

EXON SKIPPING SESSION

Improving efficiency and delivery

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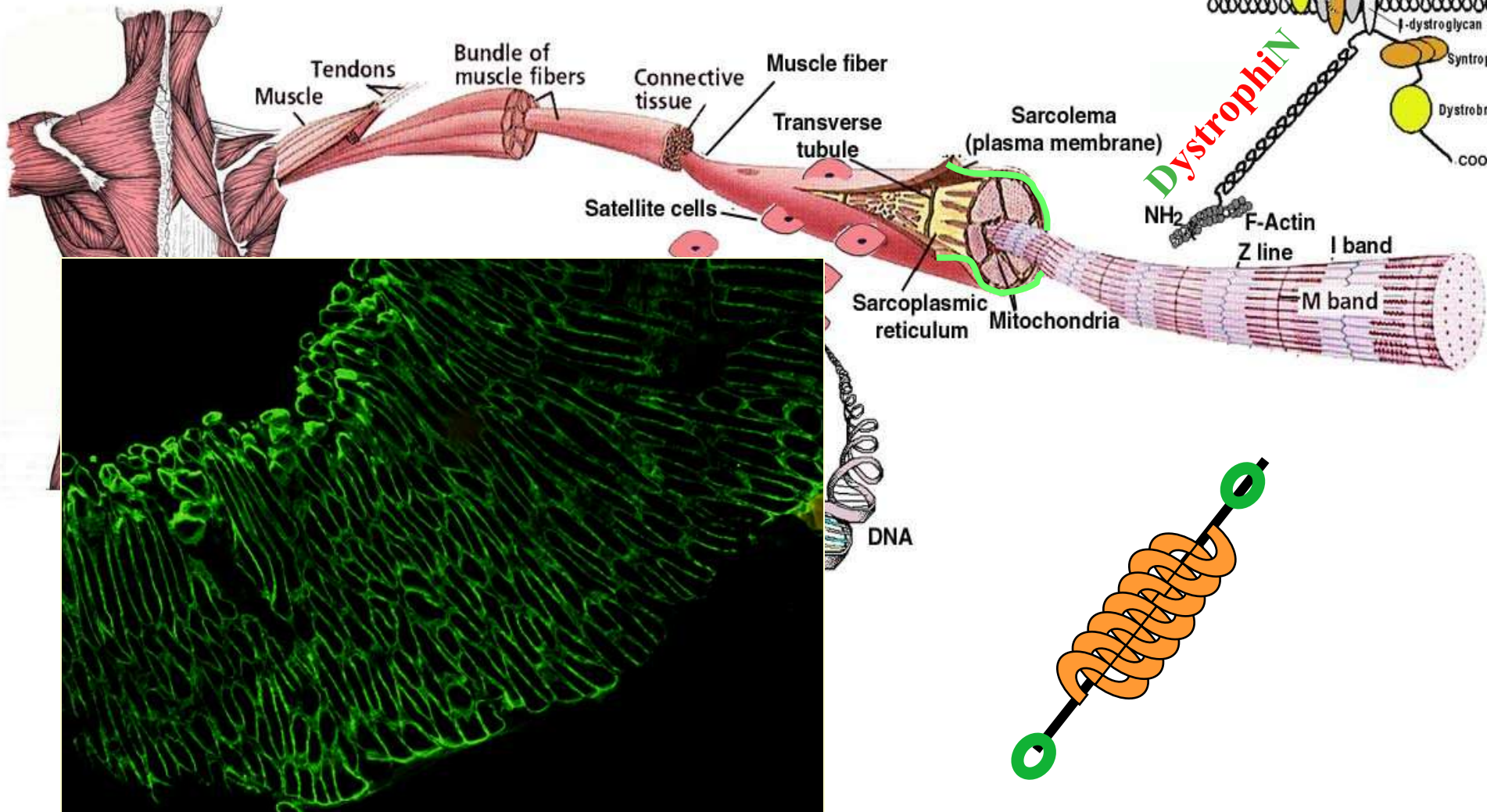


Presentation Overview

- Why, when and how of Dystrophin Exon Skipping
 - Increased oligomer efficiency
 - Identifying redundant dystrophin domains
- **Mike Gait**
 - Enhanced delivery
 - Evaluation in mouse models
- **Aurelie Goyenvalle**
 - Gene therapy application for sustained expression
 - Multi-exon skipping

Dystrophin:

a molecular “shock absorber” for muscle fibres



Dystrophin gene schematics

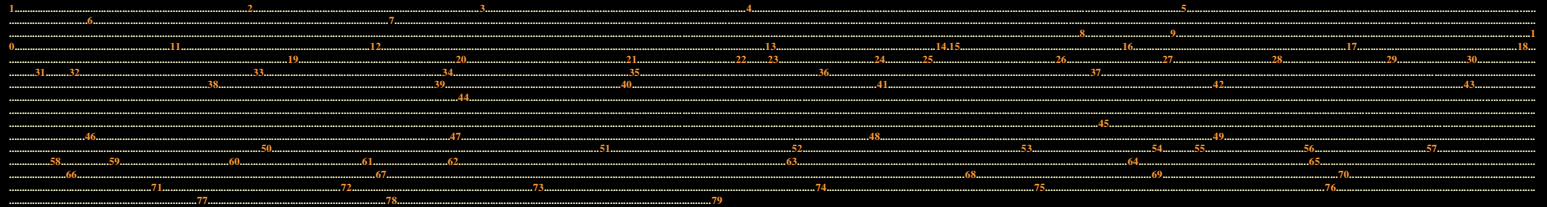
Genetic instructions to make the shock absorber

1.....2.....3.....
.....4.....5.....
.....6.....7.....
.....8.....9.....10.....
.....11.....12.....13.....
.....14.15.....16.....17.....
.....18.....19.....20.....
.....21.....22.....23.....24.....25.....26.....27.....
.....28.....29.....30.....31.....32.....33.....
.....34.....35.....36.....
.....37.....
38.....39.....40.....
41.....42.....43.....
.....44.....
.....45.....46.....
.....47.....48.....49.....
.....50.....51.....52.....53.....54.....55.....
.....56.....57.....58.....59.....60.....61.....
.....62.....63.....
.....64.....65.....66.....
.....67.....68.....69.....70.....
.....71.....72.....73.....
.....74.....75.....76.....
.....77.....78.....
.....79

