

**FACULTAT DE
FARMÀCIA**



**UNIVERSITAT DE
BARCELONA**

LES PINCES DE POLIPURINES COM A EINA D'EDICIÓ GÈNICA

Anna Solé, Carles Ciudad, Larry Chasin,
Verónica Noé

Departament de Bioquímica i Fisiologia

SUMARI

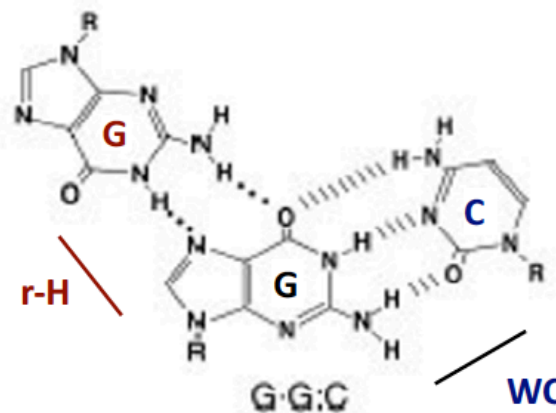
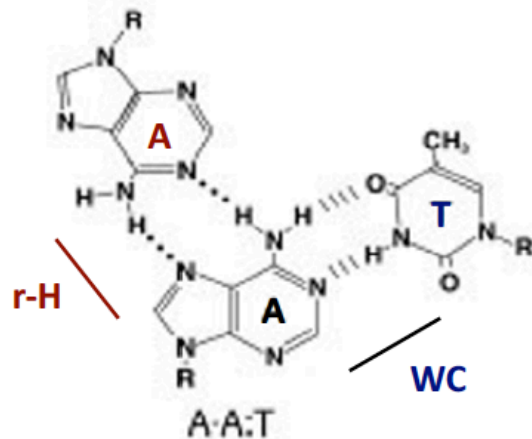
- Qué son els PPRHs?
- Quina aplicació tenen?
- Tipus de mutacions puntuals
- Repair-PPRHs
- Distrofia Muscular de Duchenne DMD
- Editing-PPRHs

PPRHs COM A EINA ALTERNATIVA EN TERAPIA GÈNICA

PPRH hairpin = PolyPurine Reverse-Hoogsteen hairpin

▪ Double-stranded DNA molecule:

- Linked by 5-T loop
- Reverse Hoogsteen bonds between antiparallel purine strands
- Watson-Crick with genomic DNA
- pH-independent, Salts required



Watson-Crick bond ★ Reverse-Hoogsteen bond



LA UNIÓ DEL PPRH AL dsDNA PROVOCA DESPLAÇAMENT DE LA CADENA

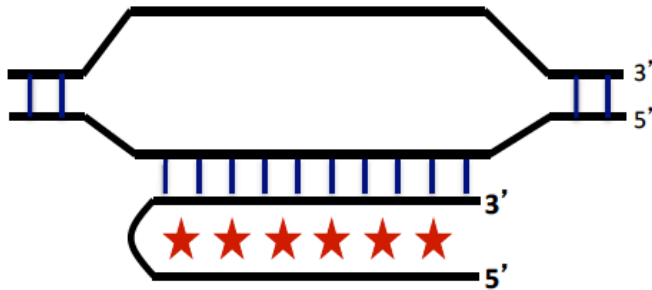
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3' TAATTTCTTACCCCGCCCCACCCTCCCCACC 5'



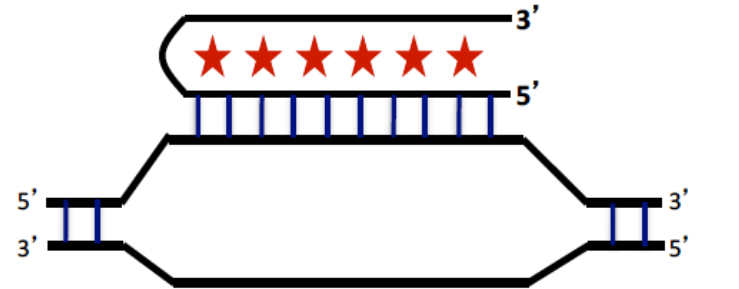
PPRHs COM A EINES DE SILENCIAMENT GÈNIC

Template PPRHs

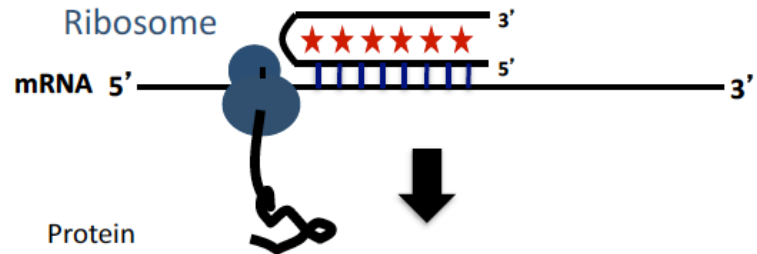


Inhibition of transcription

Coding PPRHs



Ribosome



Protein



Splicing alteration

Gene silencing tool

Watson-Crick bond ★ Reverse-Hoogsteen bond

De Almagro et al. The Journal of Biological Chemistry (2009)
Rodriguez et al. Biochemical Pharmacology (2013)
Rodriguez et al. Molecular Pharmaceutics (2015)
Villalobos et al. Nucleic Acid Therapeutics (2015)
De Almagro et al. Human Gene Therapy (2011)



OBJECTIUS

- DEMOSTRAR LA APLICABILITAT DELS REPAIR-PPRHS PER CORREGIR MUTACIONS PUNTUALS A NIVELL DEL GEN *dhfr* ENDÒGEN EN DIFERENTS LÍNIES CEL·LULARS DE MAMÍFERS.
- AVALUAR LA CAPACITAT DELS EDITING-PPRHS PER EDITAR (i.e. PER GENERAR UNA DELECCIÓ AMPLE) UN MINIGEN *dhfr* ESTABLEMENT TRANSFECTAT EN UNA LÍNIA CEL·LULAR DE MAMÍFER.

TIPUS DE MUTACIONS PUNTUALS (I)

Wild Type		Substitution			
		Synonymous	Nonsense	Missense	
				Conservative	Non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
Protein level	Lys	Lys	STOP	Arg	Thr

Wild type



BEAST

Substitution



FEAST

TIPUS DE MUTACIONS PUNTUALS (II)

	Wild type DNA sequence	Insertion	Deletion
DNA level	TTC TCG AGT	TTC GTC GAG	TTC TGA GTA
mRNA level	AAG AGC UCA	AAG CAG CUC	AAG ACU CAU
Protein level	Lys Ser Ser	Lys Gln Leu	Lys Thr His

Wild type



BEAST

Substitution



FEAST

Insertion



BR**E**AST



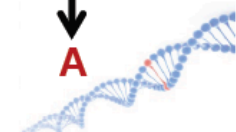
Deletion



BEST



A



MUTACIONS PUNTUALS I MALATIES

Cystic fibrosis

Cystic Fibrosis Transmembrane
conductance Regulator (CFTR)

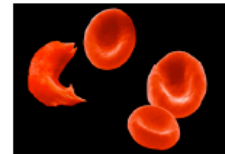
F508del-CFTR



Sickle cell anemia

β -globin gene

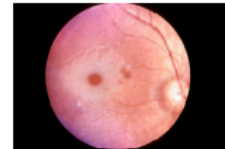
20A>T
Glu6Val



Tay-Sachs disease

Hexaminidase gene (HEXA)

