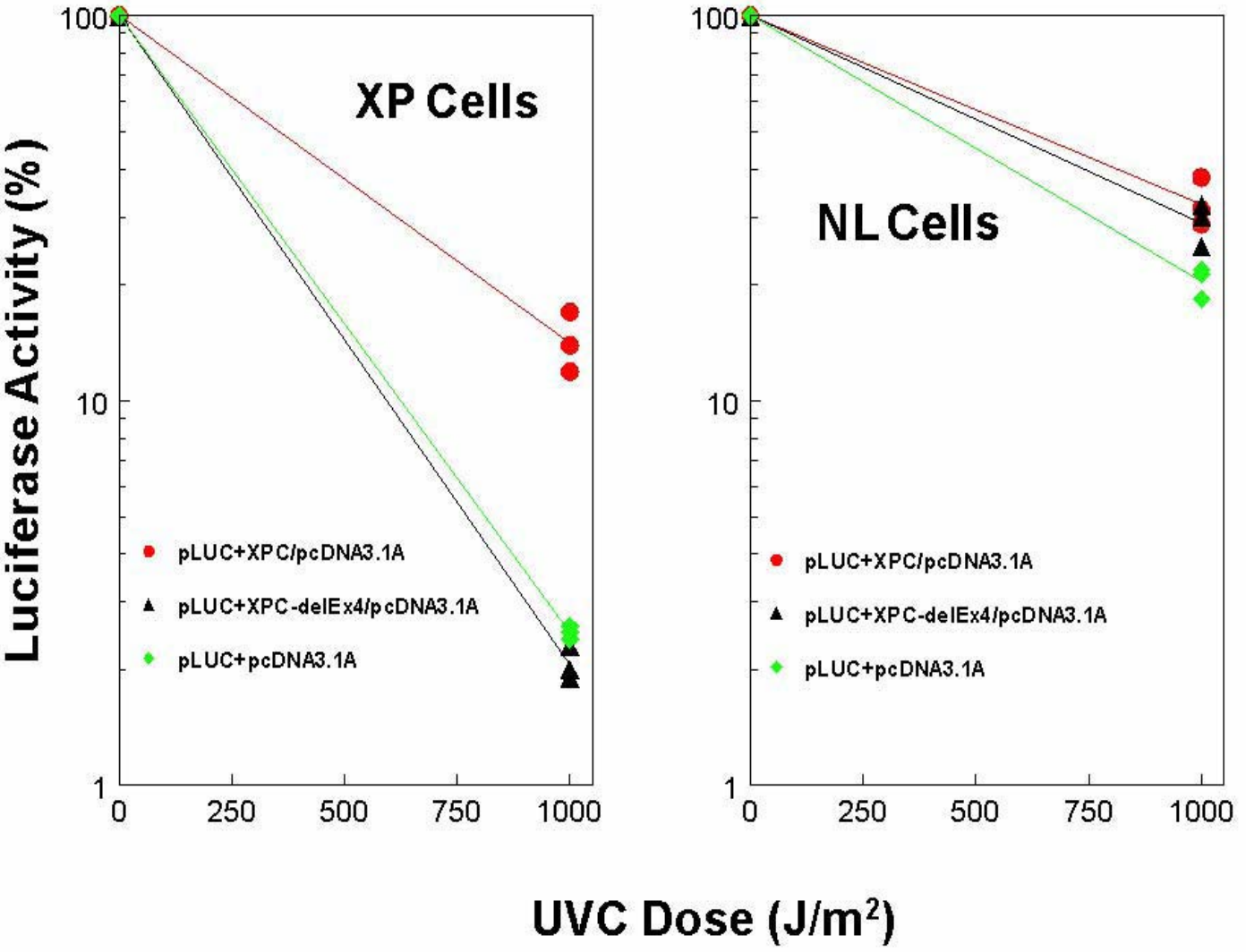
XP72TMA	5.5 (3%)[2]	24.7 (6x)[3]	25.8 (15%)	52.8 (22%)
XP73TMA	6.0 (3%)	38.7 (9x)	27.7 (16%)	51.9 (22%)
XP123TMA	8.2 (5%)	46.8 (11x)	44.0 (25%)	81.5 (34%)
KR06057 (NL)	171.0 (100%)	4.3 (1x)	177.0 (100%)	240.0 (100%)