Dystrophin gene expression

DNA transcribed > pre-mRNA

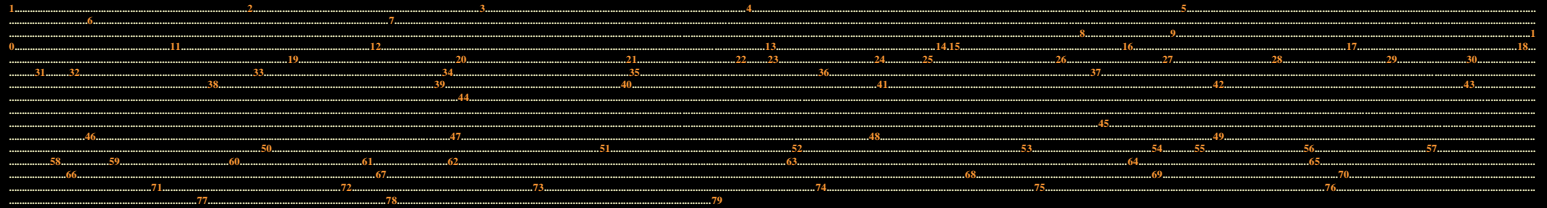
DNA
instructions



Dystrophin gene expression

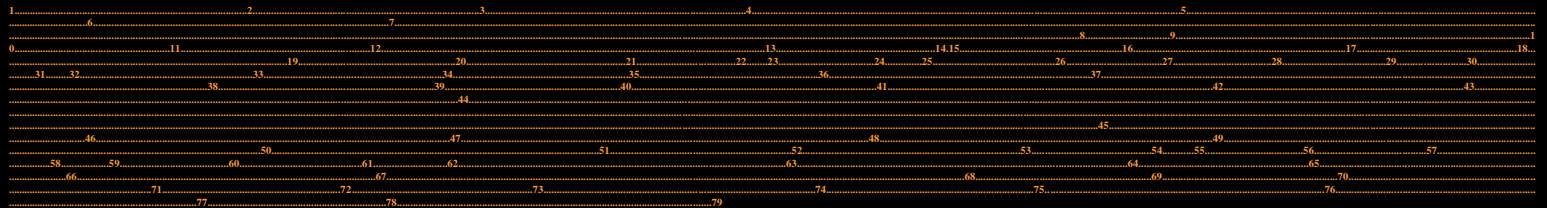
DNA transcribed > pre-mRNA

DNA
instructions



Transcribed

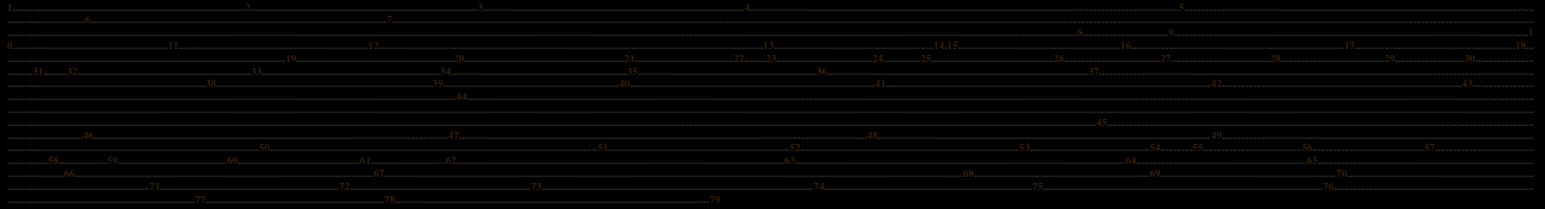
pre-mRNA



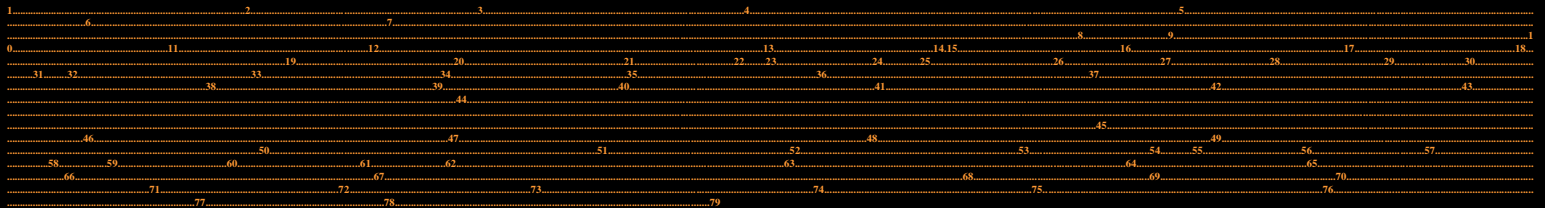
Dystrophin gene expression

pre-mRNA spliced > mRNA

DNA



pre-mRNA



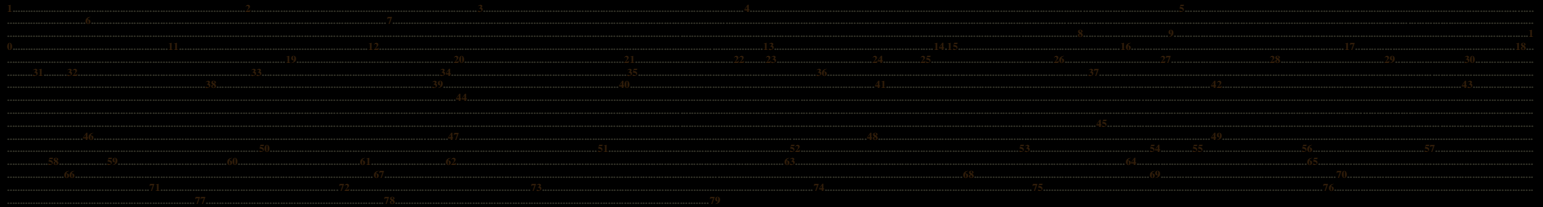
Spliced

mRNA

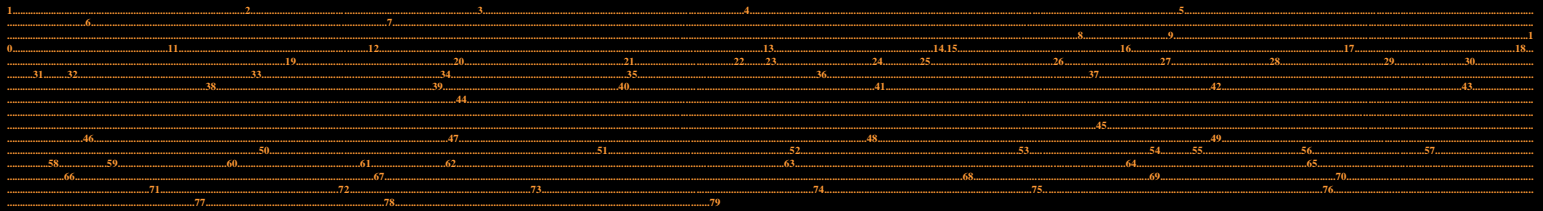
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Exon skipping: a genetic therapy