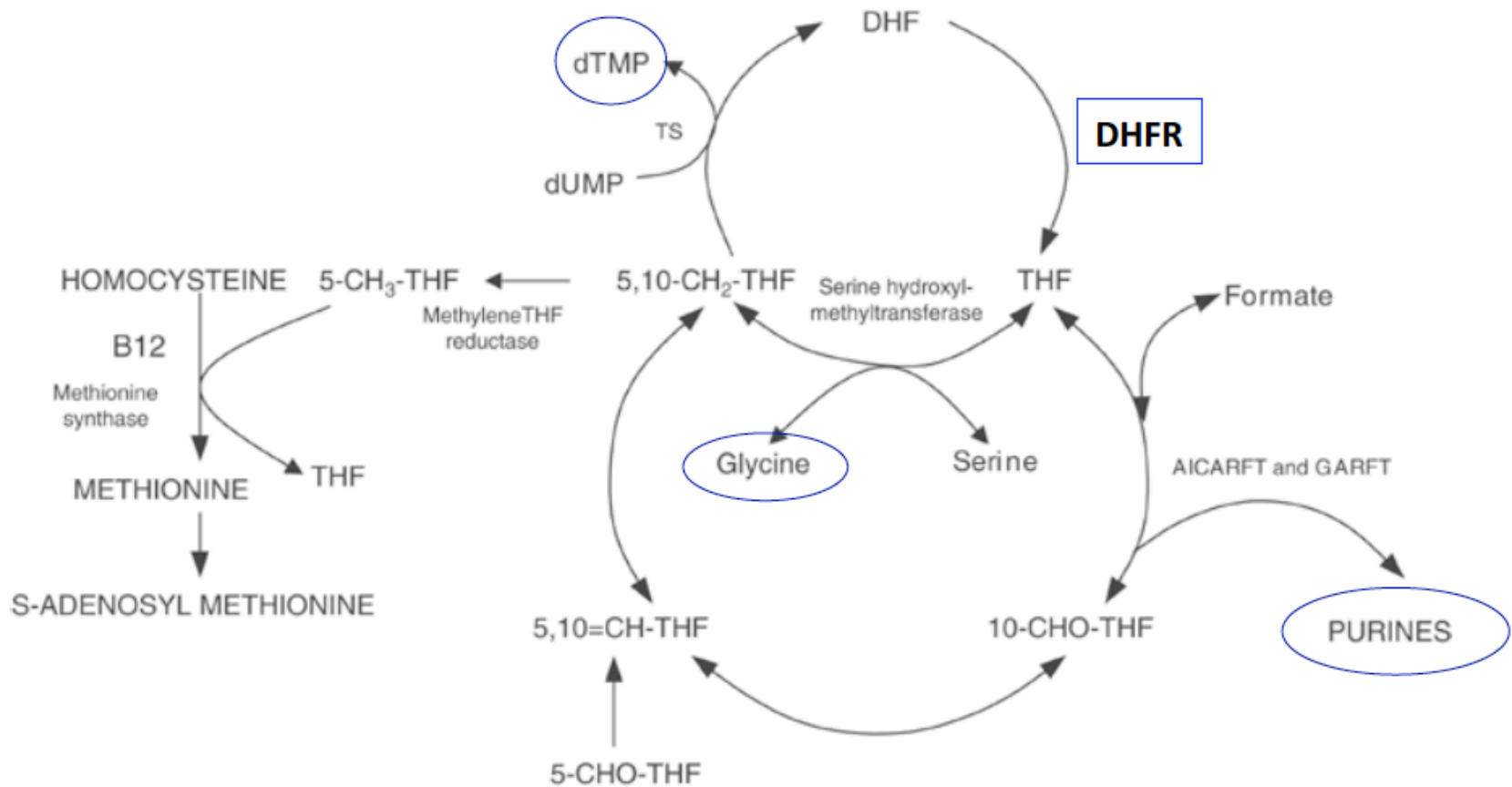
Gly269Ser



Color blindness
Haemochromatosis
Haemophilia
Phenylketonuria
Polycystic kidney disease
Fabry disease
Cancer



EL MODELO DE LA DIHIDROFOLAT REDUCTASA



LÍNIES CEL·LULARS MUTANTS

UA21: One copy of the *dhfr* gene

Cell line	Position	Alteration	Base change	Coding change (normal termination is at 562)
DA5	541 (exon 6)	Deletion (-G)	GAA > -AA	Opal at 584 in exon 6
DA7	235 (exon 3)	Substitution	GAG > TAG	Amber in place
DI33A	493 (exon6)	Insertion (+G)	GGG / GGGG	Opal at 505
DP12B	370 – 2 (intron 4)	Substitution	ag > tg	Exon 5 skipped Opal at 504
DU8	136 +1 (exon2/ intron2)	Double substitution	G/gt > A/at	Exon 2 skipped Opal at 139
DF42	541 (exon 6)	Substitution	GAA > TAA	Ochre in place



REPAIR-PPRHs

Sequences of repair-PPRHs against the *dhfr* gene.

Sequence	Total length (nt)	Name	Cell line
5' CATCAAGTATAAAATTT G AAGTCTATGAGAAGAAAGGCTAACAGAAAAGA) 5T 3' GAGAAGAAAGGCTAACAGAAAAGA)	76	HpDE6rep	DA5/DF42
5' GATATAATTAAGACAGGAAA) 5T 3' GAATGAACTCGAGA G ATGACTCTTGATATAATTAAGACAGGAAA)	69	HpDE3rep	DA7
5' GCCTGGCTGATTCATGGCTTCC T TTAAAATAAAAATAGAAGG) 5T 3' AAAATAAAAATAGAAGG)	62	HpDI4-1rep	DP12B
5' CTGATTCATGGCTTCC T TTAAAATAAAAATAGAAGGATAGTGAGAGGAA) 5T 3' ATAGTGAGAGGAA)	66	HpDI4-2rep	
5' TGATTCATGGCTTCC T TTAAAATAAAAATAGAtttttGAAAGACAAAGAGACTAGAAGA) 5T 3' GAAAGACAAAGAGACTAGAAGA)	85	SDR-HpDI4-3rep	
5' GGACTTCAGAAAGGACCCCTGGGTACCTGGAAAAGAAAAGGA) 5T 3' GGAAAAGAAAAGGA)	61	HpDE6-4rep	DI33A
5' CCTTTTCTTTTCAGGTACCCAGGGTCTTTCTGAAGTCCAGGAGGAAAAAGGCA) 5T 3' AGGAGGAAAAAGGCA)	76	HpDE6-5rep	
5' CTTAACCCCAAATTA CC TTCCACTGAGGAGGTGGTGG) 5T 3' GAGGAGGTGGTGG)	55	HpDE2-1rep	DU8
5' CCTCAGTGGA GG TAATTTGGGGTTAAGATGAGGA) 5T 3' AAGATGAGGA)	50	HpDE2-2rep	
5' ATCAAGTATAAAATTT G AAGTCTATGAGAAGAttttAGAGATGGGAGCAGGTGGAGGA) 5T 3' AGAGATGGGAGCAGGTGGAGGA)	85	LDR-HpDE6-1rep	DF42

The names and sequences of the repair-PPRHs are indicated, as well as the cell line used. The corresponding corrected nucleotide in the repair-PPRHs is in bold and bigger size. Bulleted regions (•) represent reverse-Hoogsteen bonds.

