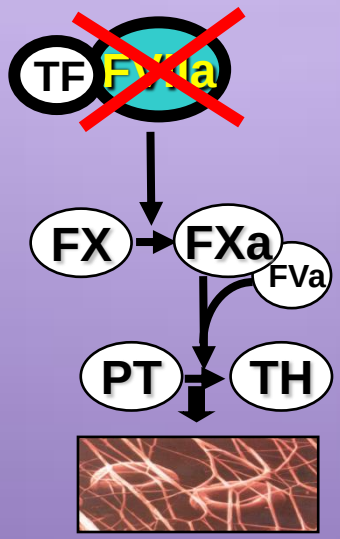
Università degli Studi di Ferrara

UTILIZZO DI U1-snRNA PER LA CORREZIONE DEL DIFETTO SEVERO DI FATTORE VII DELLA COAGULAZIONE

FVII

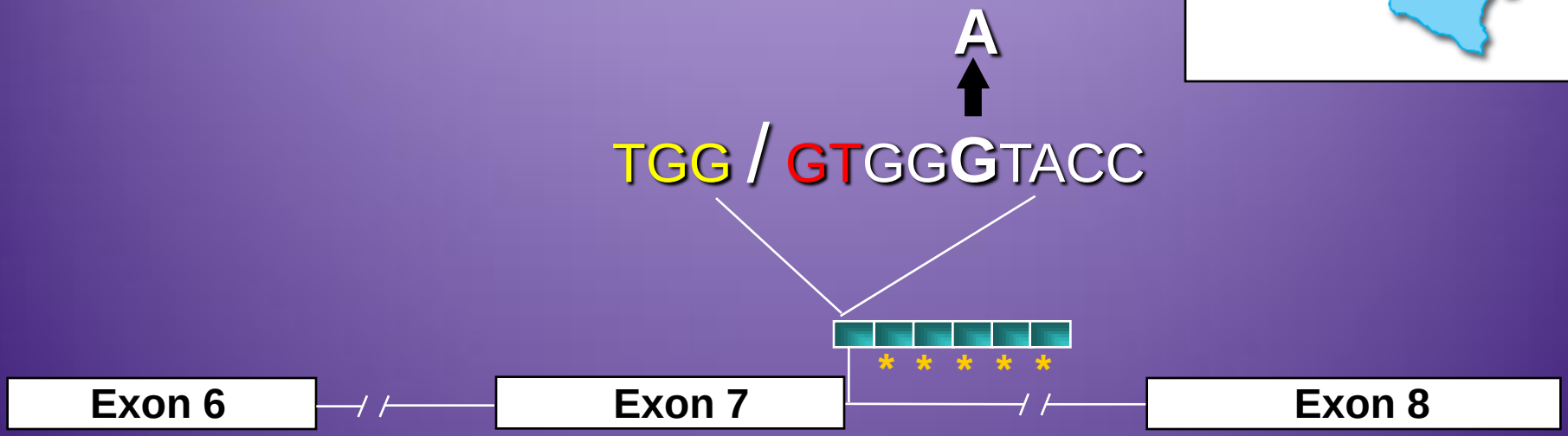


Deficienza congenita di FVII

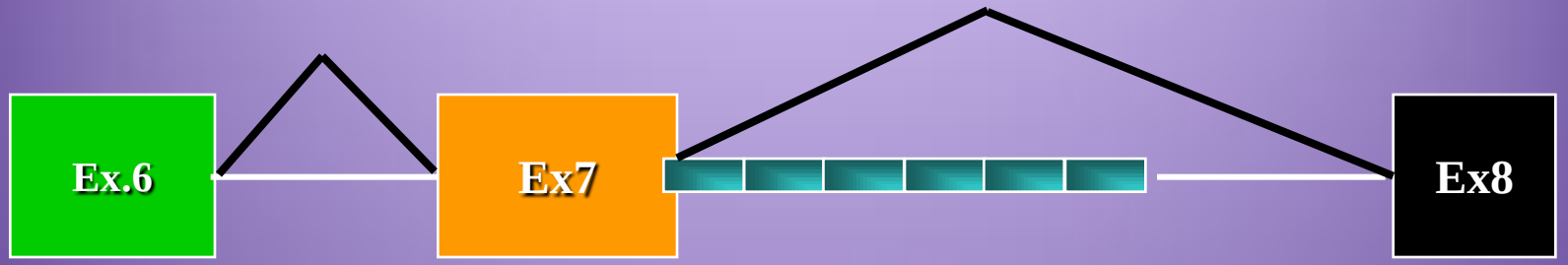
mutazione nel gene del FVII

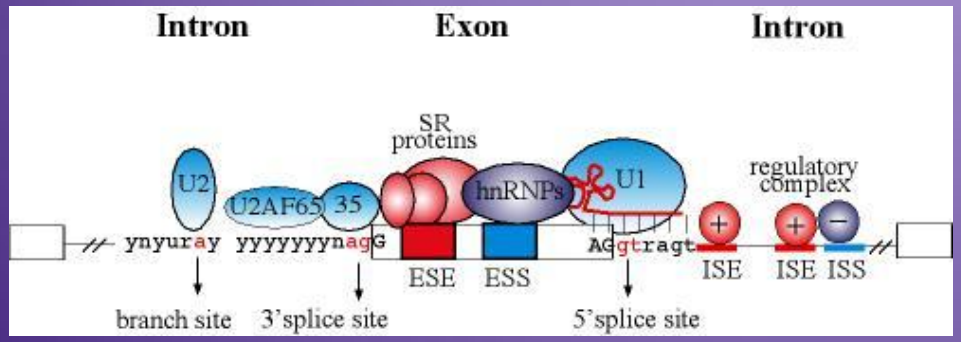