DNA



pre-mRNA



SPLICING INTERVENTION – EXON SKIPPING

mRNA

01.02.03.04.05.06.07.08.09.10.11.12.13.14.15.16.17.18.19.20.21.22.23.24.25.26.27.28.29.30.31.32.33.34.35.36.37.38.39.40.41.42.43.44.45.46.47.48.49.50.51.52.53.54.55.56.57.58.59.60.61.62.63.64.65.66.67.68.69.70.71.72.73.74.75.76.77.78.79

Coupled pre-mRNA transcription & splicing



+ + + + + - - - - - + + + + +

Assembly of splicing machinery



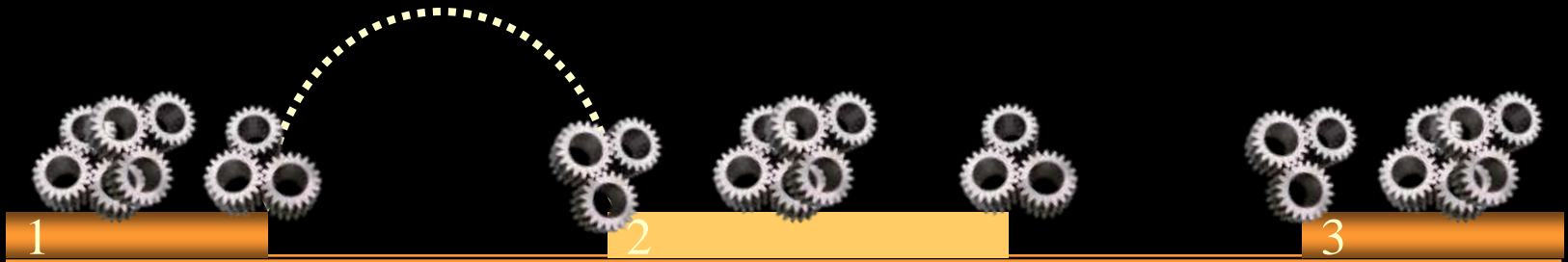
+ + + + + - - - - - + + + - + + + + +

Assembly of splicing machinery



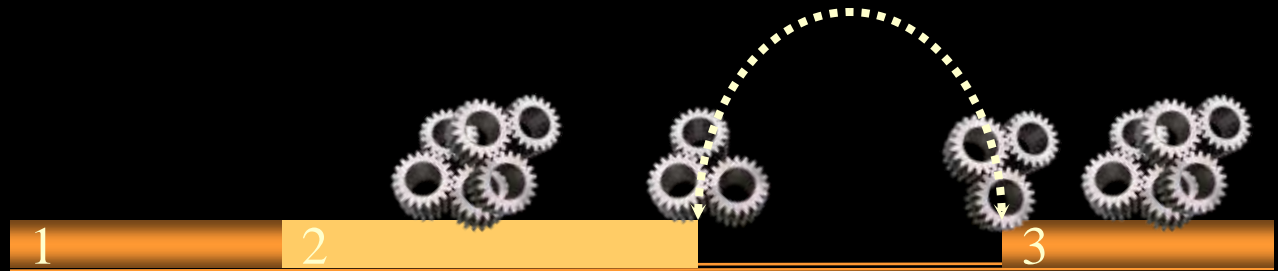
+ + + + + - - - - - - - - - + + + - + + + + + + - - - - - + + +

pre-mRNA splicing: joining exons



+ + + + + - - - - - + + + - + + + + + - - - - - + + + + + + + + + + +

pre-mRNA splicing: joining exons



mRNA processing and transport



CAP 1 2 3 AAAAAA



mRNA translation: a simple message



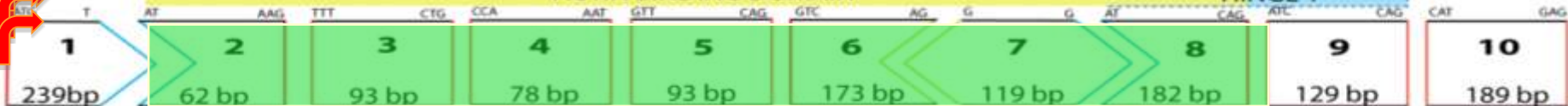
CAP 1 2 3 AAAAAA

THE OLD DOG RAN AND RAN AND RAN AND ATE THE FAT CAT END Normal

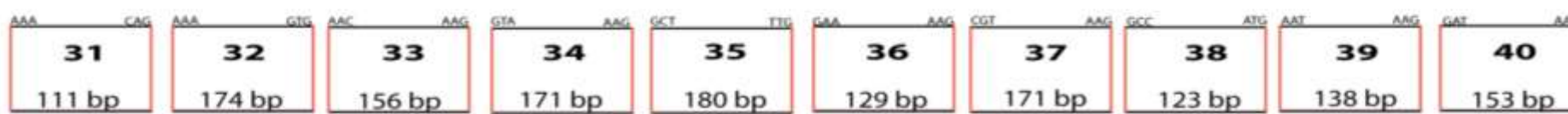
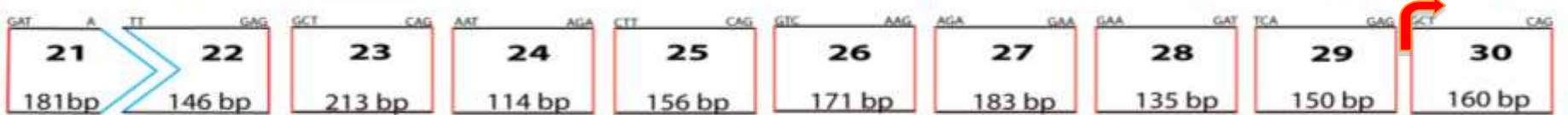
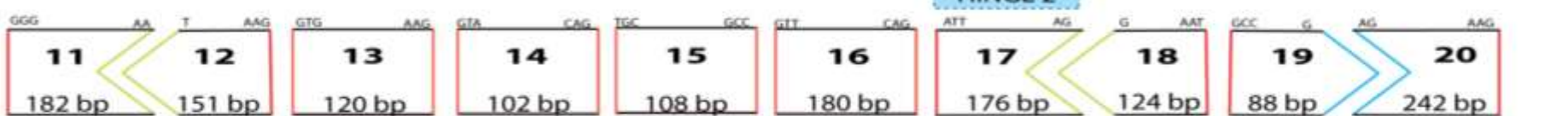