HIPÒTESI DE TREBALL

Dup*

Dup*

Dup*

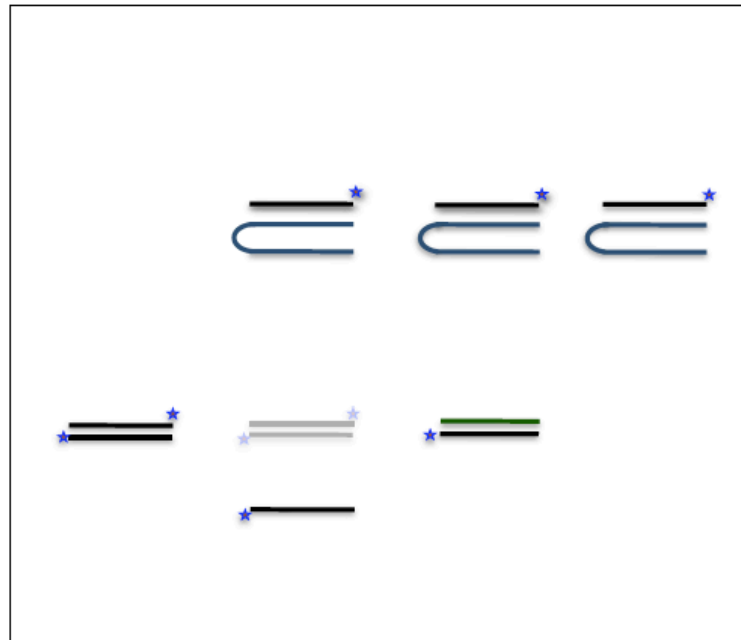
pPy*

PPRH

PPRH

PPRH

RO

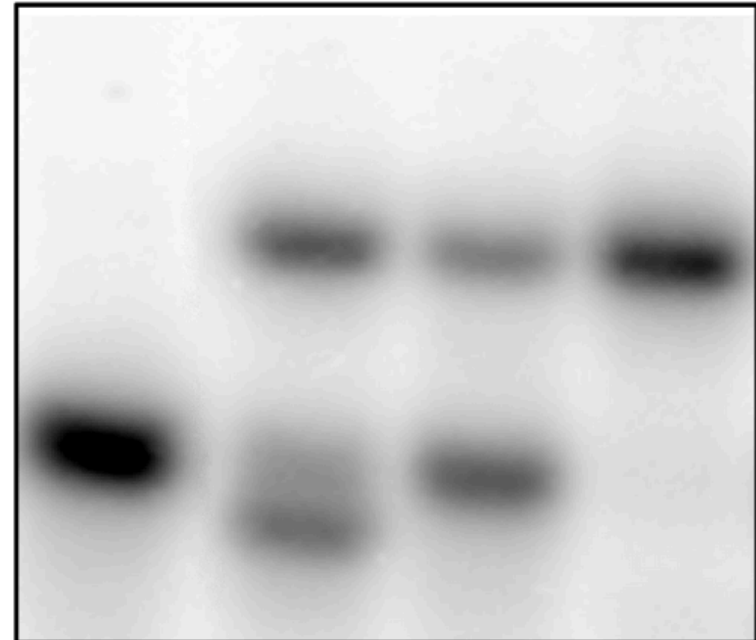


1

2

3

4



1

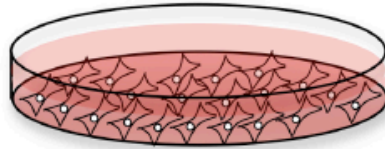
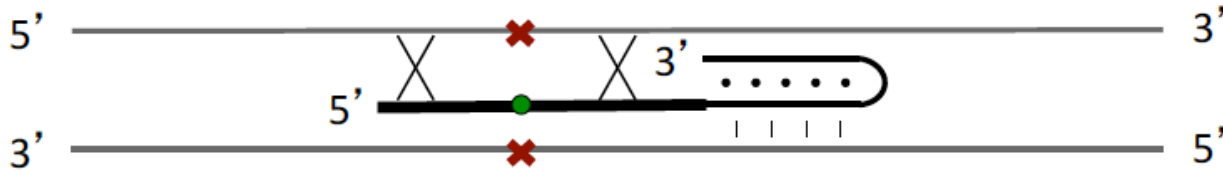
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3

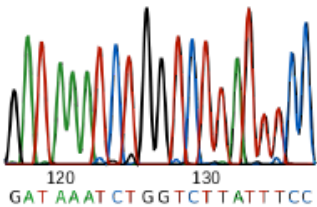
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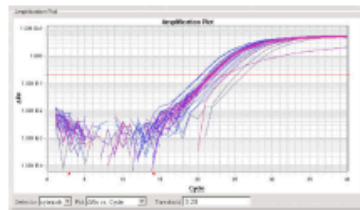
DISSENY EXPERIMENTAL



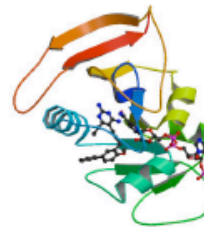
Sequencing



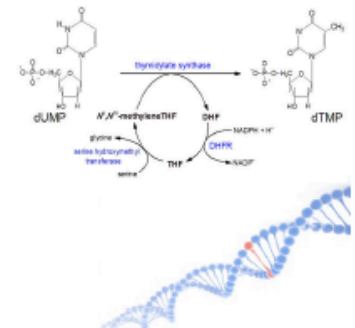
DHFR mRNA levels



DHFR protein levels



DHFR activity

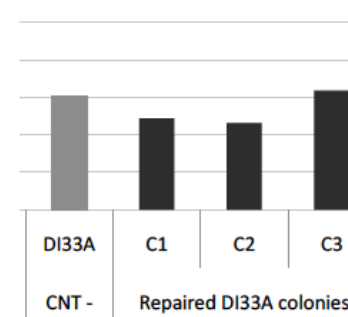
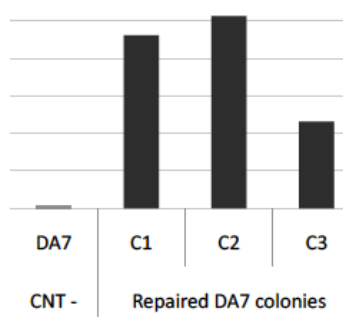
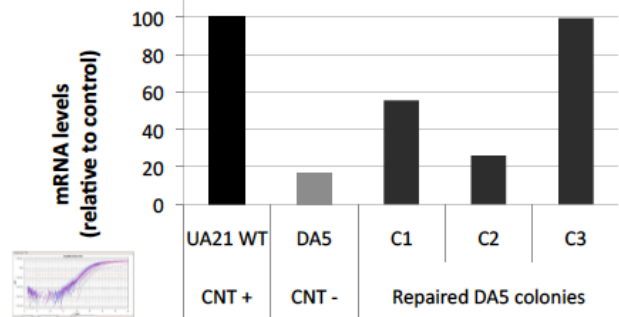
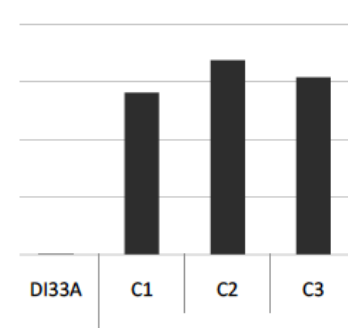
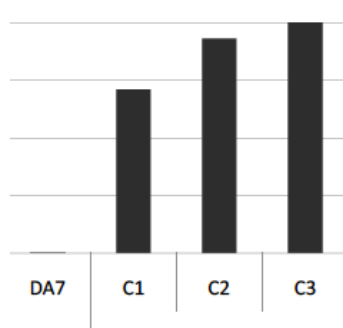
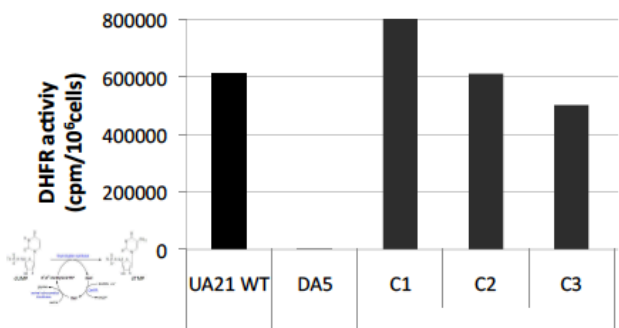
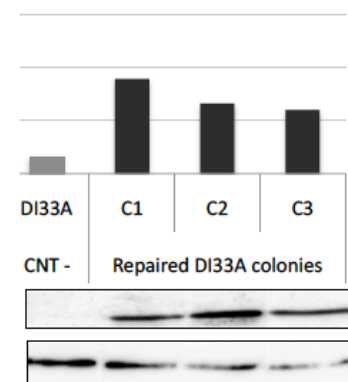
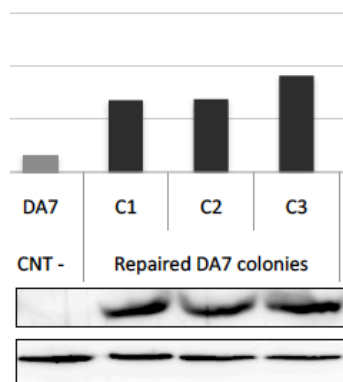
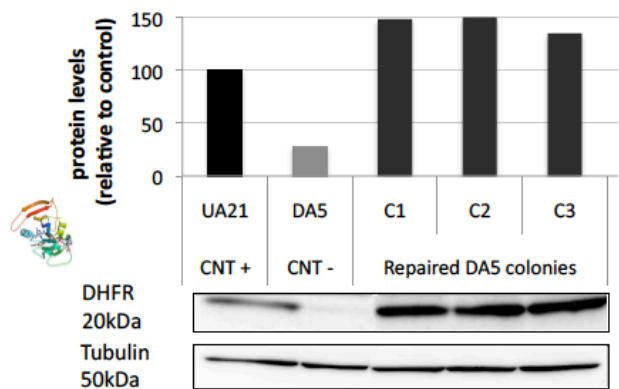