malattia autosomica (cr.13) recessiva a penetranza variabile con frequenza 1/500.000

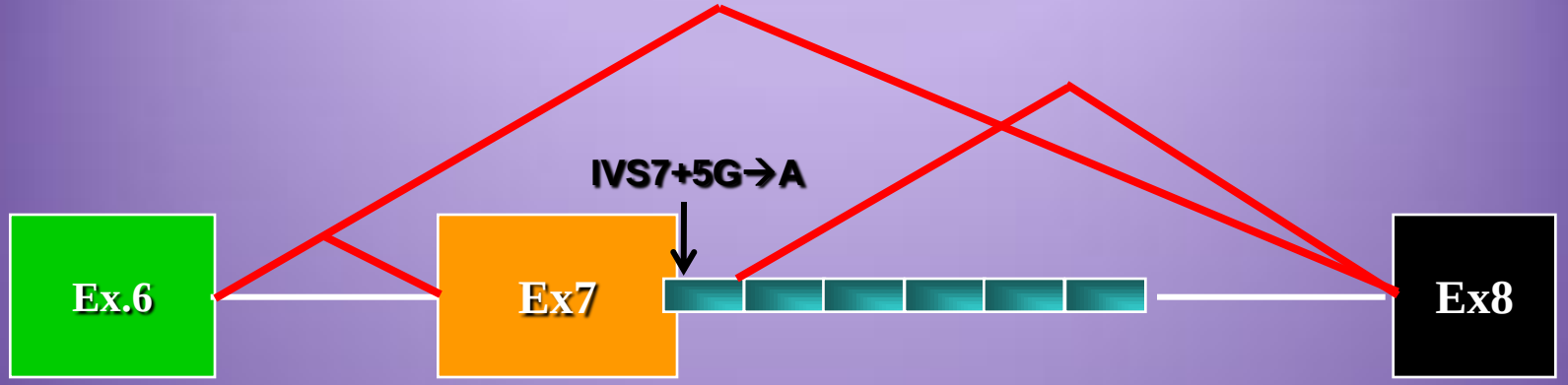
IVS7 +5G→A (FVII Lazio)



IVS7 5'ss is in the 1st of highly homologous 37bp repeats containing identical cryptic 5'ss (*)


Ex.6
Ex7
Ex8


Intron
Exon
Intron
SR proteins
U2
U2AF65
35
hnRNPs
U1
regulatory complex
ynyuray
yyyyyyynagG
AGgtragt
ESE
ESS
ISE
ISE
ISS
branch site
3'splice site
5'splice site