ACTIN BINDING DOMAIN

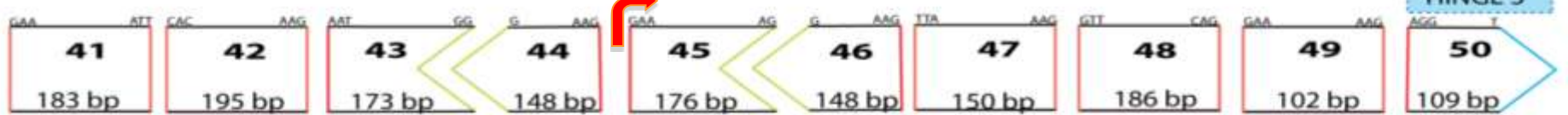
HINGE 1



HINGE 2



HINGE 3

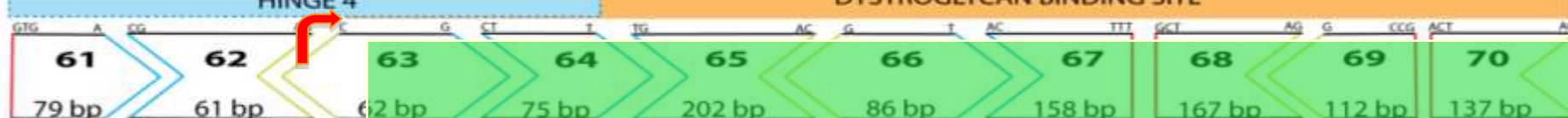


HINGE 3

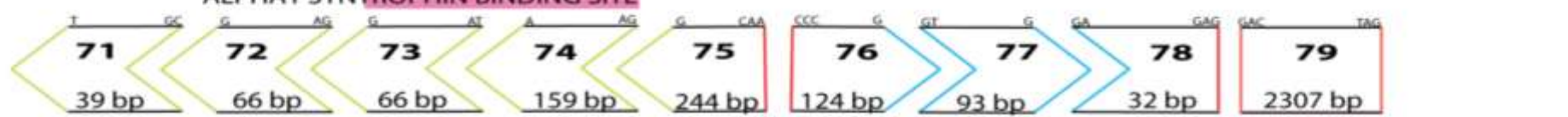


HINGE 4

DYSTROGLYCAN BINDING SITE



ALPHA1 SYNTROPHIN BINDING SITE



BETA 1 SYNTROPHIN BINDING SITE

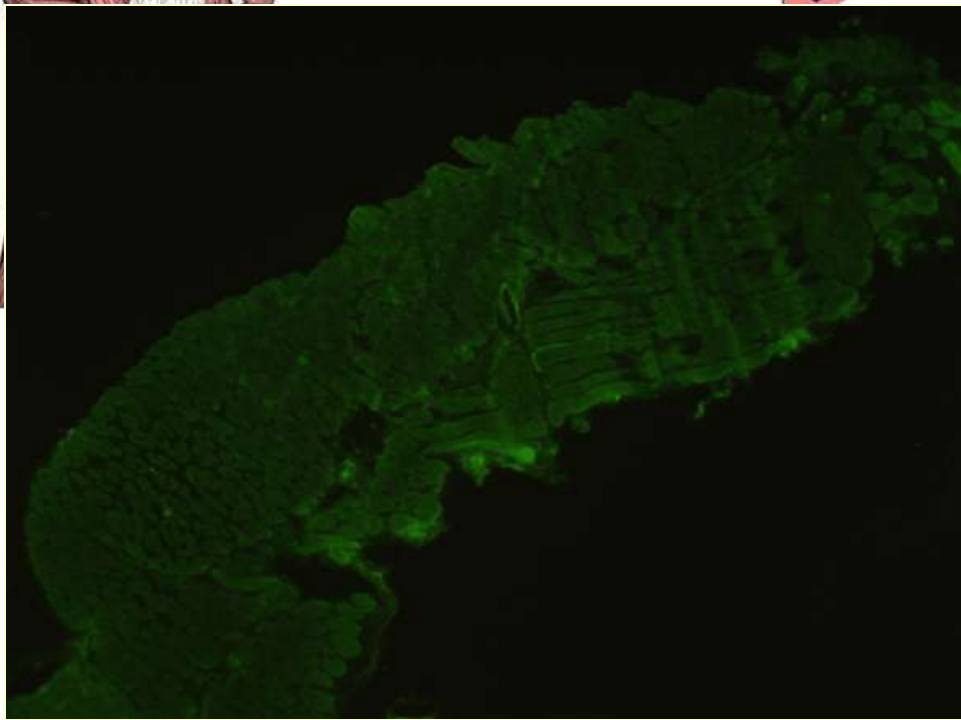
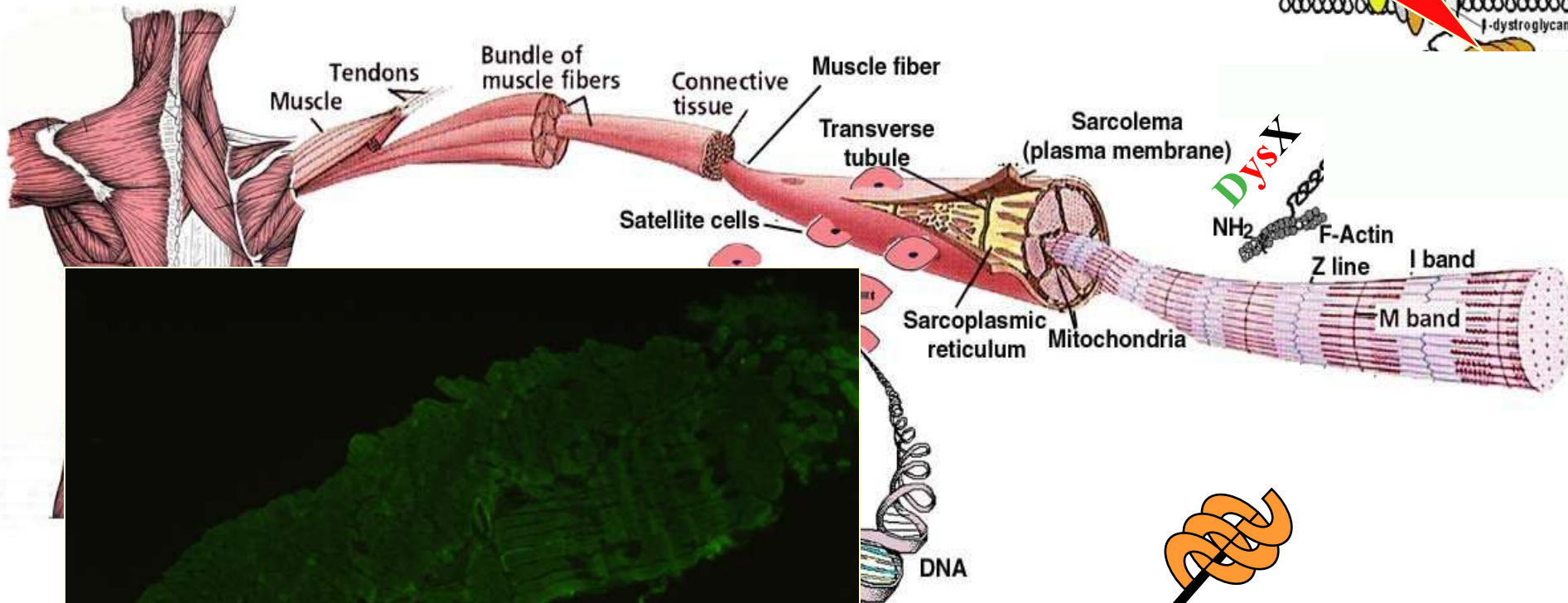
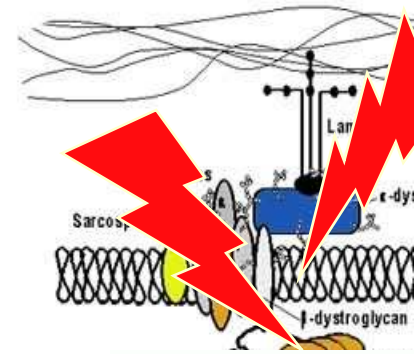
DMD mutations: a corrupted message