REPARACIÓ DE MUTACIONS PUNTUALS A LA SEQÜÈNCIA CODIFICANT

Deletion

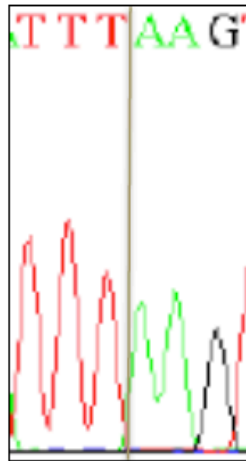
Substitution

Insertion



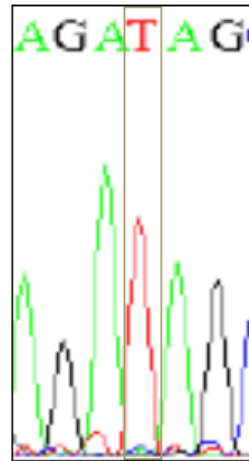
RESULTATS DE LA SEQÜENCIACIÓ DEL DNA

Deletion



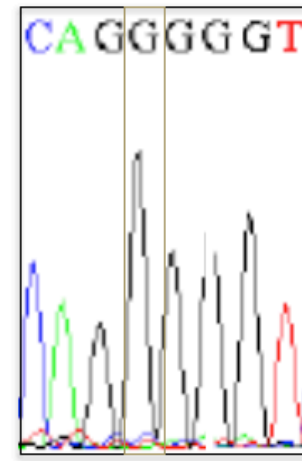
Mutant DA5

Substitution

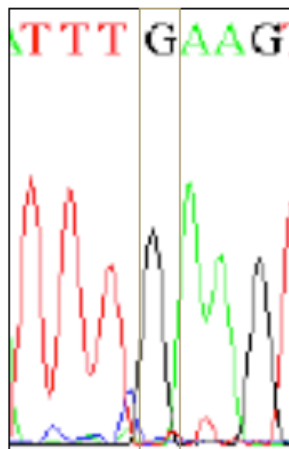


Mutant DA7

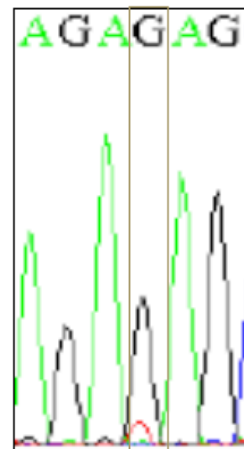
Insertion



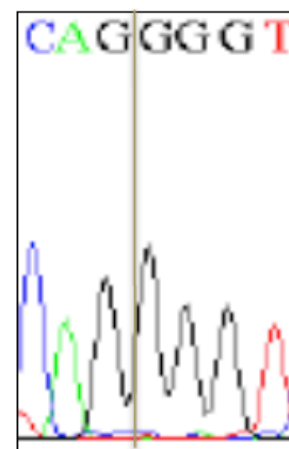
Mutant DI33A



Repaired DA5



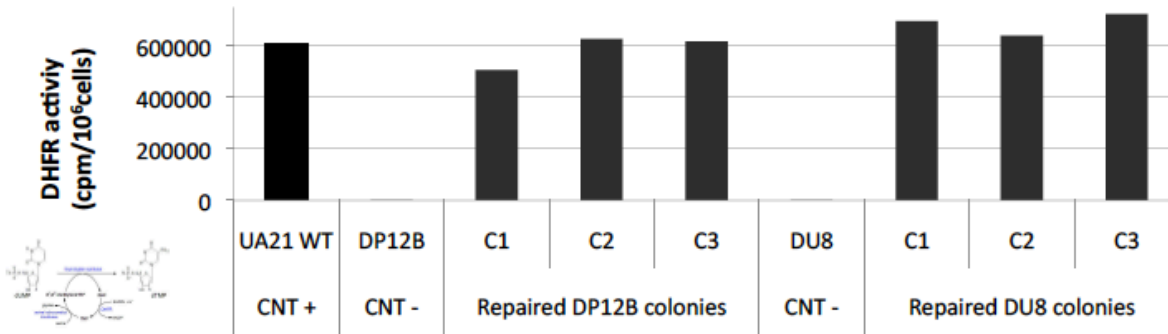
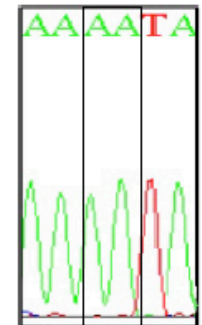
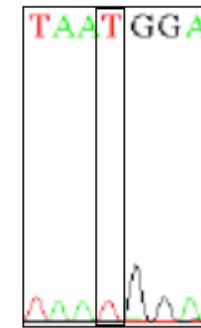
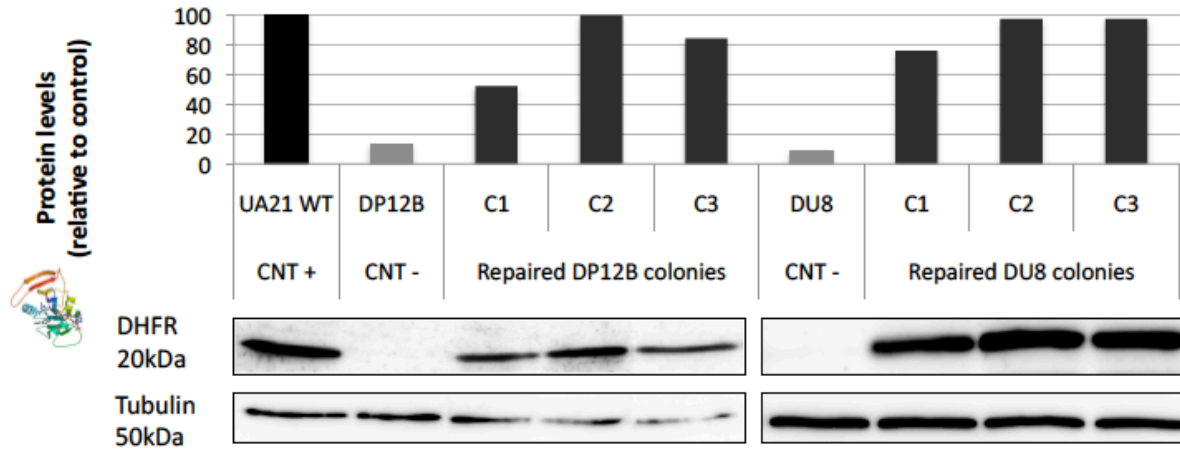
Repaired DA7