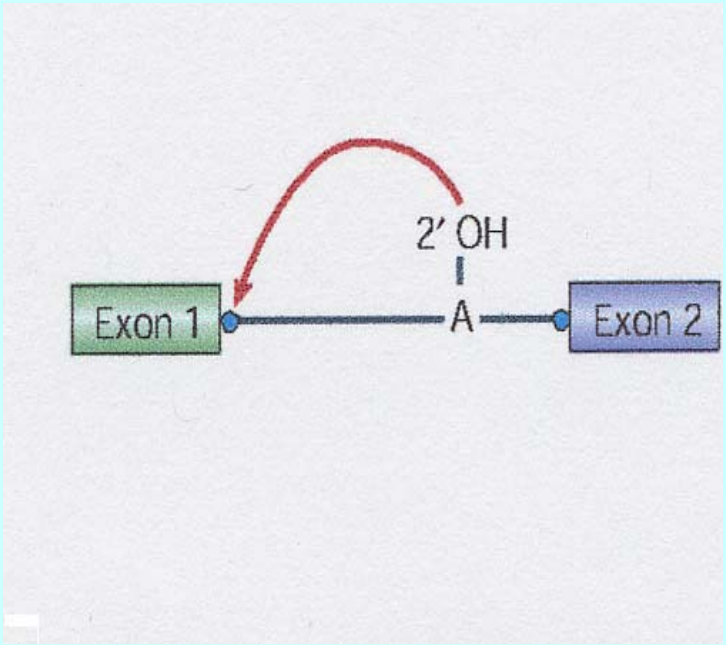
Differential expression of XPC protein in cells from severely- and mildly-affected XP patients



DELETION OF *XPC* EXON 4 ABOLISHES DNA REPAIR FUNCTION



PRE-mRNA SPLICING OCCURS BY TWO SEQUENTIAL *TRANS*-ESTERIFICATION REACTIONS



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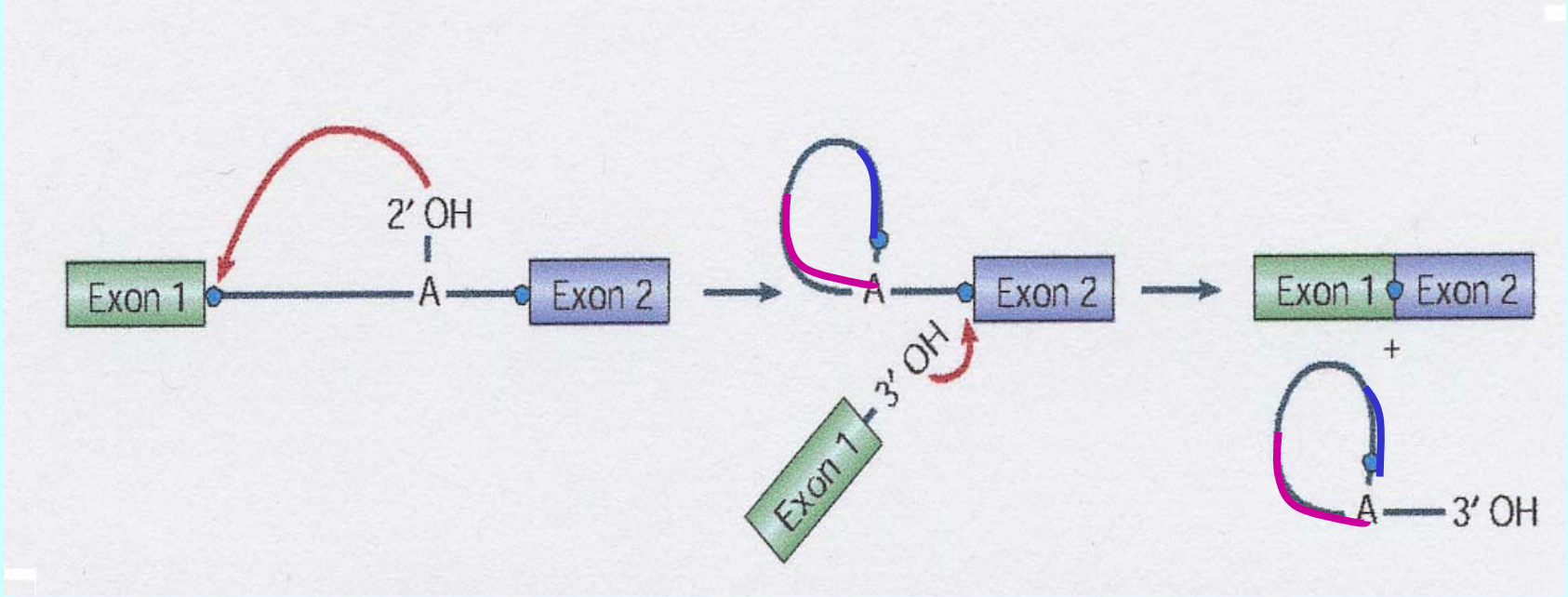