DMD

- THE OLD DOG RAN AND RAN AND RAN AND ATE THE FAT CAT END Normal
- THE OLD DOG RAN **END** RAN AND RAN AND ATE THE FAT CAT END Nonsense
- THE OLD DOG RAN ^(A) NDR ANA NDR ANA NDA TET HEF ATC ATE ND FS Deletion
- THE OLD DOG RAN **A**AN DRA NAN DRA NAN DAT ETH EFA TCA TEN D FS Duplication

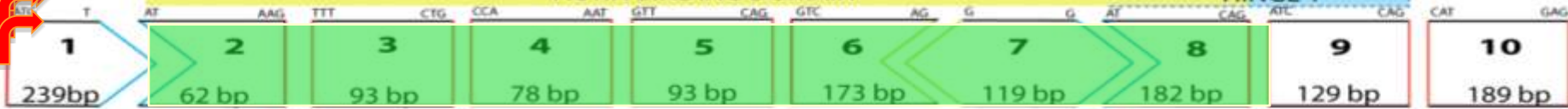
Duchenne MD: defective dystrophin



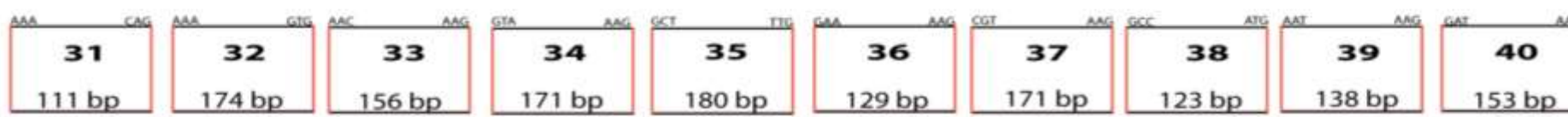
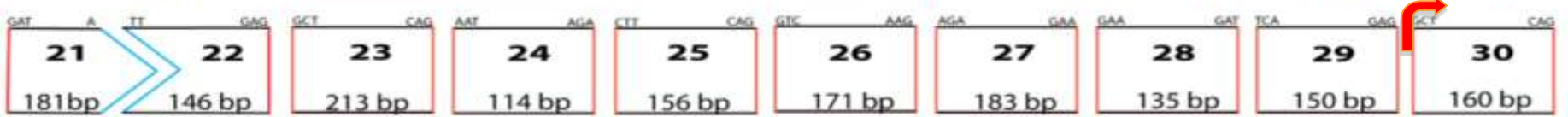
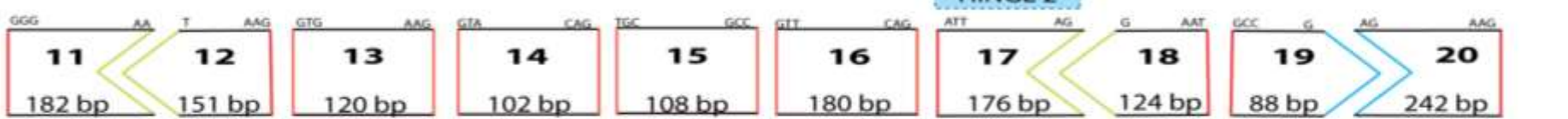