Repaired DI33A

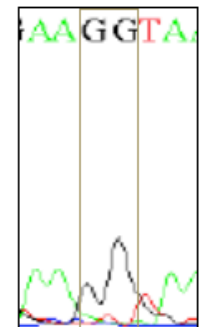
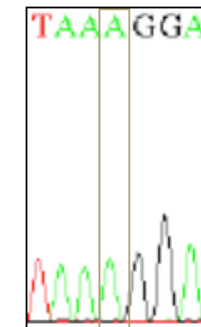
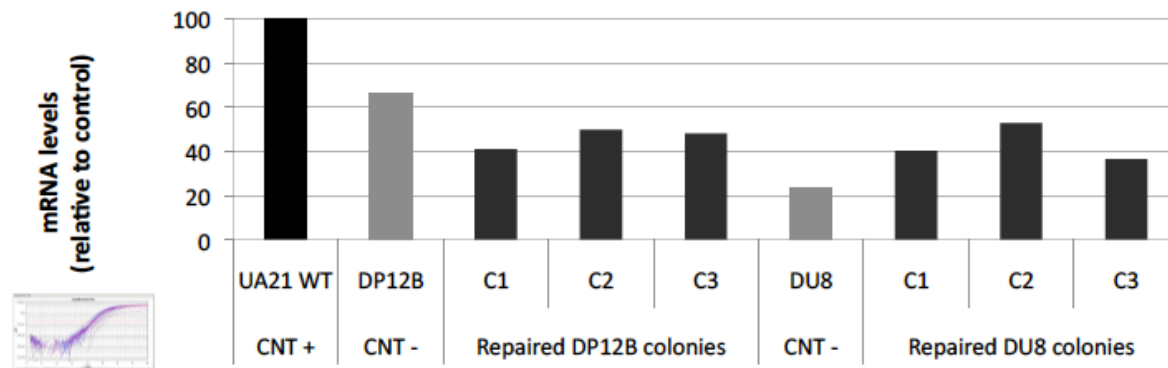
Substitution in an intron

Double substitution



Mutant DP12B

Mutant DU8



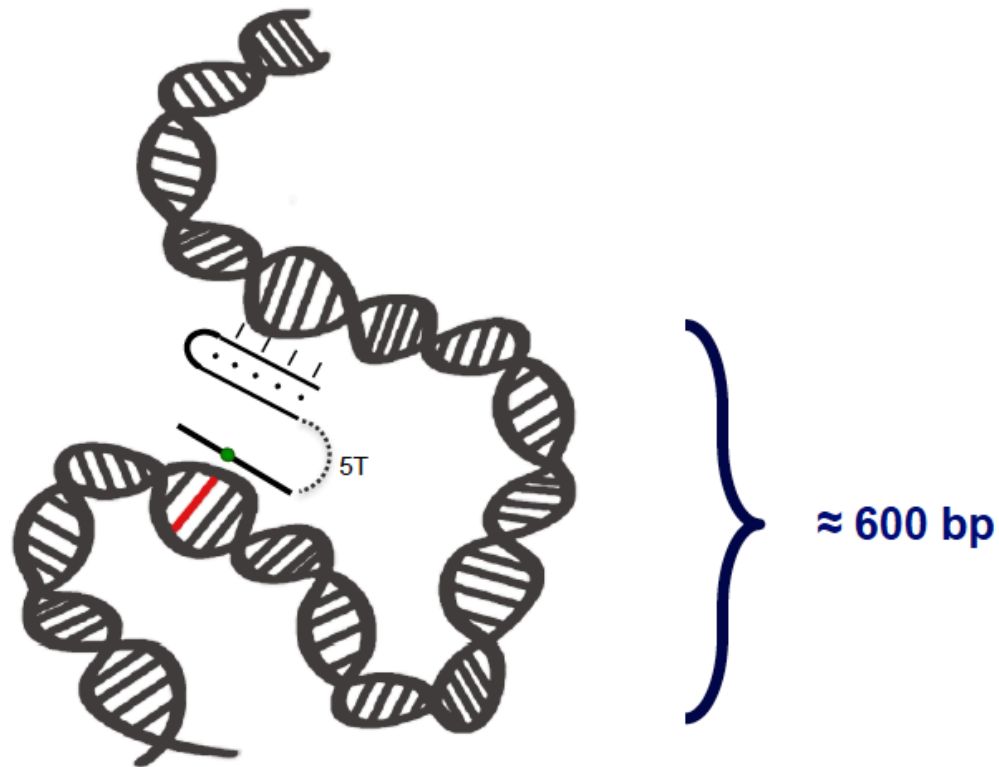
Repaired DP12B

Repaired DU8



DISSENY DE REPAIR-PPRHs A DISTÀNCIA

Long Distance Repair-
PPRH (LDR-PPRH)

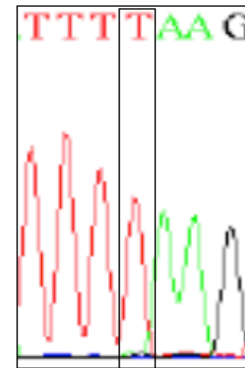
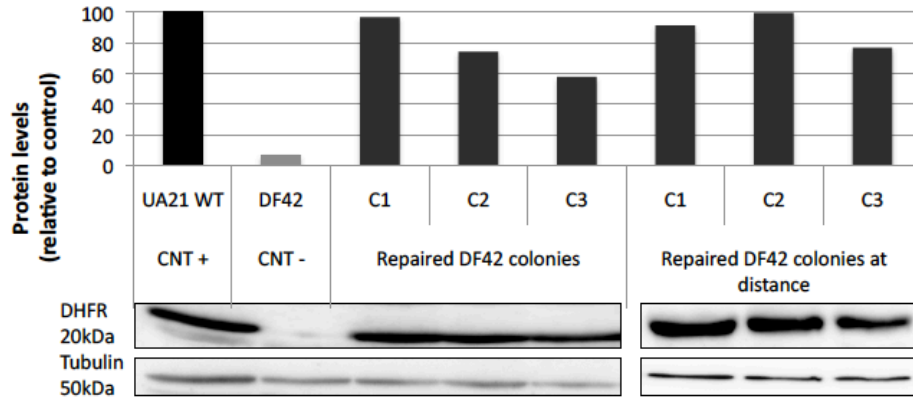


REPAIR-PPRHs A DISTÀNCIA

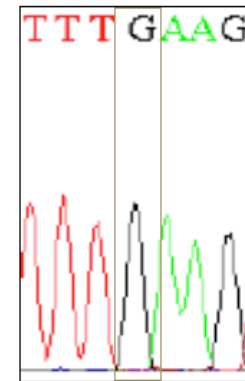
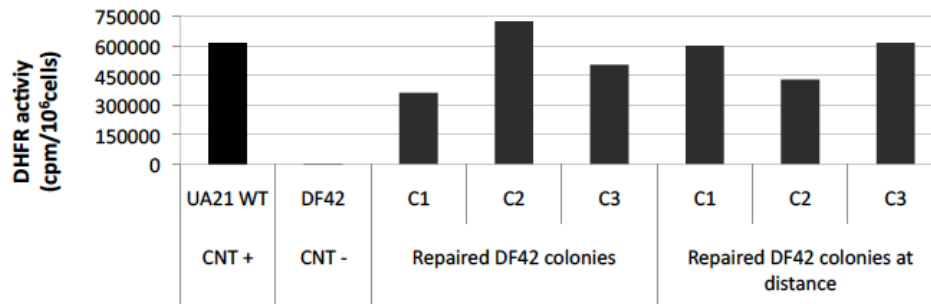
Substitution

