Mutagenesis of Triplex Targeted Crosslinks

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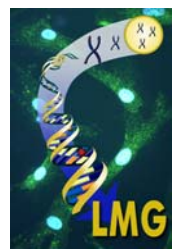


Experimental systems for analysis of DNA repair and mutagenesis in mammalian cells

Treatment of cells with damaging agent-random modification

- Survival
- strand breakage
- immunofluorescence
- mutagenesis across a marker gene

Plasmids- site specific modification

- survival
- mutagenesis
- inhibition of transcription

covalent modification of a specific chromosomal site

GENE TARGETING

- **Find and bind a target sequence in living mammalian cells**
  - **Cells must survive**

- **Influence Transcription**
- **Change genomic sequence**

- **Introduce DNA damage**

Sequence recognition in chromosomal DNA

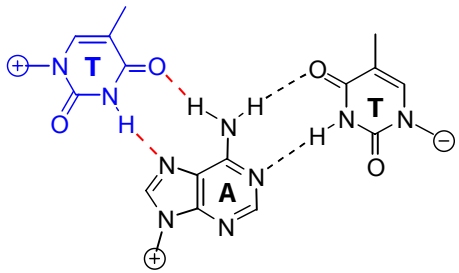
denatured: single strand

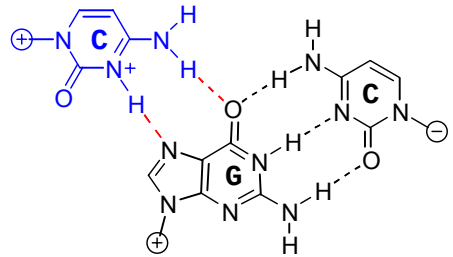
major groove

minor groove

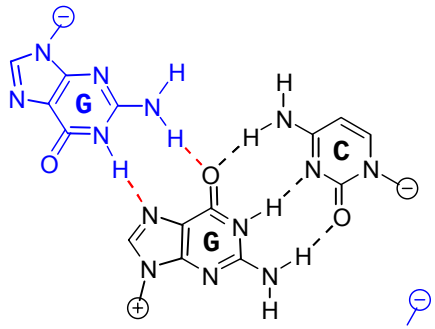
Two Modes of Triple Helix Formation

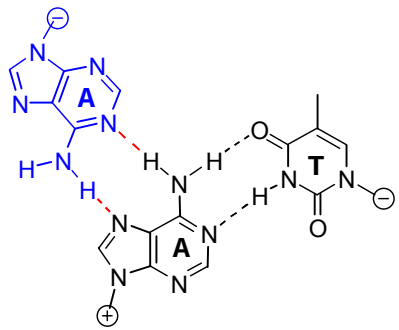
Parallel Triplets

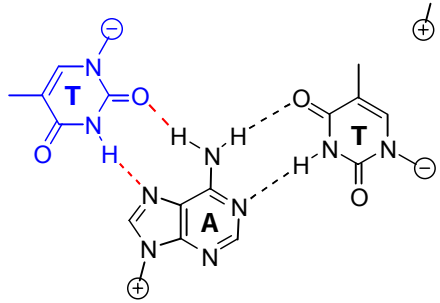




Antiparallel Triplets

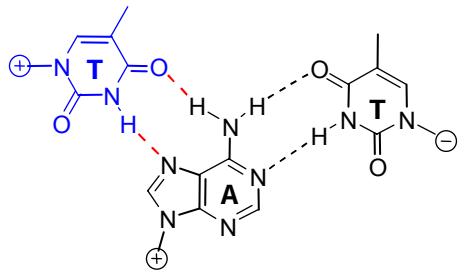


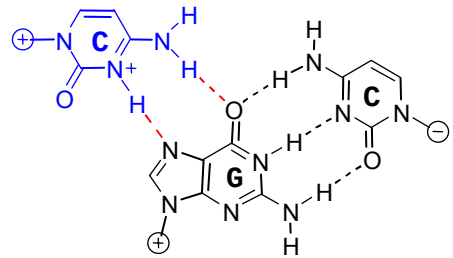




DNA Triple Helix

Pyrimidine motif





- homopurine:homopyrimidine sequences in duplex DNA
  - Abundant in mammalian genomes
  - Common in promoters and introns
- Inherent property of nucleic acids
- Triplex formation: kiss and zip
- Very stringent with respect to sequence

Challenges to Bioactivity of Triplex Forming Oligonucleotides

Oligonucleotide:duplex

Poor activity of Pyrimidine motif TFOs in physiological pH.

Nuclease sensitivity of oligonucleotides

Conformational constraint required for triplex formation

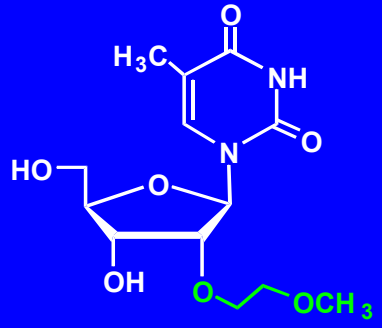
Charge repulsion between TFO and target duplex
Low Mg^{++} in cell

Genomic targeting

Chromatin-target access

Adoption of 3'-*endo* (North) Conformation by 2'-*O*-modified Nucleosides

(A)



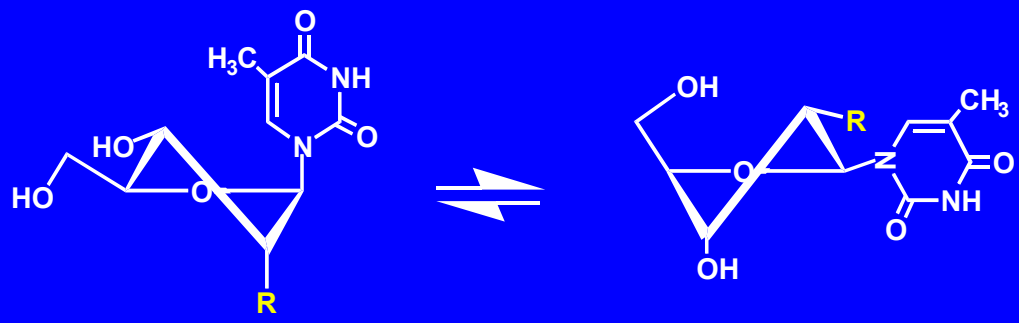
(B)

(C)

(D)

Some Salient Features about 2'-O-(2-Aminoethyl) Nucleotides:

* C-3'-*endo* conformation
* Confers nuclease stability
* Extra H-bond with phosphate