ACTIN BINDING DOMAIN

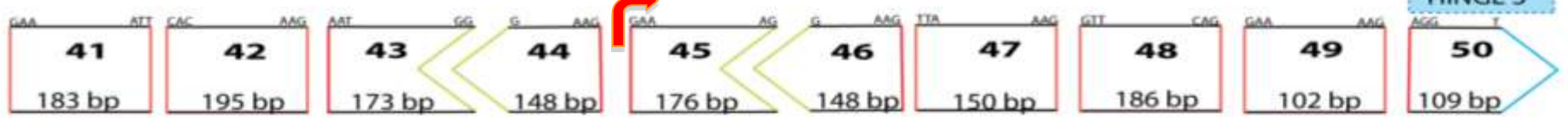
HINGE 1



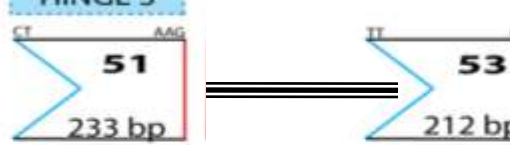
HINGE 2

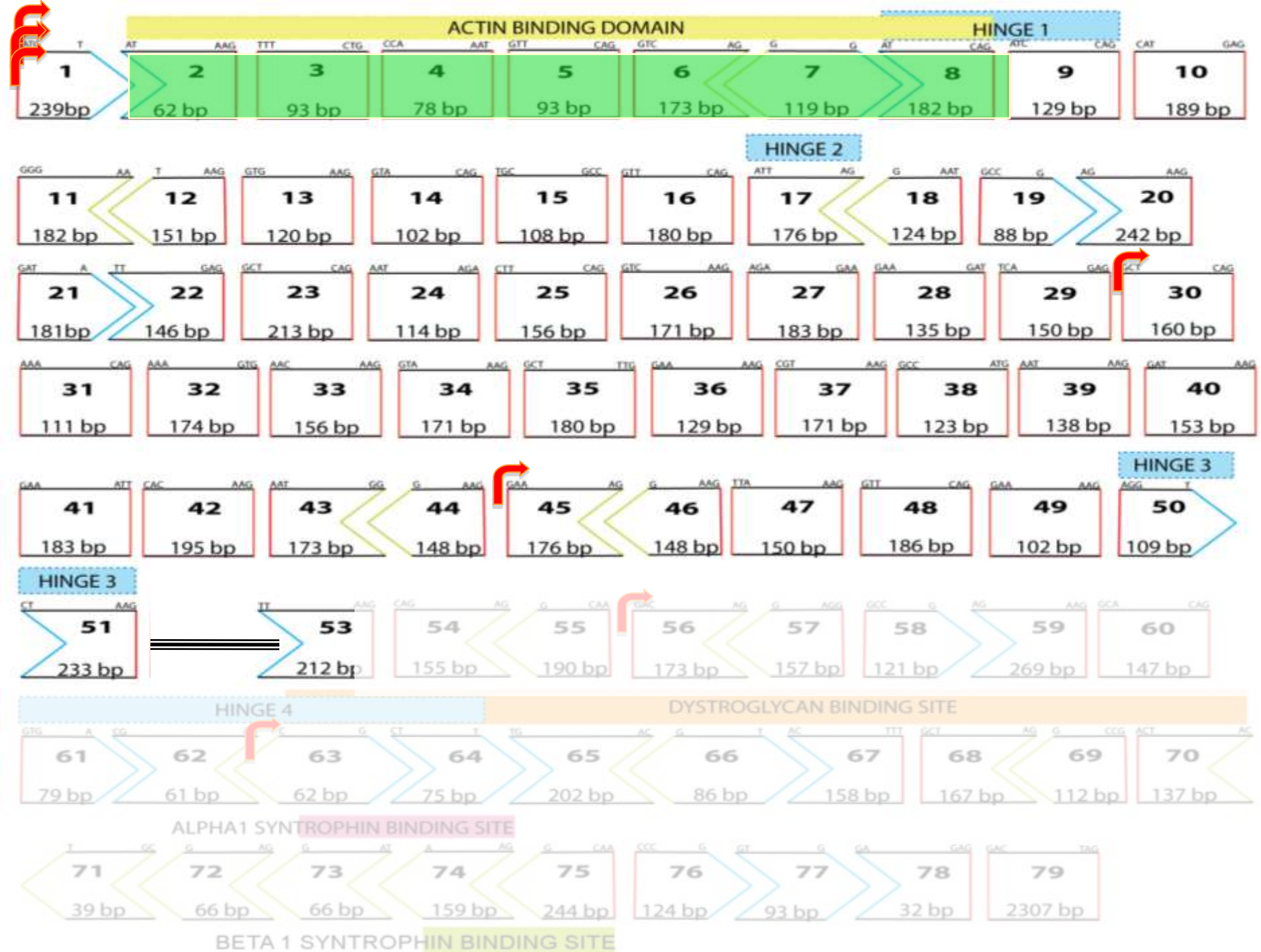


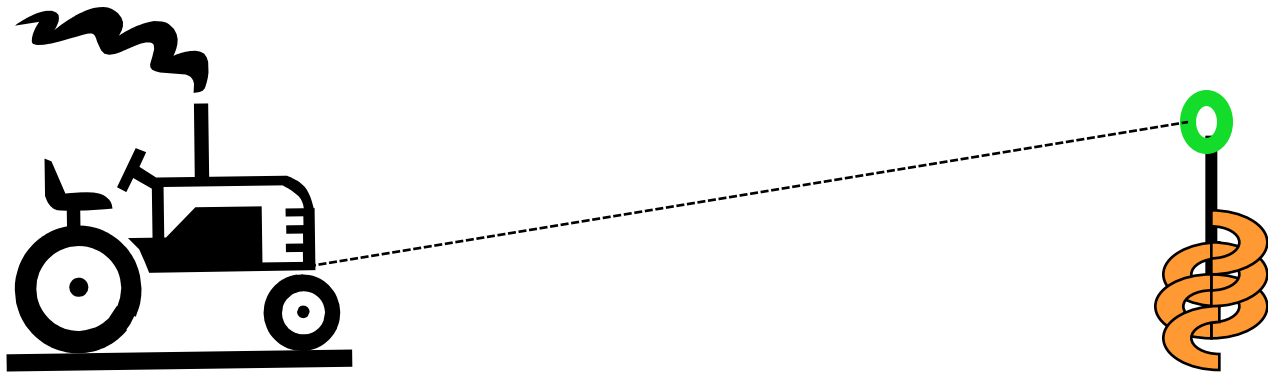
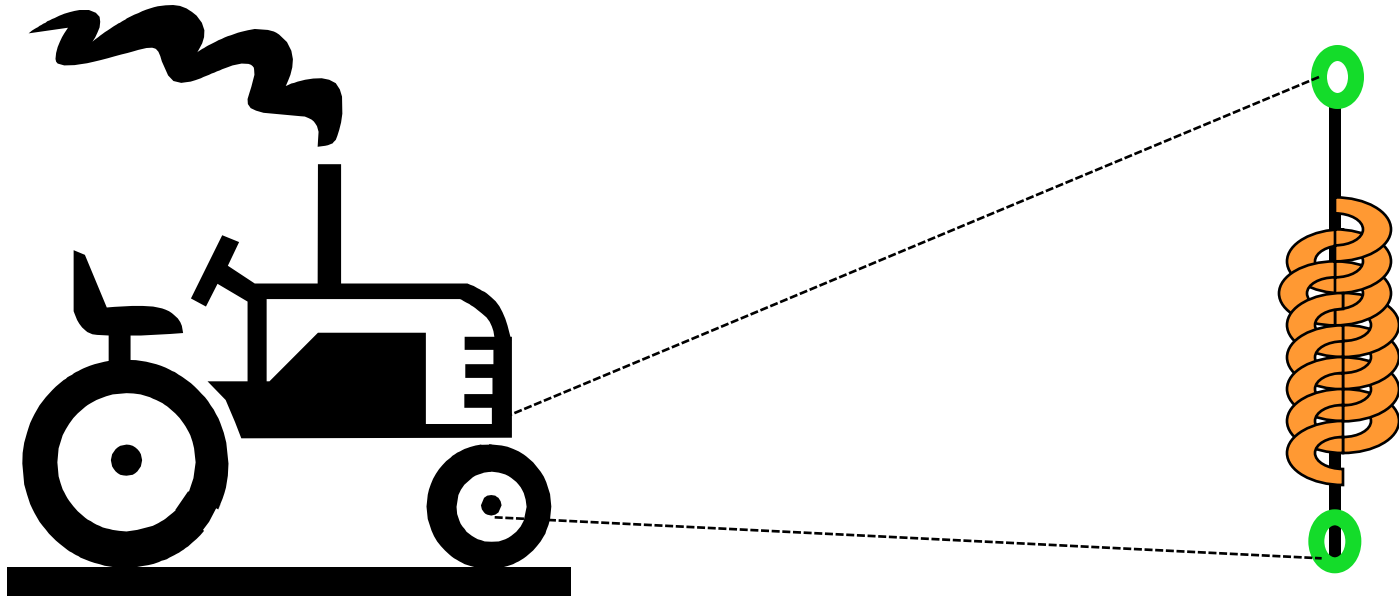
HINGE 3