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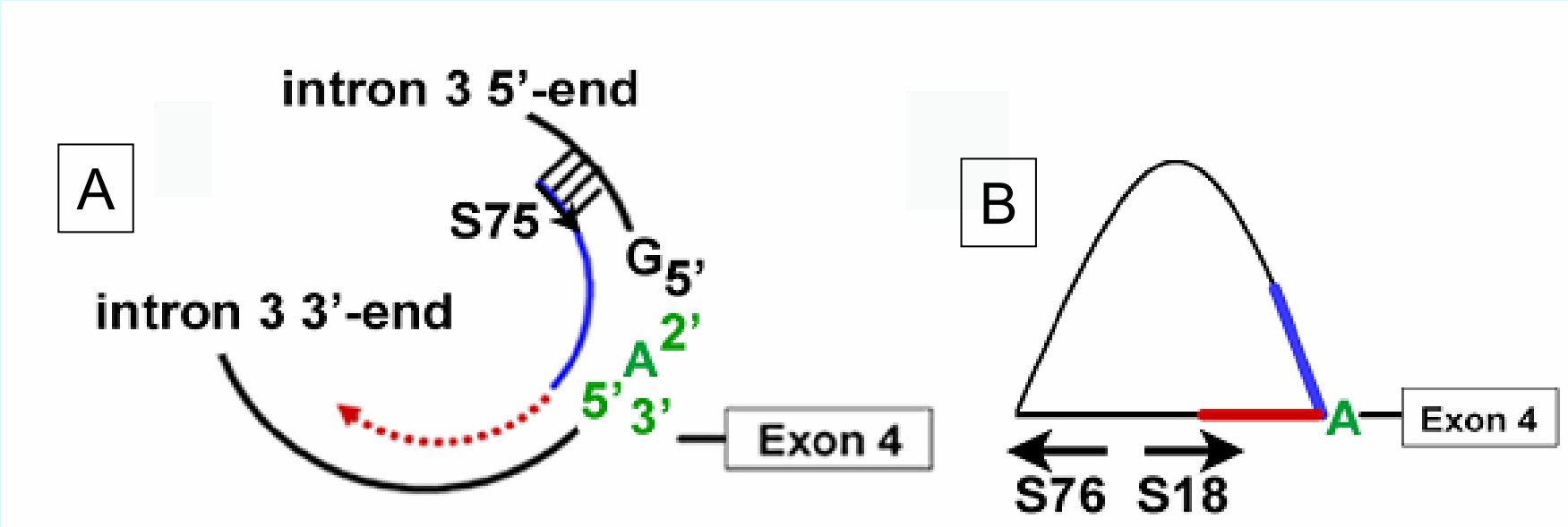
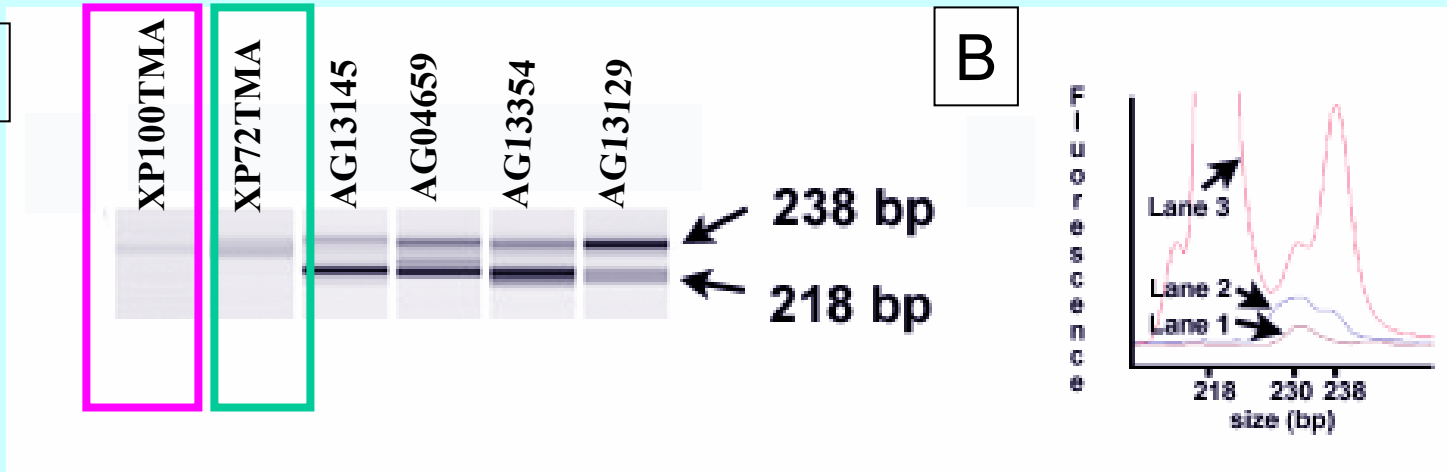
MAPPING OF THE BRANCH POINT SEQUENCE