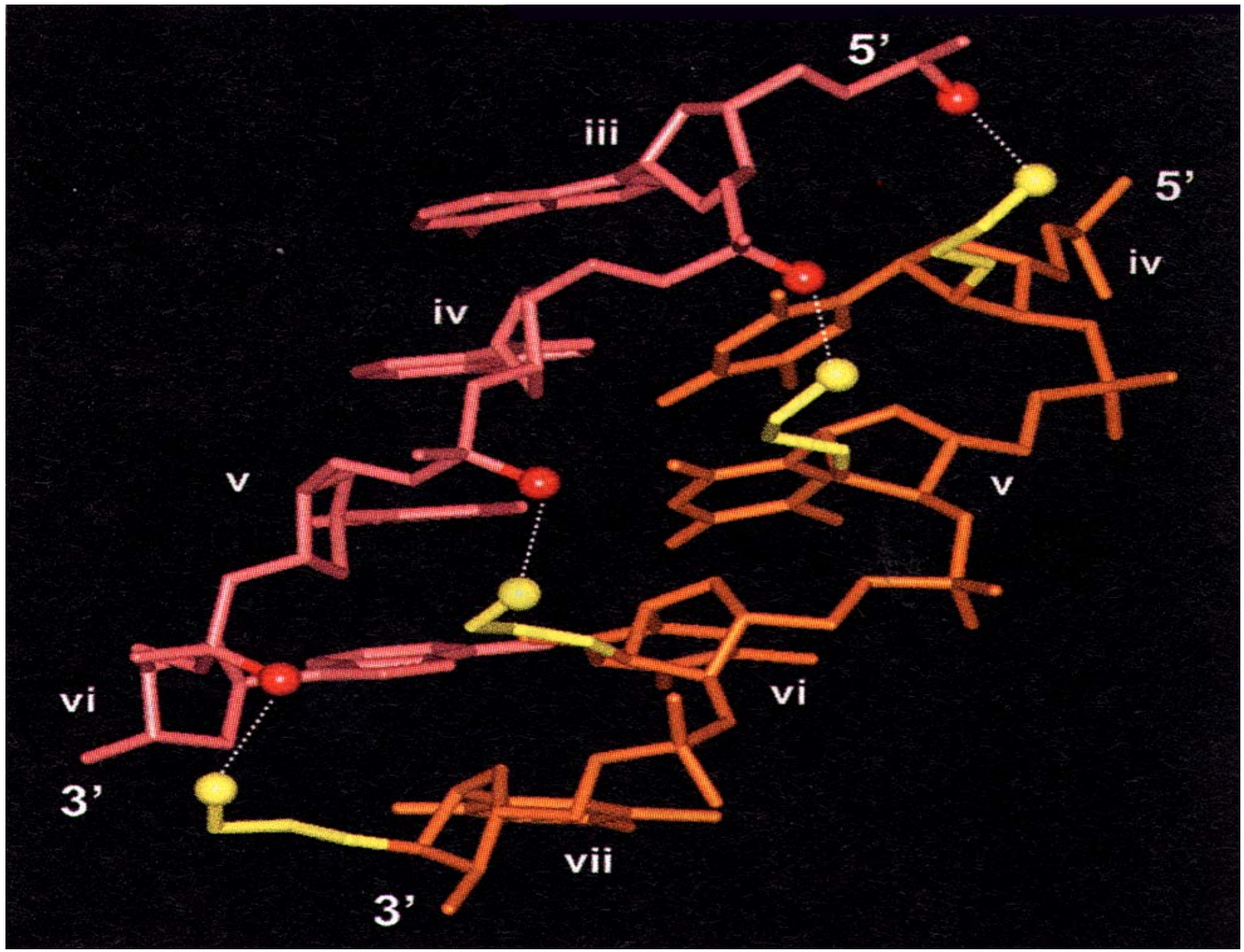


R = OH : North/South > 1

R = H : North/South < 1

Angew. Chem. Int. Ed. **1998**, *37*, 1288-1291

NMR Structure : 2'-*O*-(2-Aminoethyl) TFOs

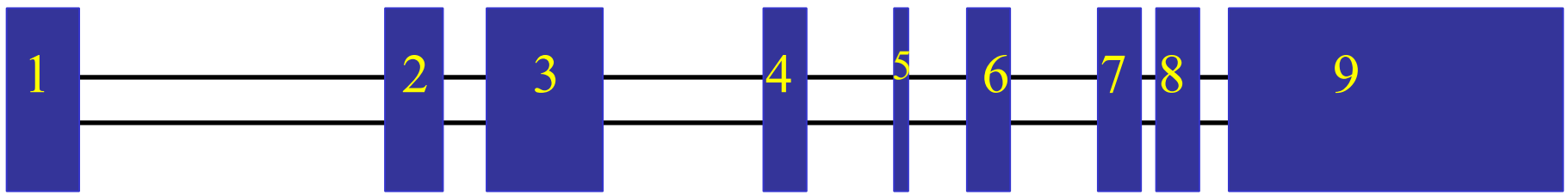


TTTCTCTTTTTTTCTTCT-pso TFO