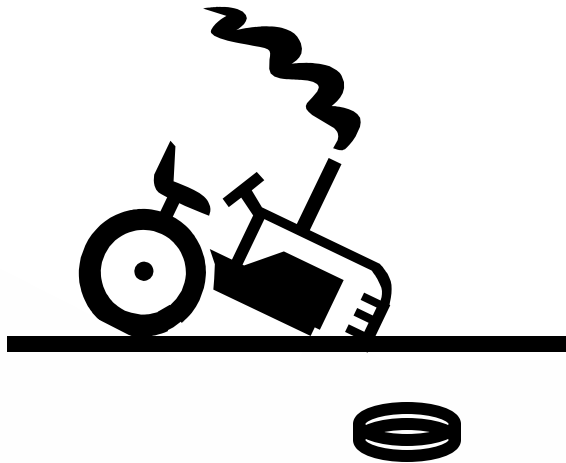
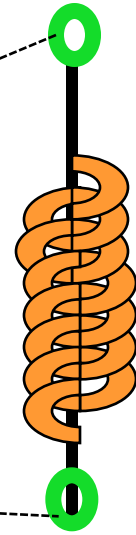
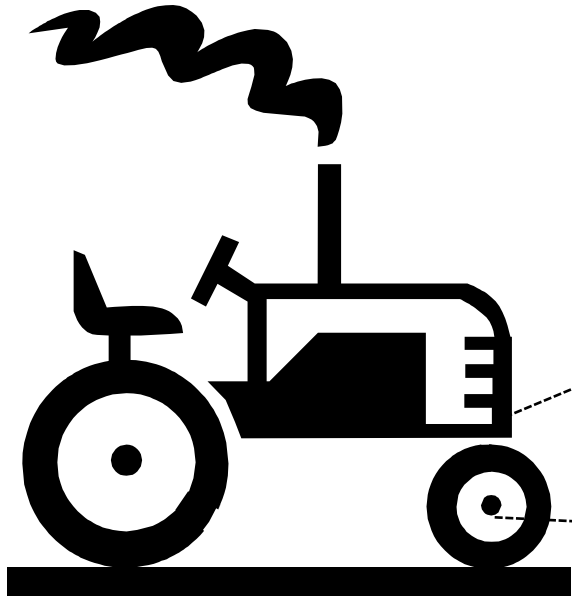