Substitution at distance

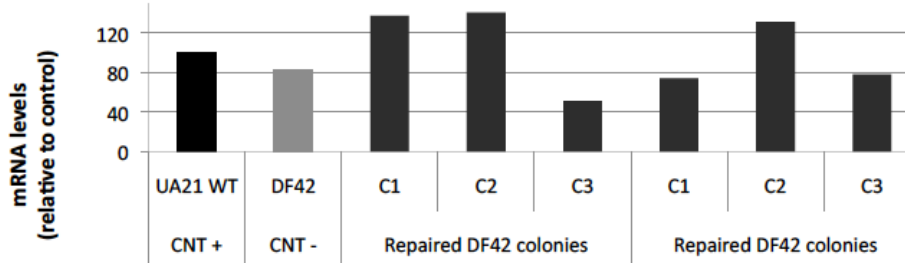
RESULTS



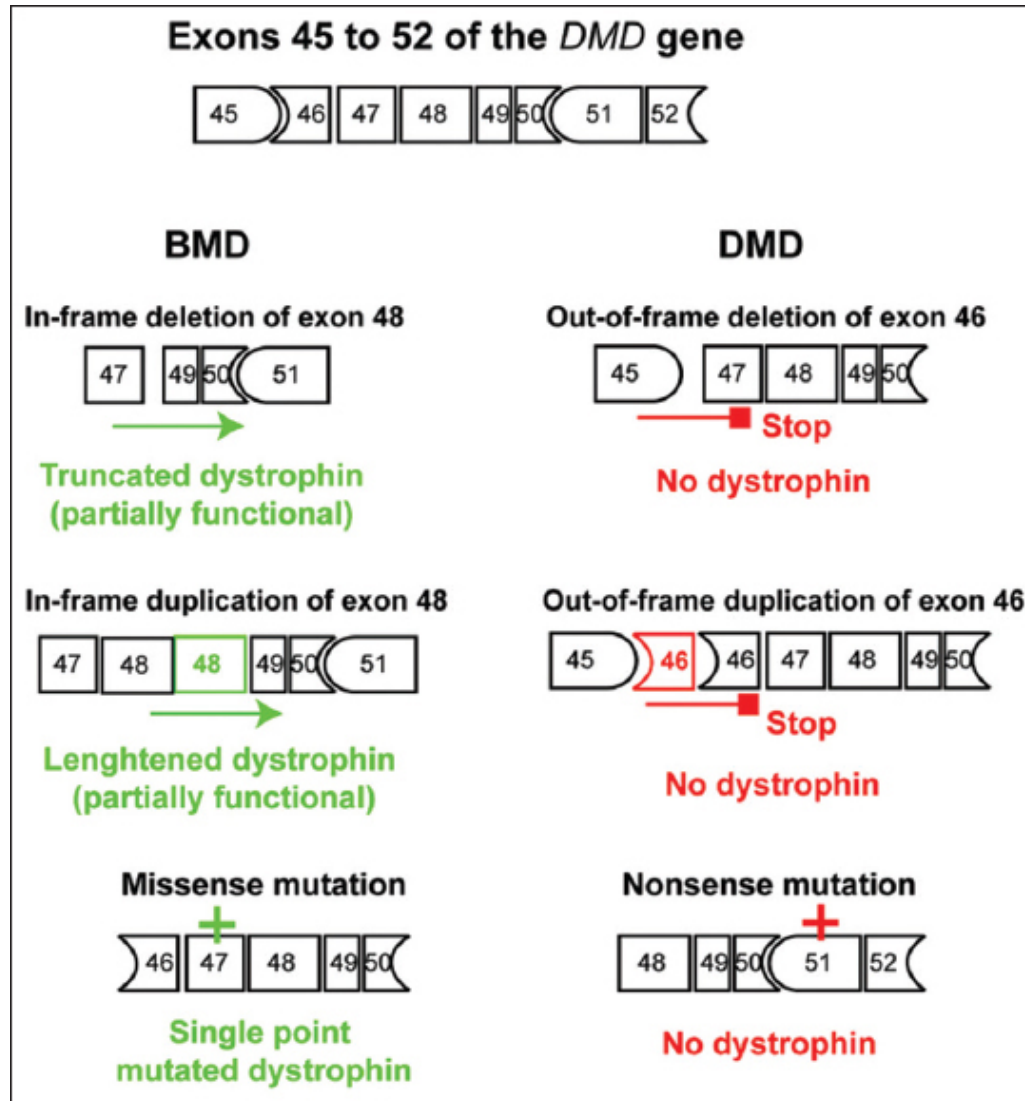
Mutant DF42



Repaired DF42

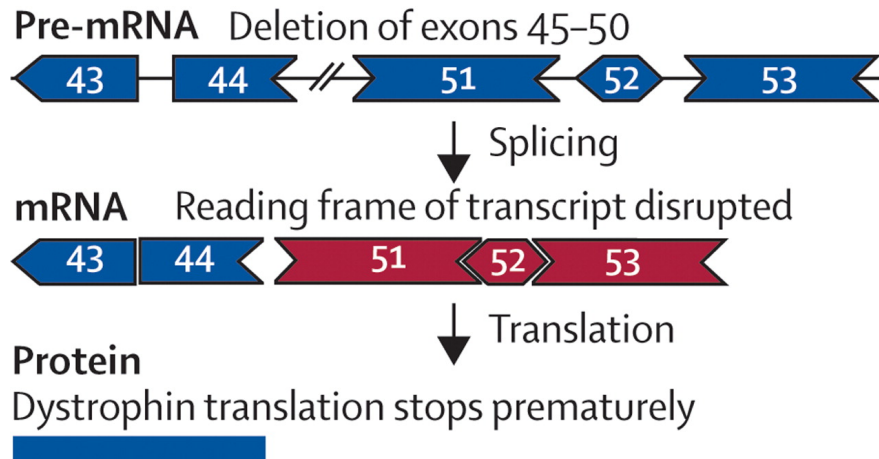


DISTROFIES MUSCULARS DE DUCHENNE I DE BECKER

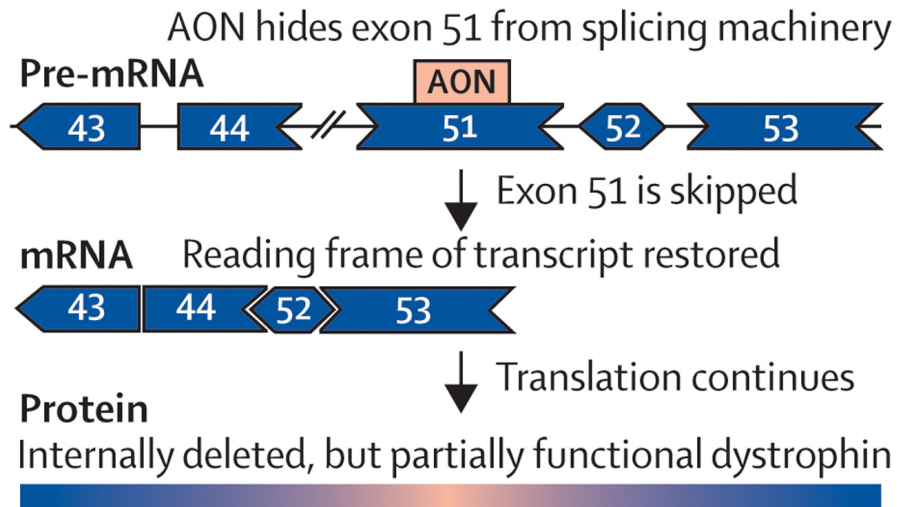


ESTRATÈGIA D'EXON SKIPPING COM A TERAPIA PER DMD

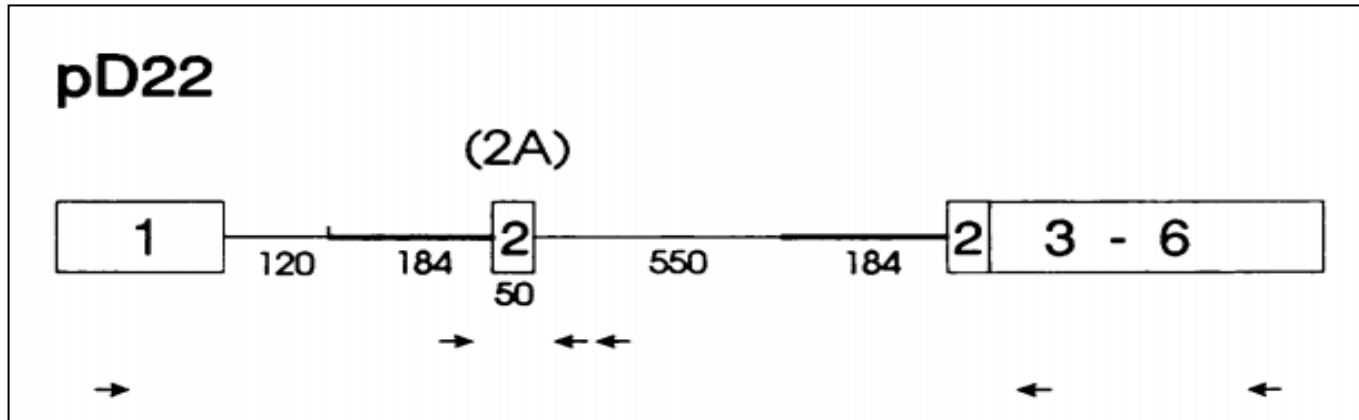
Duchenne muscular dystrophy



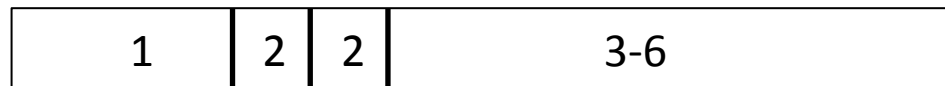
Exon skipping to reframe transcripts



ESTRUCTURA DEL MINIGEN *dhfr* pD22



↓ TRANSCRIPTION



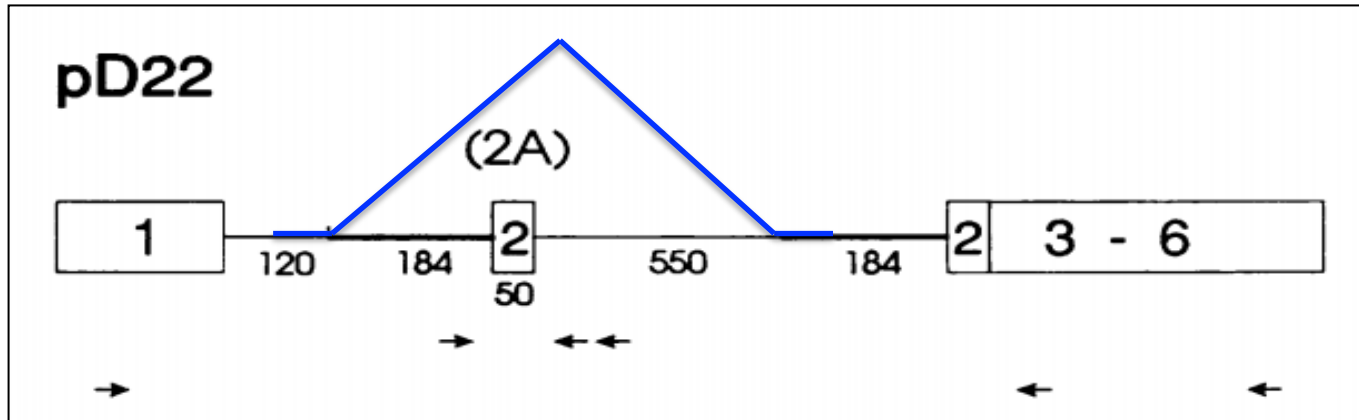
↓ UNEFFICIENT TRANSLATION

NO DHFR PROTEIN



CELL DEATH IN SELECTIVE MEDIUM -GHT