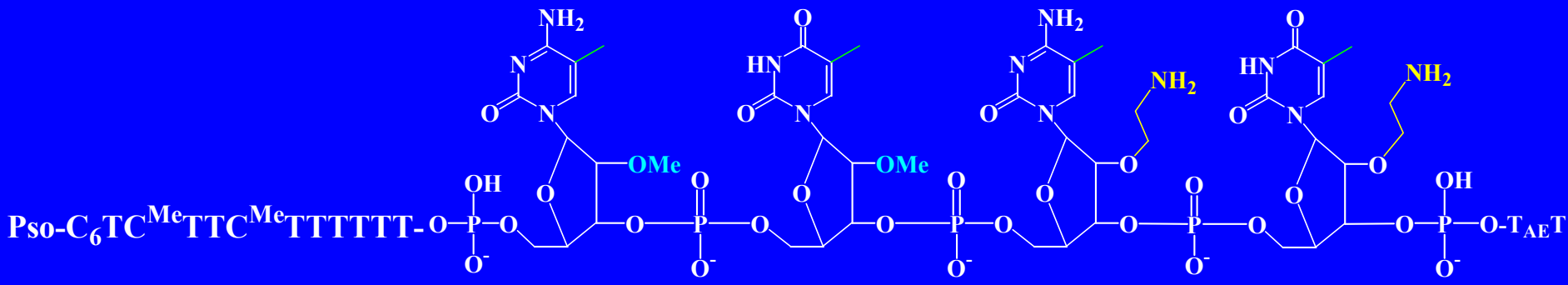
*Xba*I

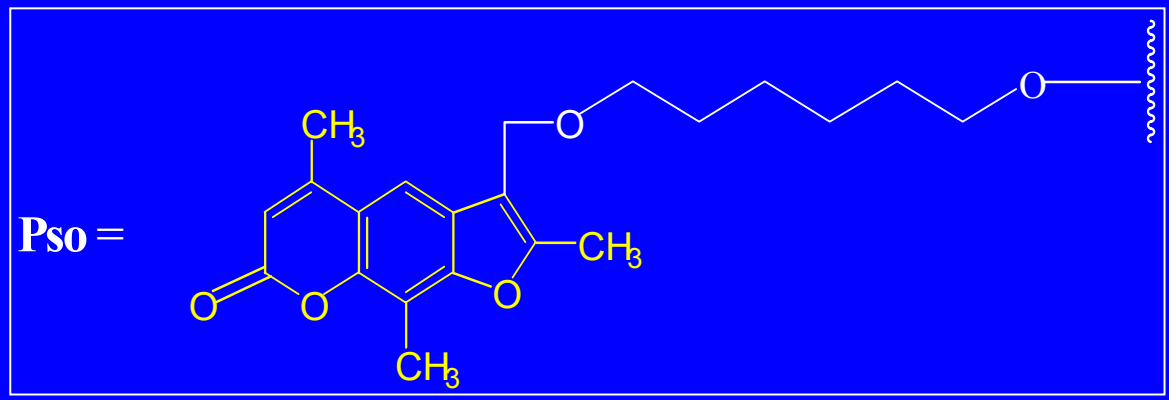
5’ TTTTCATTTCTCTTTTTTTCTTCTAGaatgt

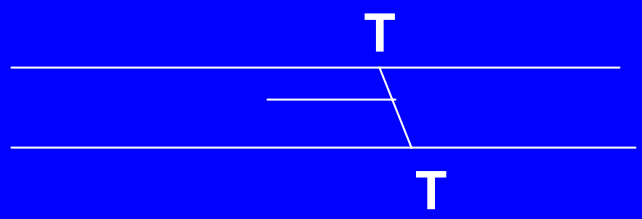
HPRT



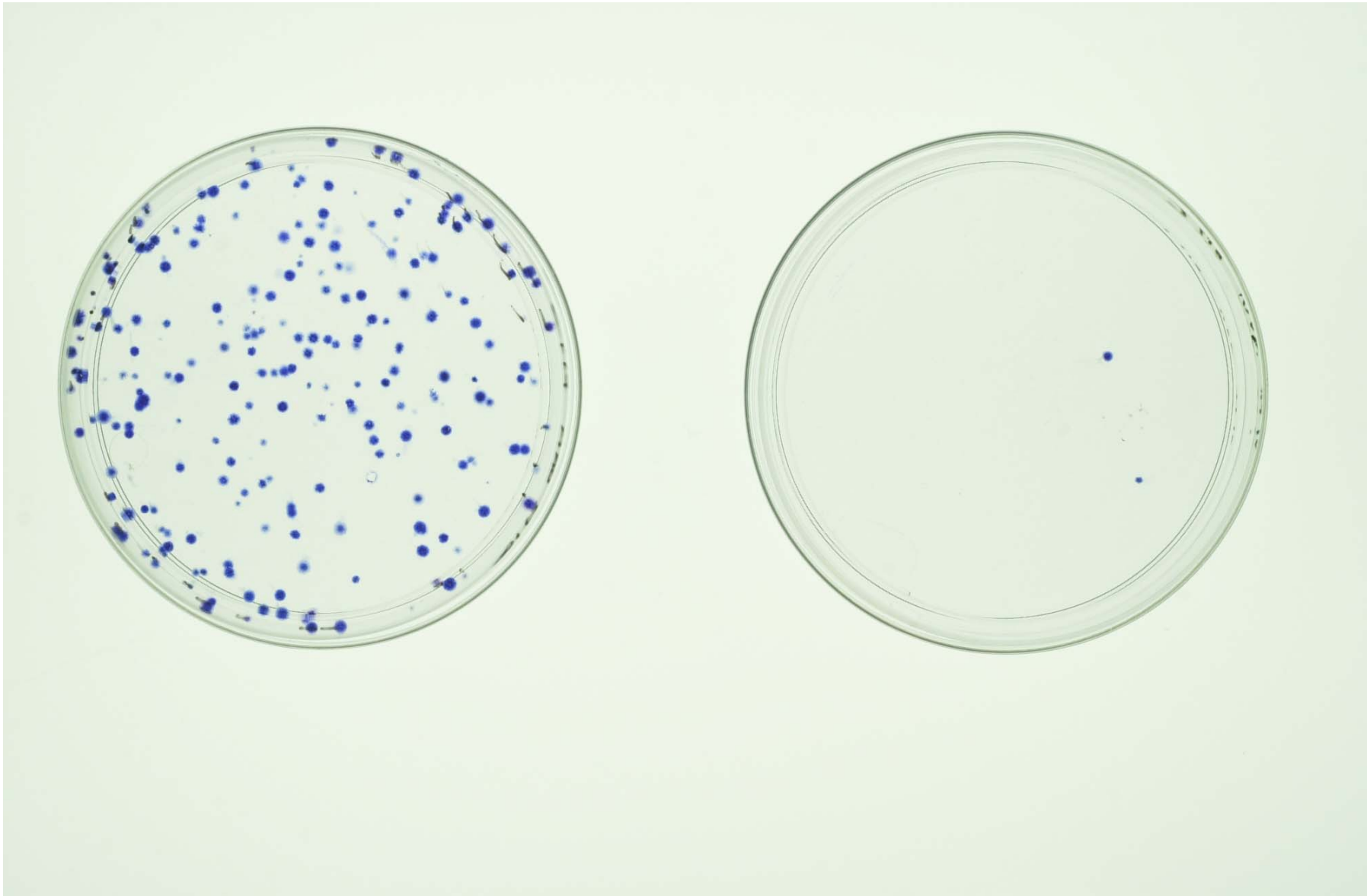
5’-Pso-2’-*O*-(2-Aminoethyl) Chimeric TFO