A) **Diagram of an intron-containing splicing intermediate in a lariat configuration in which the 'G' at the 5' end of the intron is linked by a 2' – 5' phosphodiester bond (RNA branch) to a single adenosine residue near the 3' end of the intron.**

B) **Diagram of a primer pair designed for inverse PCR and sequencing.**

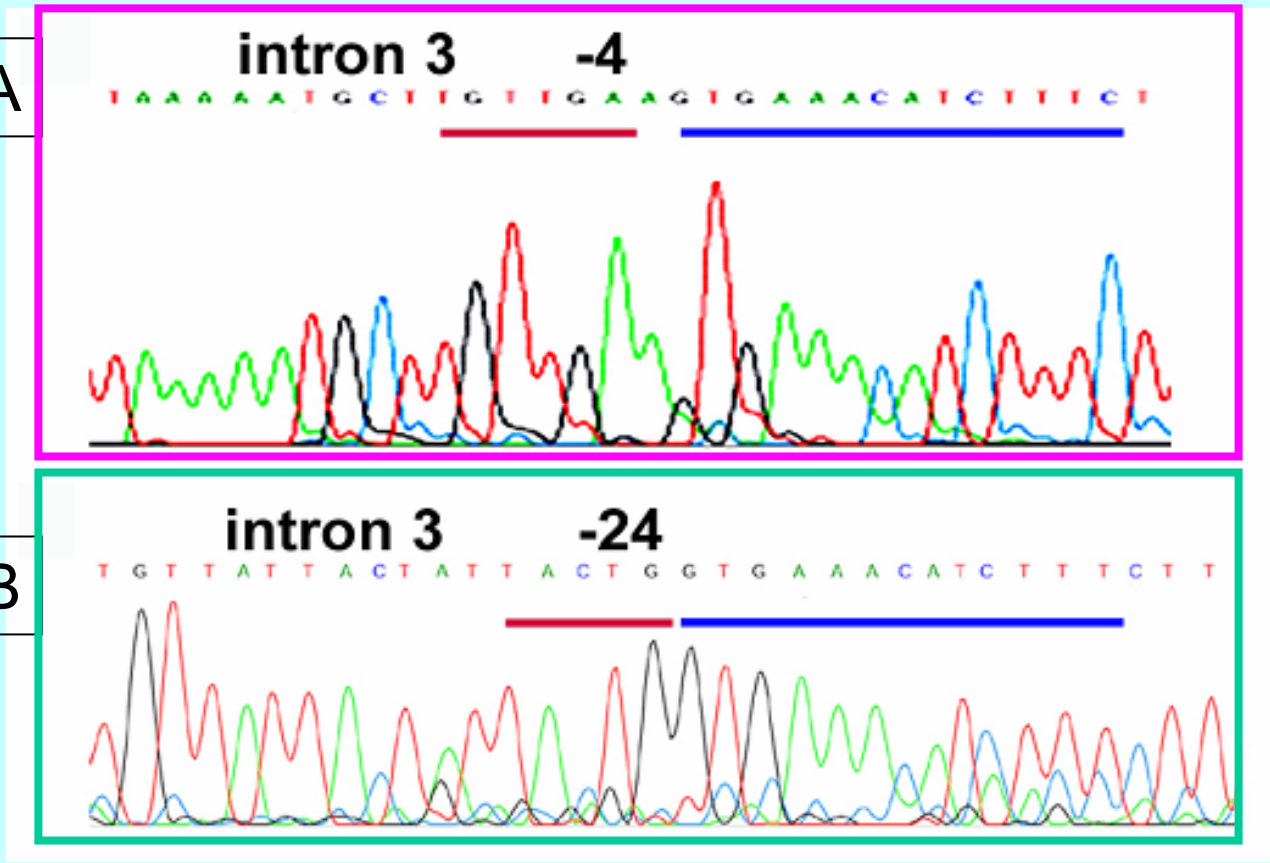
MAPPING OF THE BRANCH POINT SEQUENCE

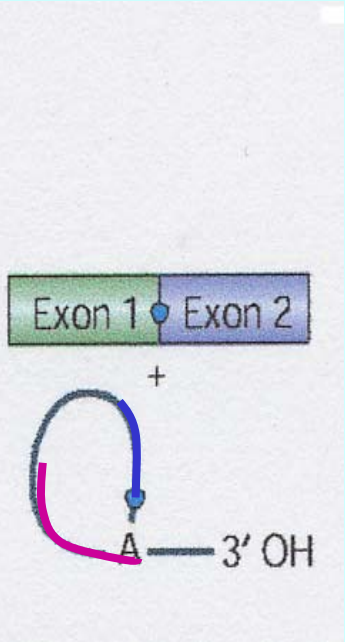


A) RT using a gene-specific primer followed by PCR with inverse primers generated two lariat-specific bands of 238 and 218 bps (separated by an Agilent 2100 Bioanalyzer) with RNA from normal fibroblasts

B) The electropherogram data for lanes 1- 3 were used to create the image that reveals non-specific 230 bp peak but no both specific peaks in XP100TMA