HIPÒTESI DE TREBALL



↓ TRANSCRIPTION

1-2-3-4-5-6

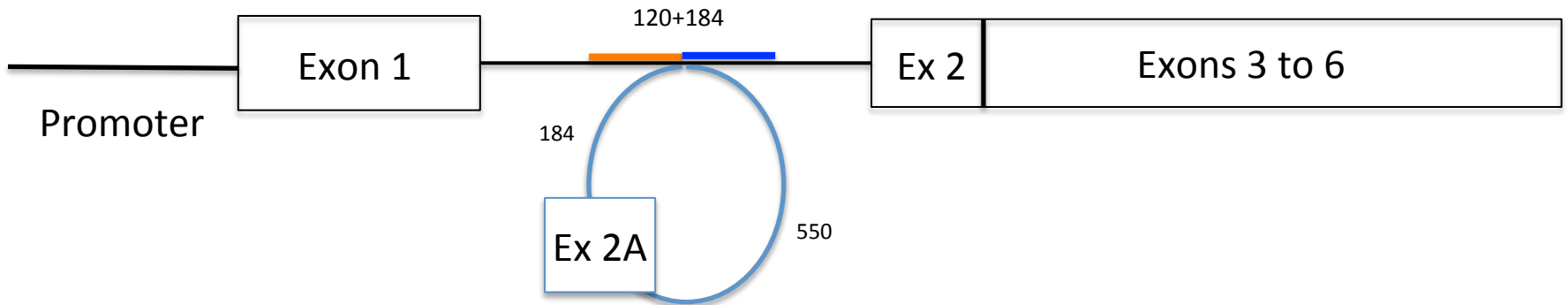
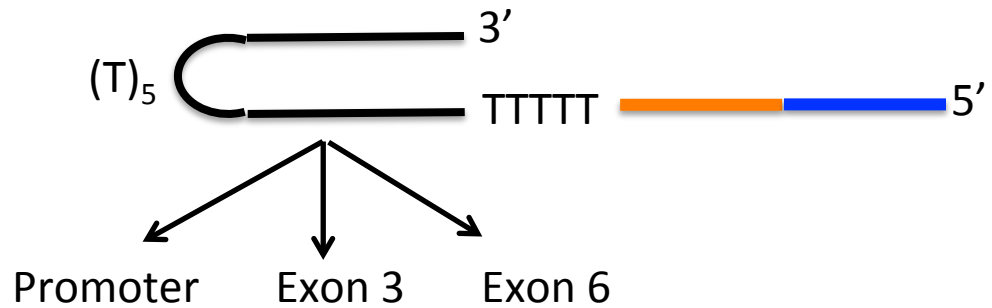
↓ TRANSLATION

DHFR PROTEIN



CELL SURVIVAL IN SELECTIVE MEDIUM -GHT

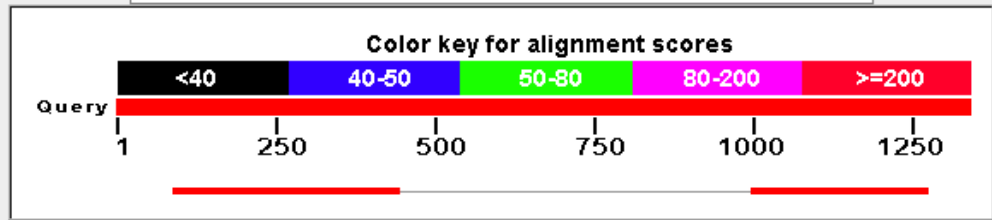
DISSENY DELS EDITING-PPRHS



CARACTERITZACIÓ DE LES COLÒNIES EDITADES

Distribution of 2 Blast Hits on the Query Sequence

Mouse over to see the define, click to show alignments



Download Graphics Sort by: E value

151023-48_D21_1587ZAA050.ab1

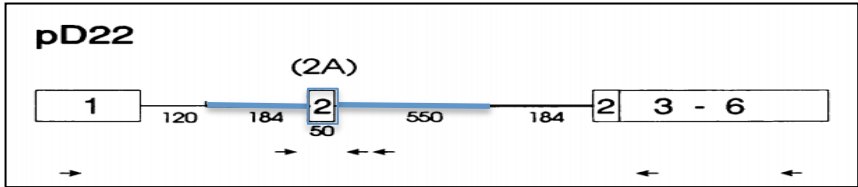
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Range 1: 12 to 364 Graphics Next Match Previous Match