Thioguanine resistant colonies following treatment with pso-TFO

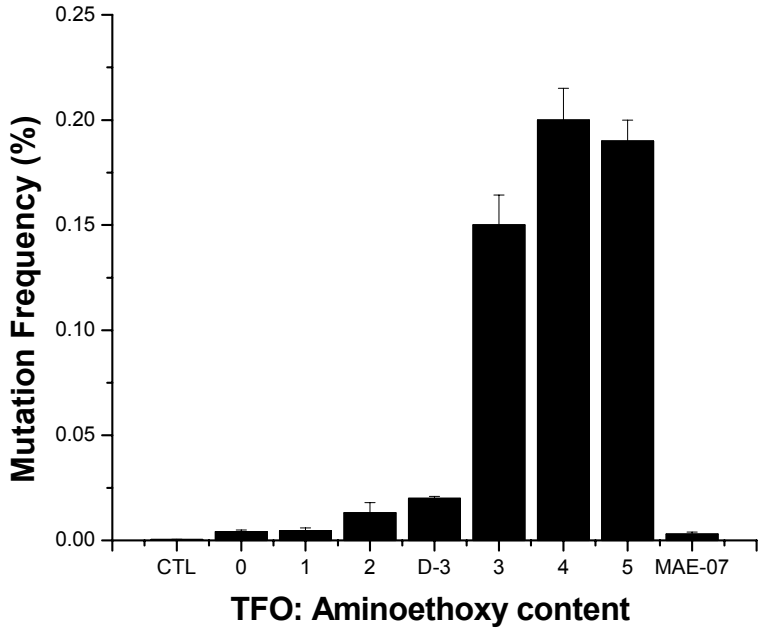


TFOs with a cluster of aminoethoxy residues are bioactive

tTTCTCTTTTTTCTTCT-pso TFO

Bioactivity requires clustered AE residues

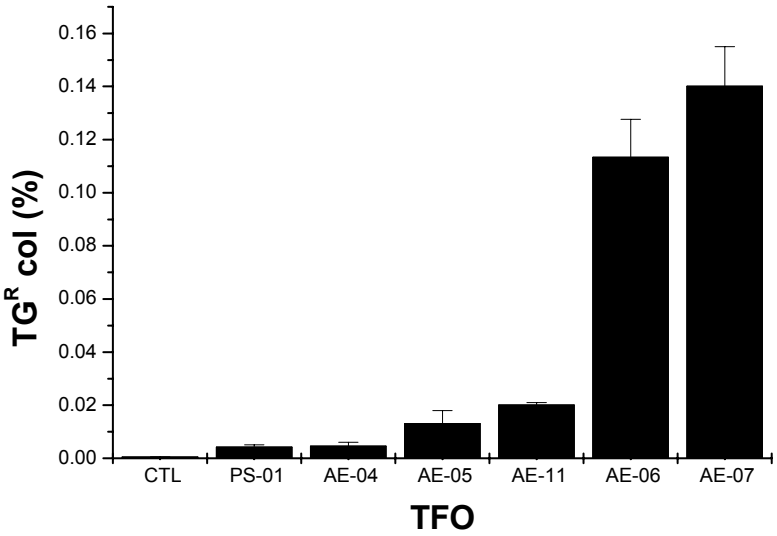
A single mismatch is inactivating



Triplexes are much less stable in cells than in vitro

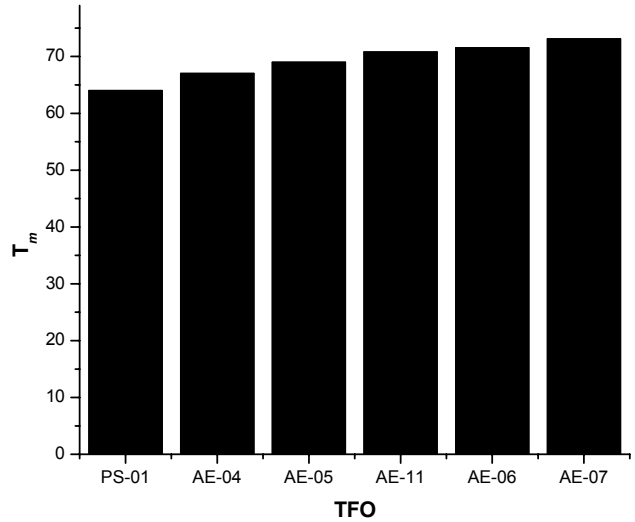
Triplexes are unwound by chromosomal helicases and translocases

Bioactivity



0 1 2 D3 3 4

Thermal stability

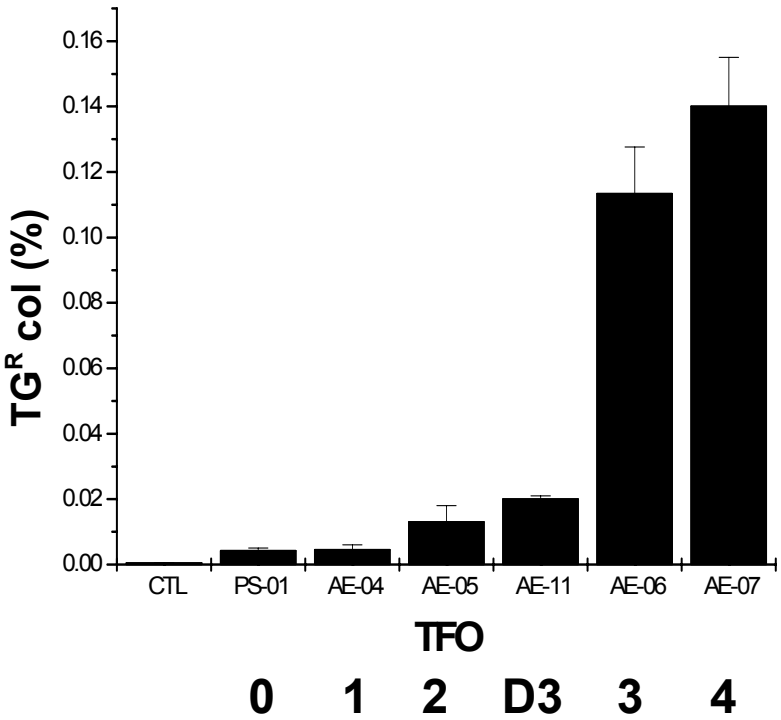


0 1 2 D3 3 4

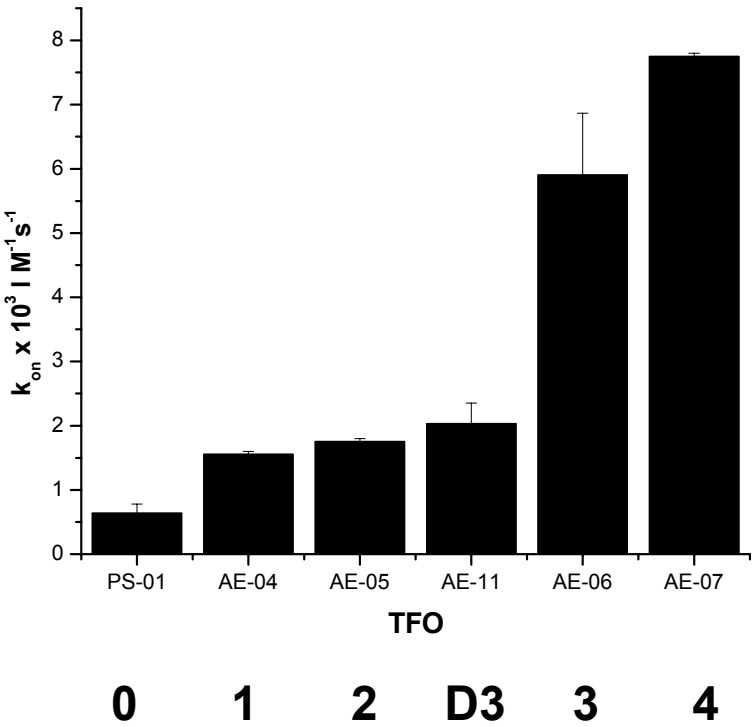
tTTCTCTTTTTTCTTCT-pso TFO

T_m does not distinguish active and inactive TFOs

Bioactivity



Association rate



AE cluster supports faster association (kiss)

Association rate appears important for bioactivity

Why would Association Rate Matter?