IVS7+5G→A
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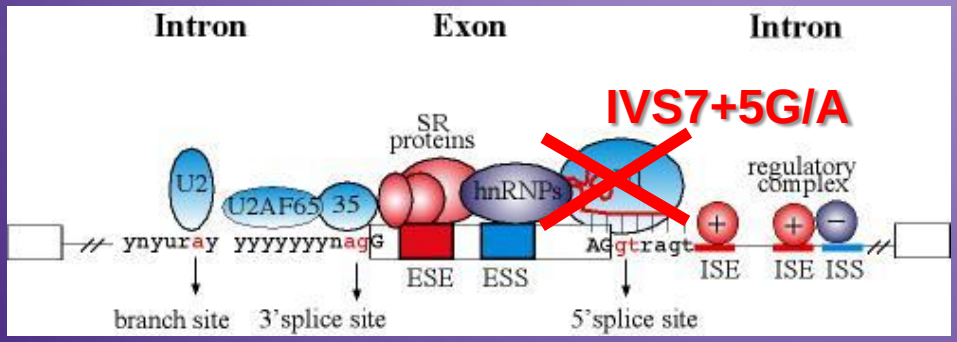
Intron
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IVS7+5G/A
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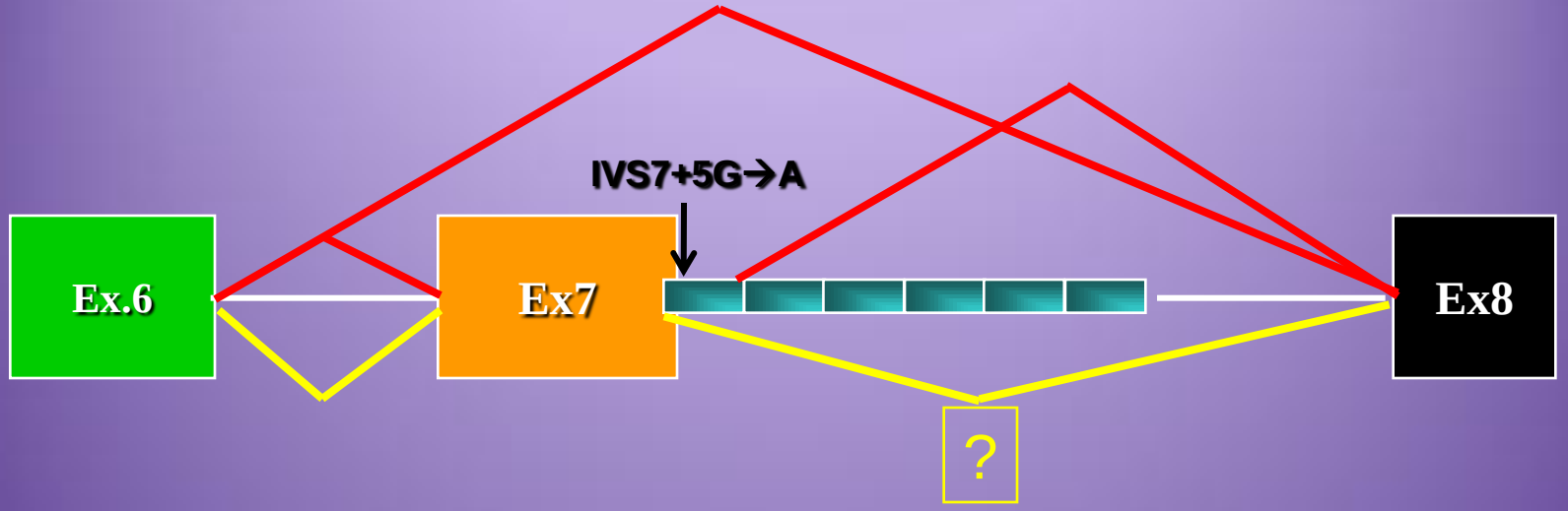
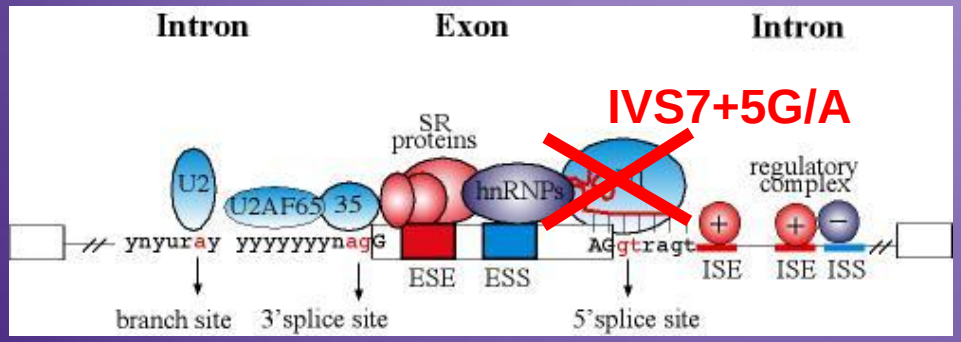
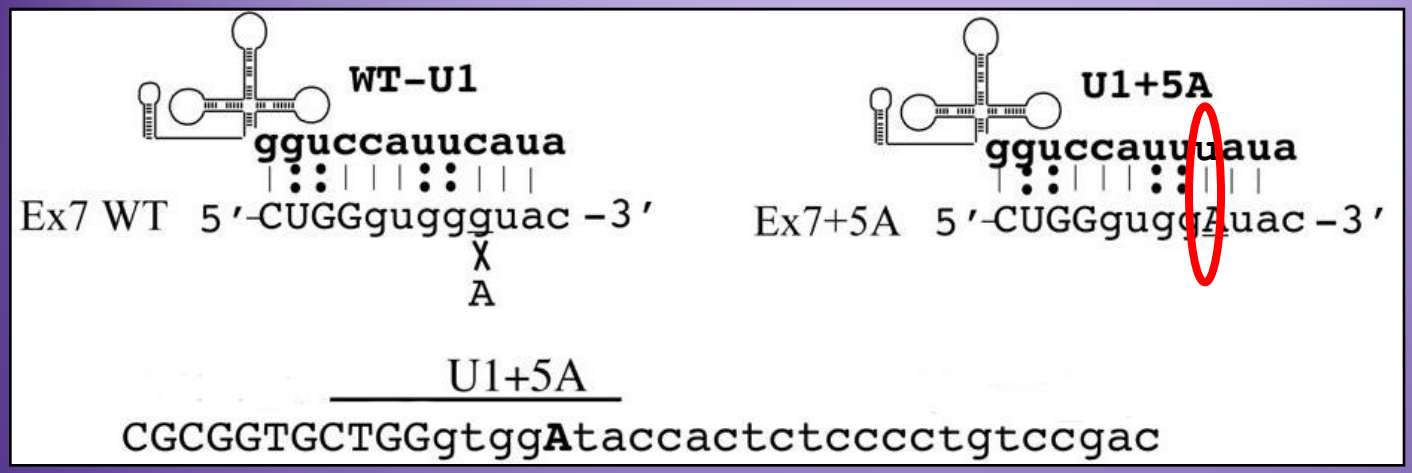
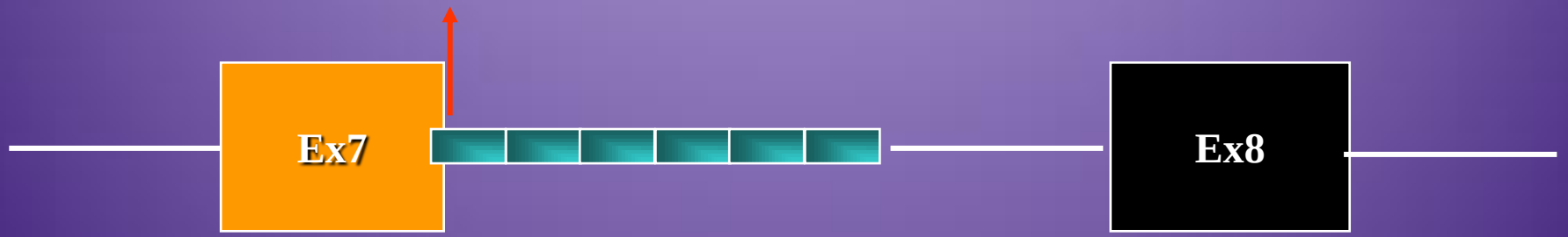