MAPPING OF THE BRANCH POINT SEQUENCE



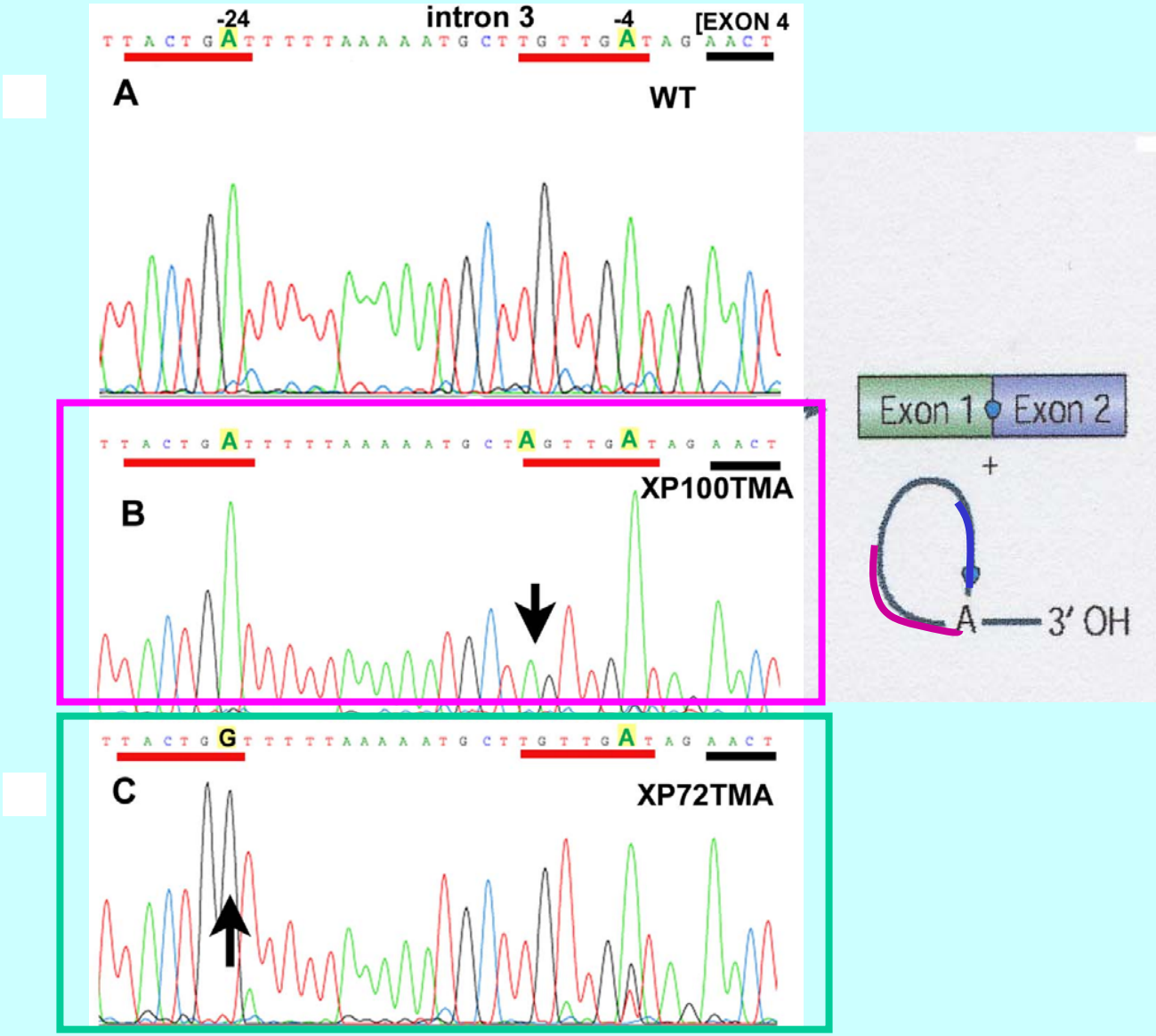


Sequencing the *XPC* intron 3 lariat - specific PCR products in normal fibroblasts:

A) The branch point adenosine 'A' was mapped at – 4 position

B) The adenosine 'A' of the upstream BPS was mapped at –24 position

MUTATIONAL ANALYSIS OF THE *XPC* GENE IN CELLS FROM PROBAND OF EACH XP-C FAMILY FROM TURKEY



CONCLUSIONS / SPECULATIONS

- **Mutations identified in the two BPS of the *XPC* intron 3 resulted in alternative splicing that impaired DNA repair function.**
- **Both of these BPS are essential but not redundant for accurate and efficient splicing of the *XPC* pre-mRNA.**

- **Cells from the severely affected patients had no measurable full length *XPC* mRNA and no measurable XPC protein.**
- **A low but measurable amount of normal *XPC* message containing exon 4 and XPC protein was present in the mildly affected XP-C patients.**
- **The amount of normal *XPC* message (and therefore normal XPC protein) in the patient's cells can be a major determinant of clinical phenotypes and can provide partial protection against skin cancers.**