Triplexes are stable in vitro

If, after formation, triplexes were stable in cells, then waiting longer would be useful.

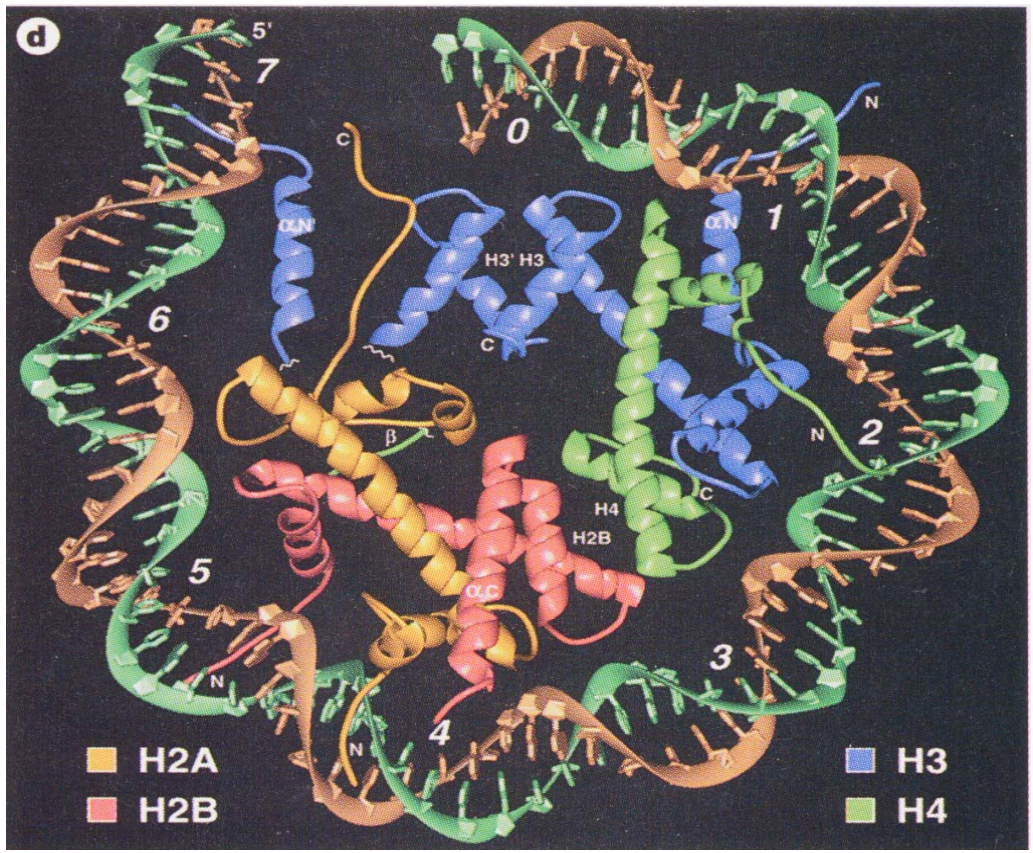
If they are not stable in cells then the association step becomes very important

Enzymes elute triplexes

- **Helicases**
- **Translocases**
- **Chromatin remodeling complexes**

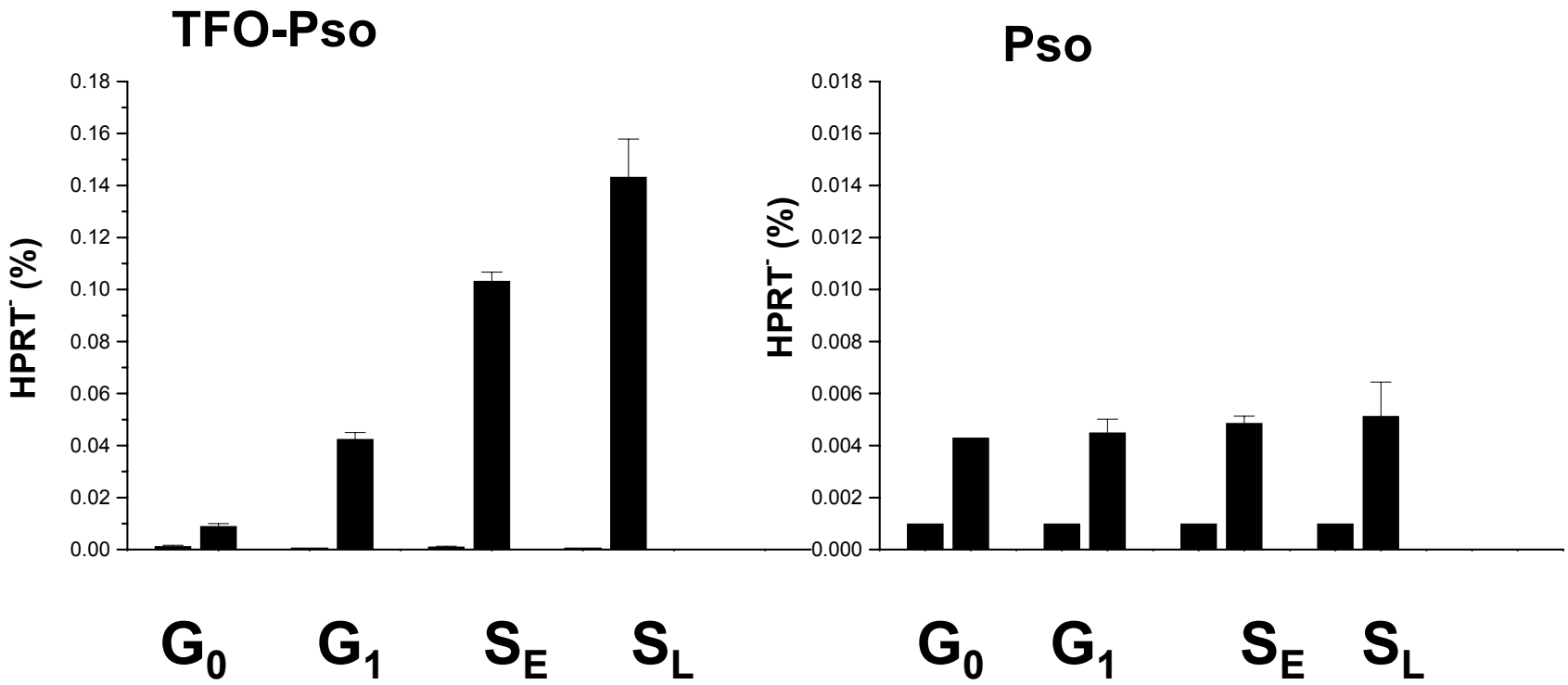
Can the biology of the cell influence target access?