L'utilizzo di una snRNA-U1 modificata può ripristinare il corretto splicing del messaggero?

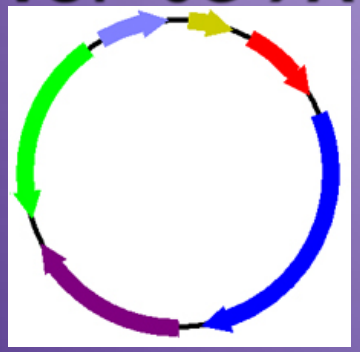




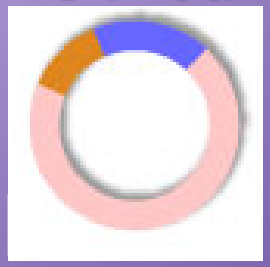
Costruzione di un vettore di espressione per la U1snRNA modificata appositamente per riconoscere il mutato 5'ss

Ripristino parziale dello SPLICING mediante snU1+5A

IVS7+5G→A



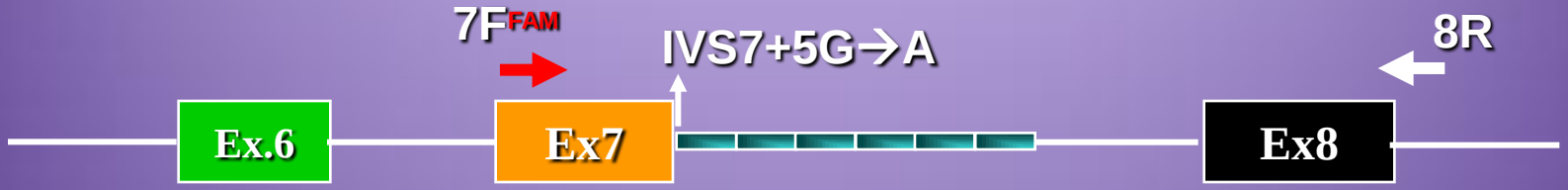
U1+5a

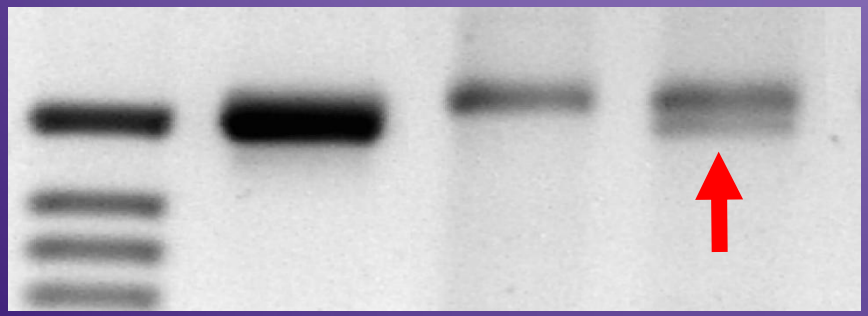


COTRASFEZIONE

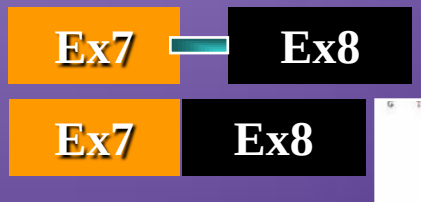
Estrazione RNA
ed RT-PCR

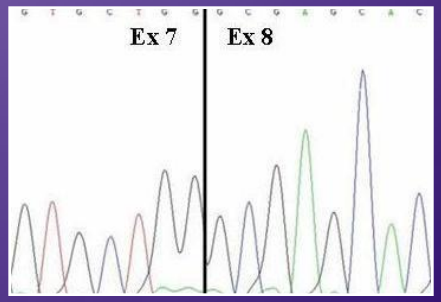
Ripristino parziale dello SPLICING mediante snU1+5A





RT-PCR 7F-8R





In seguito a trattamento con U1+5A si nota la comparsa di un messaggero normale

CONCLUSIONI

•L’ utilizzo di una U1 modificata è in grado di ripristinare parzialmente il corretto processamento del messaggero del FVII.

•Le U1snRNAs modificate possono rappresentare un approccio terapeutico mutazione-specifico nella deficienza di FVII così come per altre patologie causate da mutazioni di splicing.

IN VIVO