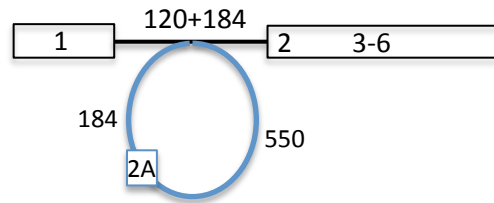
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Sbjct 12	TGGCTGGATTGGGTTAGG-AACCGAGGCGGTTTCGCTGAATCGGGTCGAGCACTTGGCGG	70		
Query 151	AGACGGCCGGCCAACTACTTAGGGACAGTCATGAGGGCTAGGCCCGCCGGCTGCAGCCC	210		
Sbjct 71	AGACGGCCGGCCAACTACTTAGGGACAGTCATGAGGGCTAGGCCCGCCGGCTGCAGCCC	130		
Query 211	TTGCCCATGCCCGGGTATCCCAATGCTGTGCCAGCCTTTGCCAGAGGCGCTCTAGCT	270		
Sbjct 131	TTGCCCATGCCCGGGTATCCCAATGCTGTGCCAGCCTTTGCCAGAGGCGCTCTAGCT	190		
Query 271	GGGAGCAAAGTCCGGTCACTGGGCAGCACACCCCGGACTTGCATGGGTAGCCGCTGA	330		
Sbjct 191	GGGAGCAAAGTCCGGTCACTGGGCAGCACACCCCGGACTTGCATGGGTAGCCGCTGA	250		
Query 331	GATGGAGCCTGAGCACACGTGACAGGGTCCCTGTTAACGCAGTGTTCCTCTAACTTTCAG	390		
Sbjct 251	GATGGAGCCTGAGCACACGTGACAGGGTCCCTGTTAACGCAGTGTTCCTCTAACTTTCAG	310		
Query 391	GAACGAATCAAGTACTTCCAAAGAATGACCACCACCTCCTCAGTGGAAAGGTAA	444		
Sbjct 311	GAACGAATCAAGTACTTCCAAAGAATGACCACCACCTCCTCAGTGGAAAGGTAA	364		

Range 2: 127 to 401 Graphics Next Match Previous Match First Match

Score	Expect	Identities	Gaps	Strand
508 bits(275)	4e-148	275/275(100%)	0/275(0%)	Plus/Plus
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Sbjct 127	GCCCTTGCCATGCCCGGGTATCCCAATGCTGTGCCAGCCTTTGCCAGAGGCGCTCT	186		
Query 1051	AGCTGGGAGCAAAGTCCGGTCACTGGGCAGCACACCCCGGACTTGCATGGGTAGCCG	1110		
Sbjct 187	AGCTGGGAGCAAAGTCCGGTCACTGGGCAGCACACCCCGGACTTGCATGGGTAGCCG	246		
Query 1111	CTGAGATGGAGCCTGAGCACACGTGACAGGGTCCCTGTTAACGCAGTGTTCCTCTAACT	1170		
Sbjct 247	CTGAGATGGAGCCTGAGCACACGTGACAGGGTCCCTGTTAACGCAGTGTTCCTCTAACT	306		
Query 1171	TCAGGAACGAATCAAGTACTTCCAAAGAATGACCACCACCTCCTCAGTGGAAAGGTAAAC	1230		
Sbjct 307	TCAGGAACGAATCAAGTACTTCCAAAGAATGACCACCACCTCCTCAGTGGAAAGGTAAAC	366		
Query 1231	AGAACCTGGTGATTATGGGCCGGAAAACCTGGTTC	1265		
Sbjct 367	AGAACCTGGTGATTATGGGCCGGAAAACCTGGTTC	401		

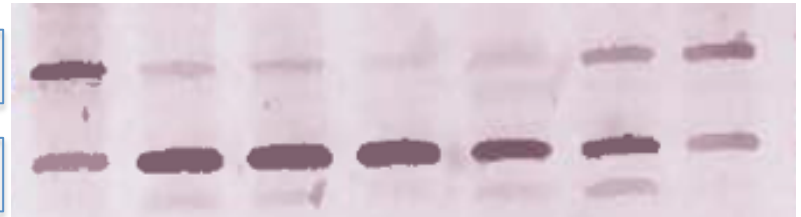


Edited sequences



1 2 2 3-6

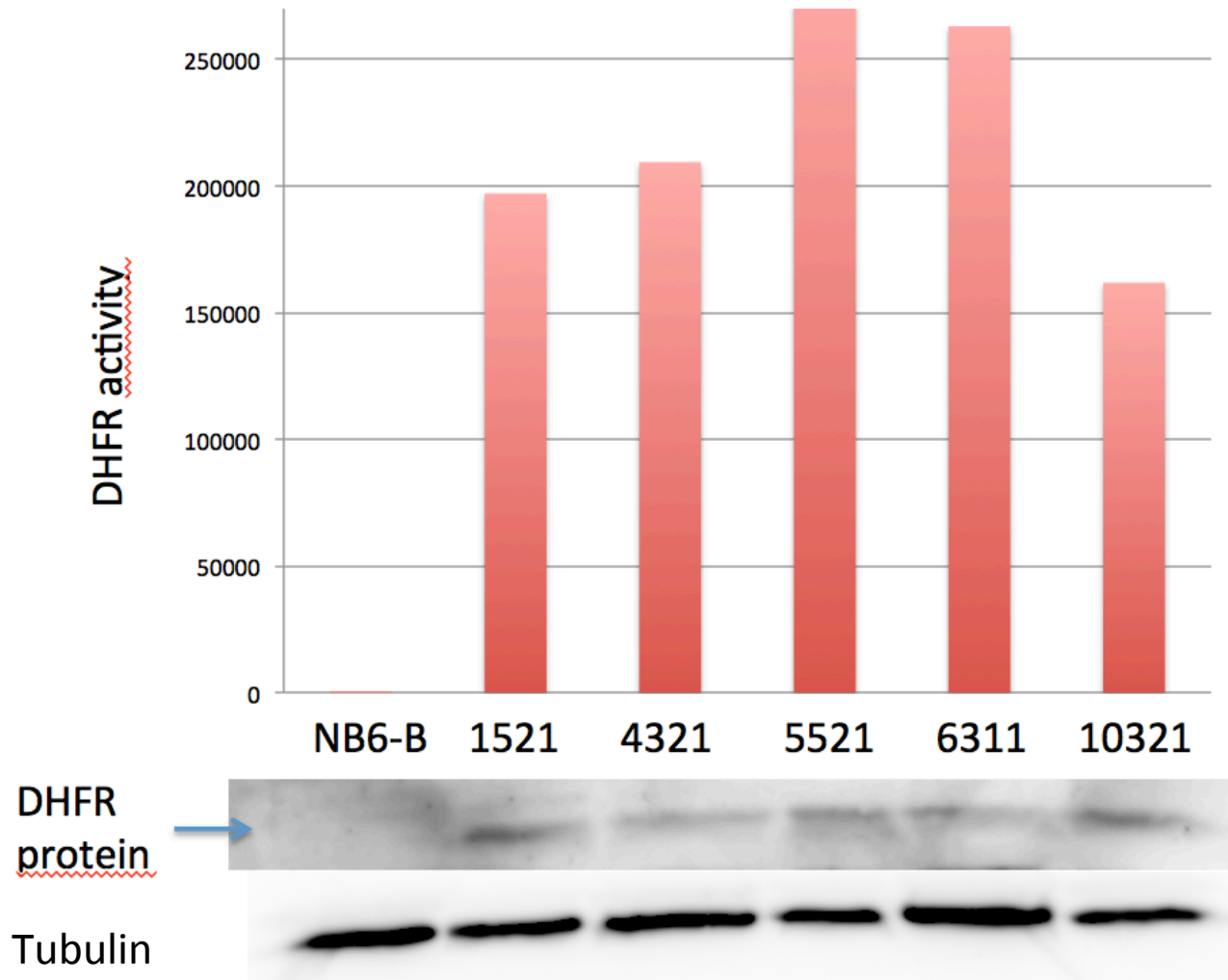
1 2 3-6



Nivells de mRNA per a la DHFR

EDICIÓ DEL MINIGEN *dhfr* EN CÈL.LULES NB6

NIVELLS DE PROTEÏNA DHFR I ACTIVITAT



CONCLUSIONS

REPAIR-PPRHs

Diferents tipus de mutacions puntuals en un gen endògen com delecions, insercions i substitucions simples o dobles es poden corregir mitjançant els Repair-PPRHs en cèl·lules de mamífer.

El nucleòtid corregit s'ha de trobar al mig de la cua reparadora i no gaire proper del cor de PPRH

Els Long-Distance-Repair-PPRHs (LDR-PPRHs) es poden emprar amb èxit en absència d'una seqüència de polipirimidines adient a prop de la mutació puntual.

EDITING-PPRHs

Els Editing-PPRHs son una tecnologia útil per provocar exon skipping a nivell del DNA d'un gen determinat.

GRÀCIES PER LA VOSTRA ATENCIÓ!