Triplex- Compatible with Chromatin Structure ?

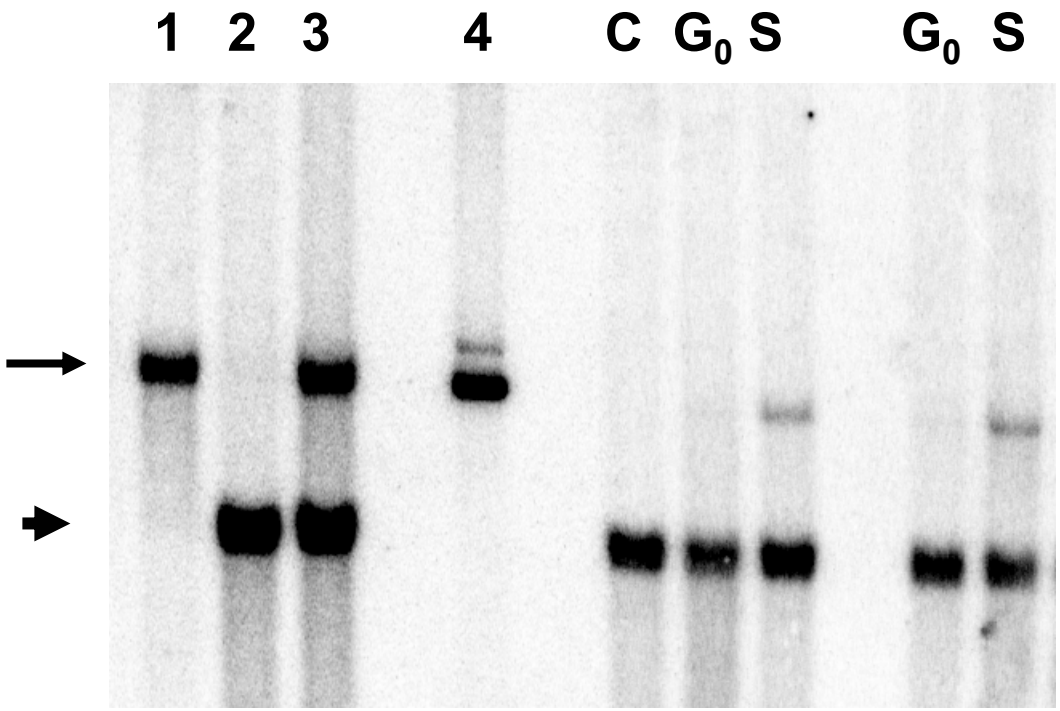


Crystal structure of the Nucleosome Core (2.8 Å)

Influence of Cell Cycle on TFO-Pso or Pso Activity



Targeted Crosslinking in vivo in S phase cells



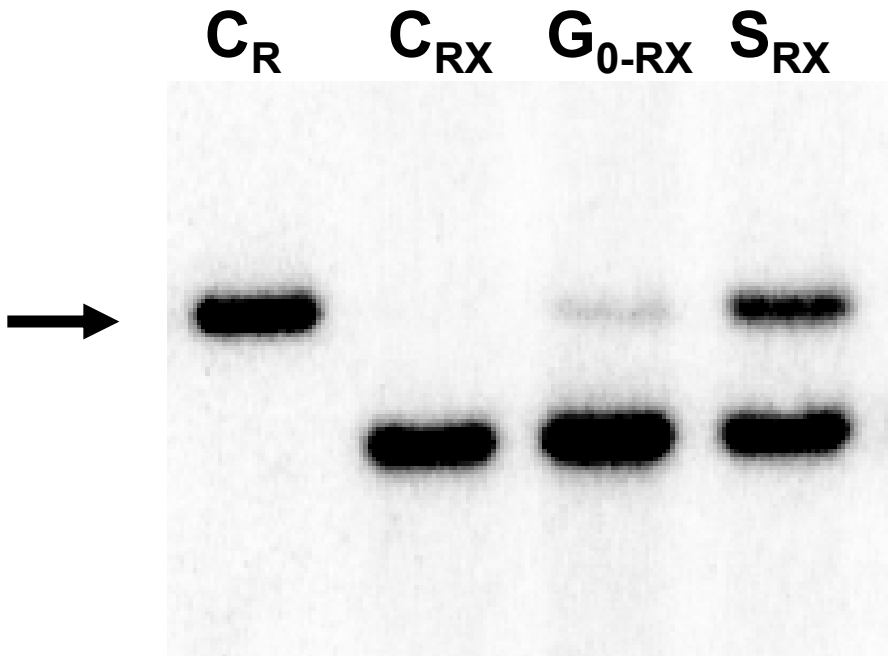
The genomic restriction fragment with the targeted crosslink is denaturation resistant

Targeted Crosslinking *in vivo* in S phase cells

XbaI Exon 5

TTTCTCTTTTTTTCT TCTAGa atgt

……A T……



Restriction Resistance demonstrates physical presence of targeted crosslink.

Efficient Gene Targeting requires an effective reagent and an available target

Glazer lab found enhanced targeting in permeabilized cells following induction of transcription in target region

We find enhanced targeting activity in S phase cells relative to quiescent cells

Target access is a critical feature of gene targeting

cellular treatments that influence chromatin structure may effect target access.

DNA Interstrand Crosslinks

Reaction of DNA and oxidation products formed during normal cellular metabolism

lipid peroxidation products

chemotherapy

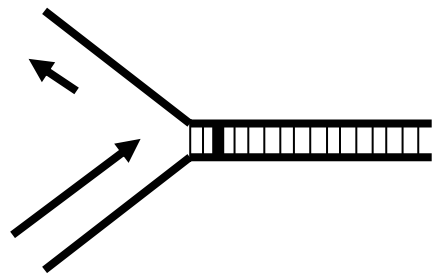
Cytotoxic lesions

more toxic than classical monoadducts (UV, Benzopyrene)

two log differential for unrepaired XL vs monoadducts

Absolute Blocks to Replication and Transcription

Failure to resolve blocked or stalled forks results in rearrangements, chromosome abnormalities, genomic instability

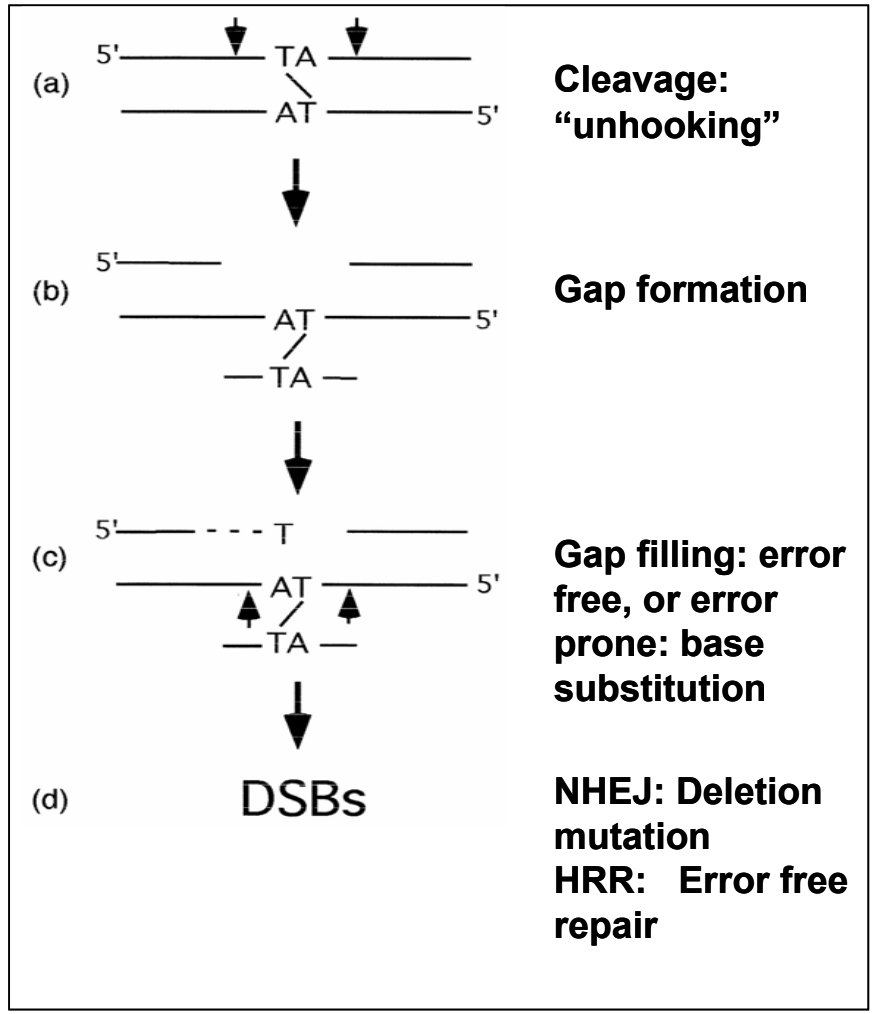


cannot be accumulated in proliferating cells

Crosslink Repair

More complex than repair of single strand adducts

Requires release of crosslinked strands and sequential gap filling, excision, and gap filling



Base Substitutions and Double Strand Breaks can be formed during crosslink repair

Crosslink hypersensitivity a feature of cells deficient in two groups of genes:

ERCC1/XPF

Recombinational repair:

BRCA1, BRCA2, Rad51, Rad51 paralogues (XRCC2, XRCC3)

essential for integrity of replication process

Mutagenic endpoints of the psoralen-TFO crosslinks

Mutagenesis by free psoralen in mammalian cells is dominated by base substitutions, few deletions

Mutagenesis by triplex-psoralen crosslinks in supF plasmids

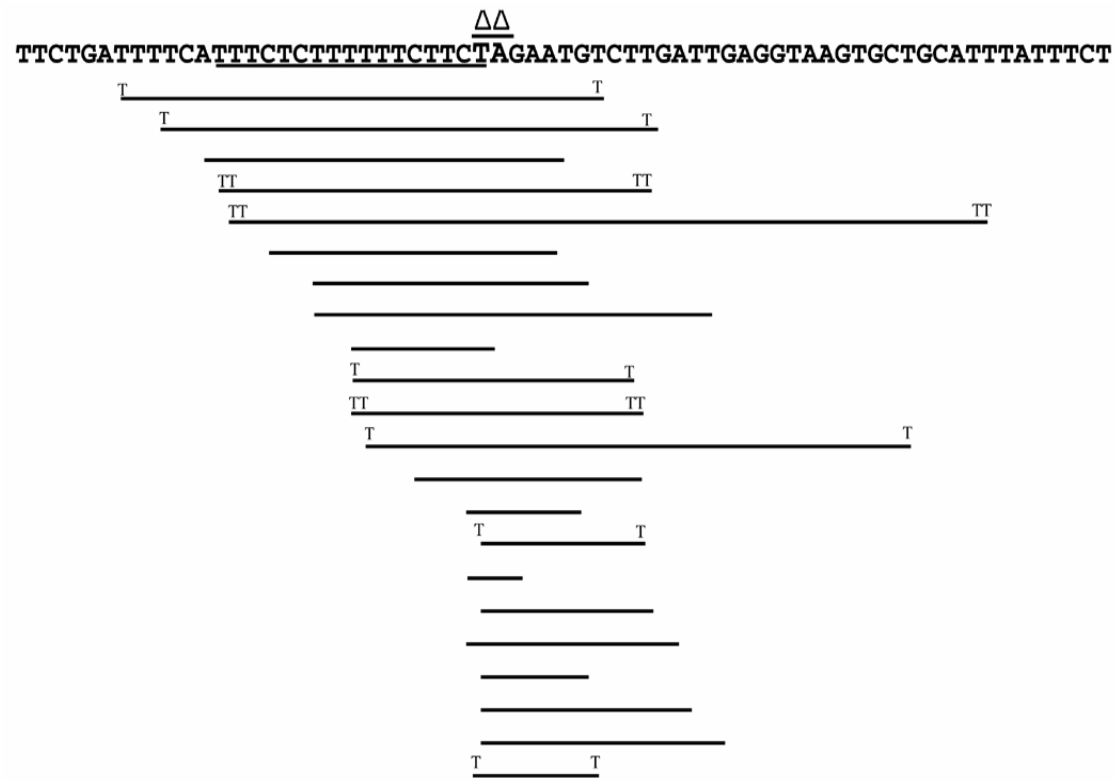
largely base substitutions as well as deletions

Deletion Mutagenesis of Chromosomal Pso-TFO Crosslinks

XbaI **Exon 5**

TTTCTCTTTTTTCT TCT A Gaatgt

……A T……

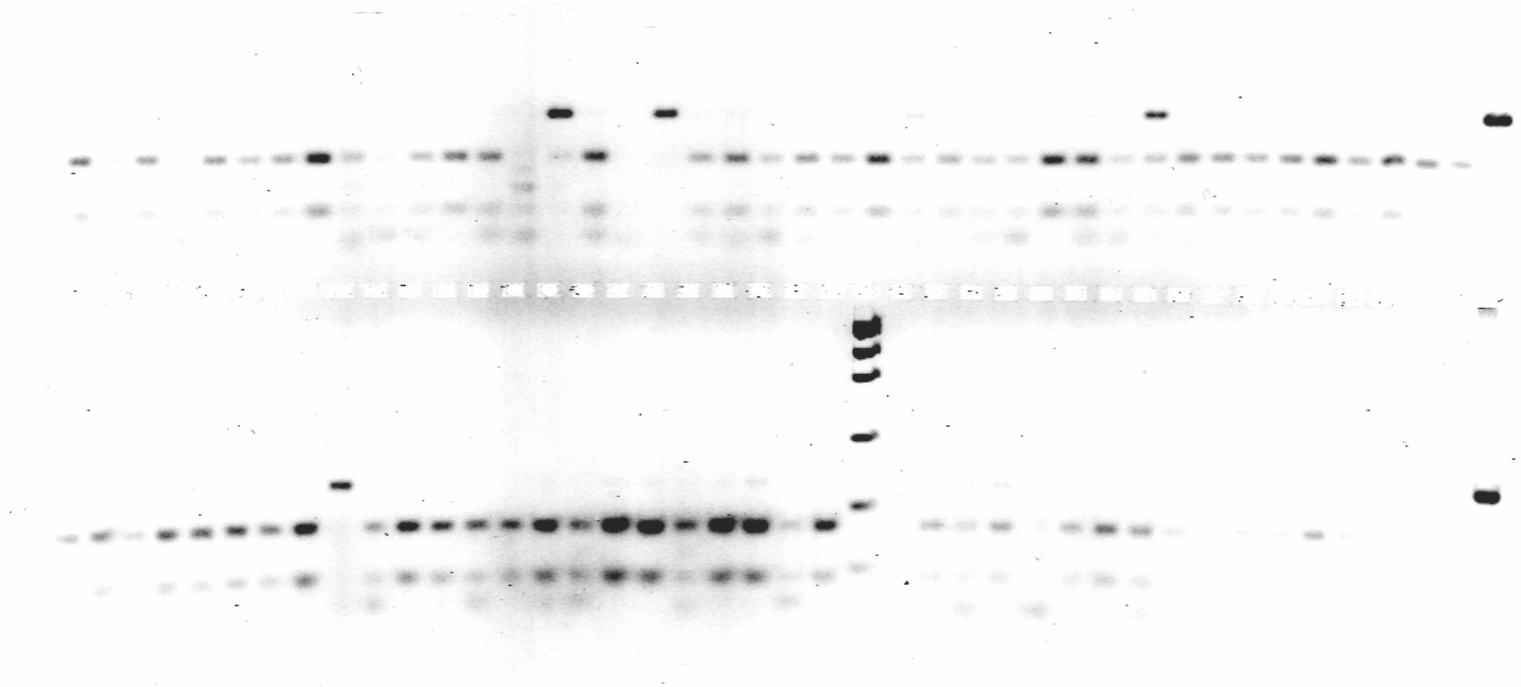


Base Substitution Mutagenesis of Targeted Crosslinks

XbaI Exon 5

TTTCTCTTTTTTCT TCT A Gaatgt

……A T……



C

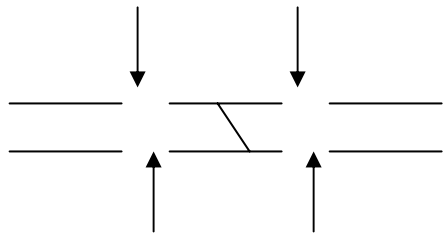
….TTTTCTTCT AGA

Events and Endpoints in TFO-Crosslink Metabolism

RECOGNITION

Single-strand incision

Dual-strand incision



Incisions on template strand for transcription or leading strand synthesis

Targeted XL

25%

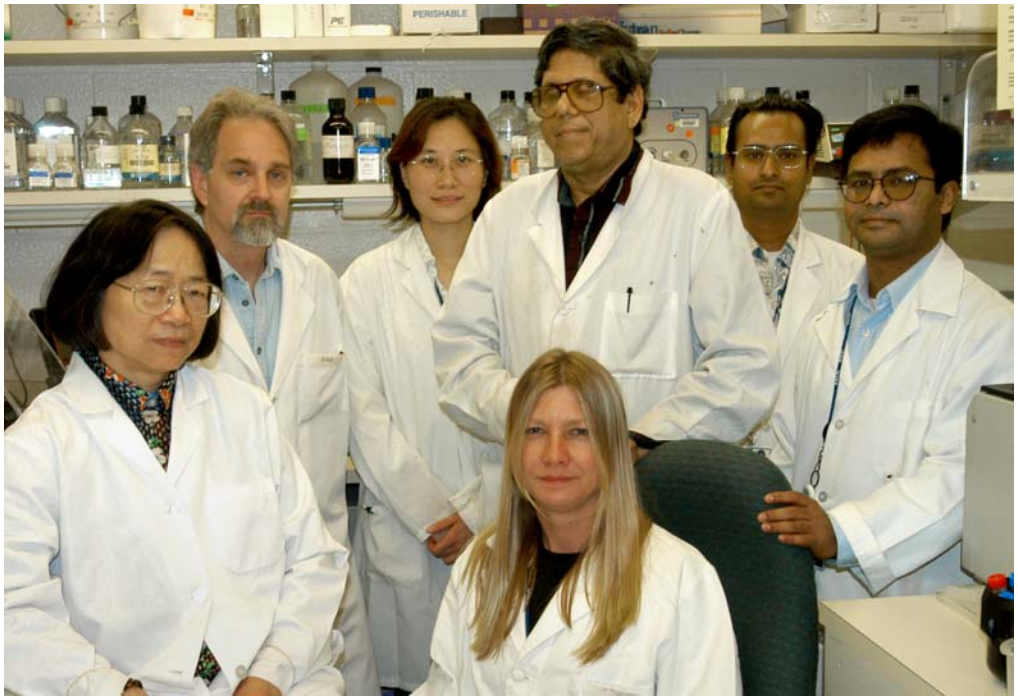
BASE SUBSTITUTION

5%

DELETION

0.2%

Section on Gene Targeting



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