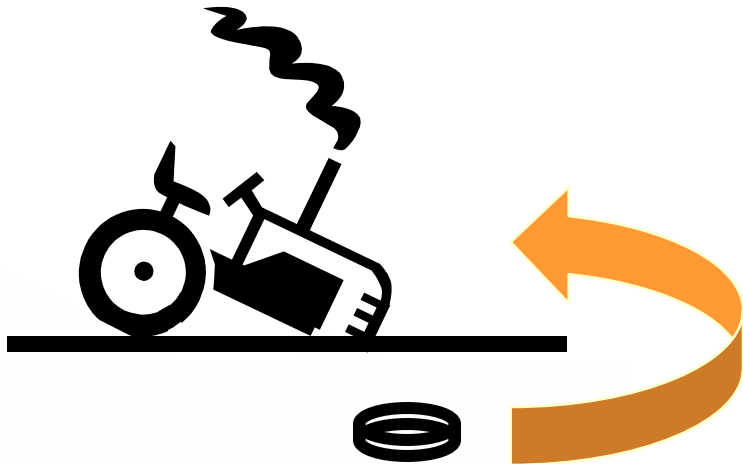
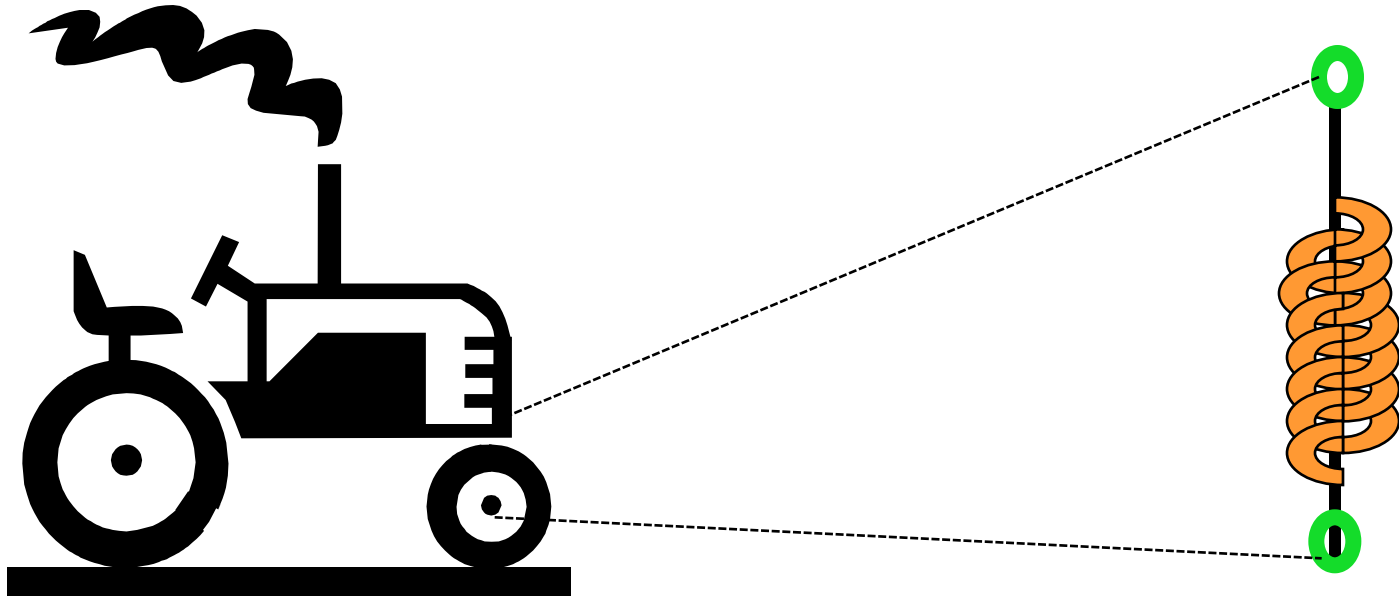
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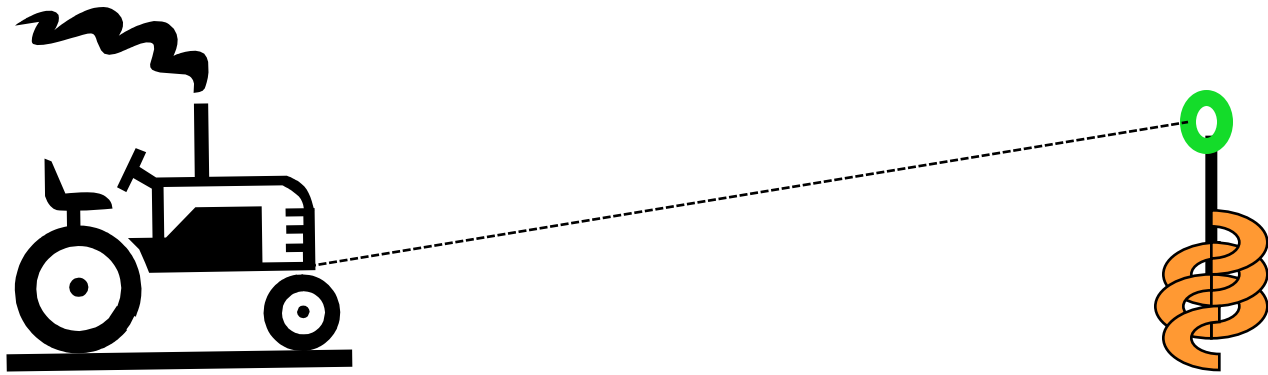
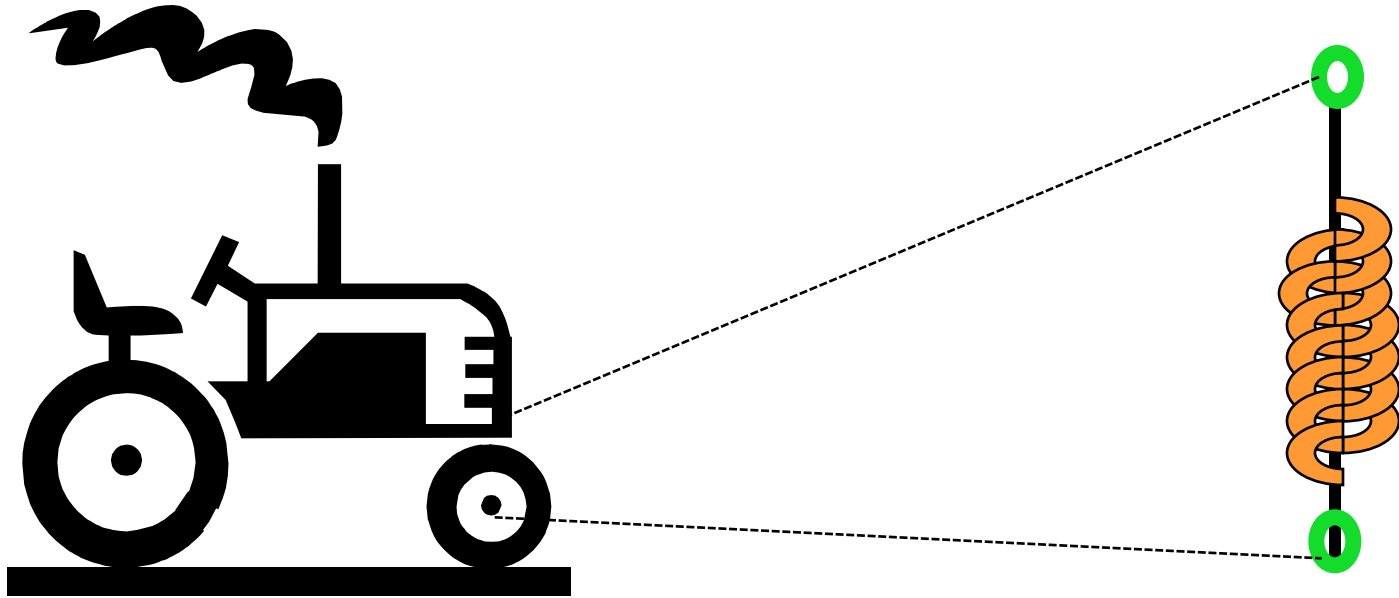


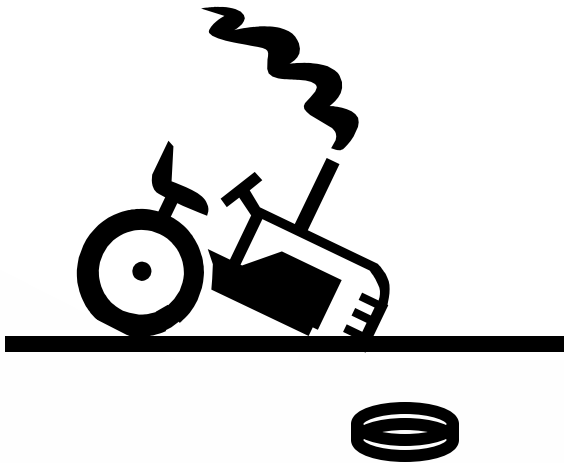
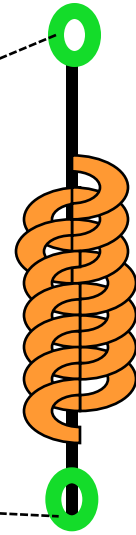
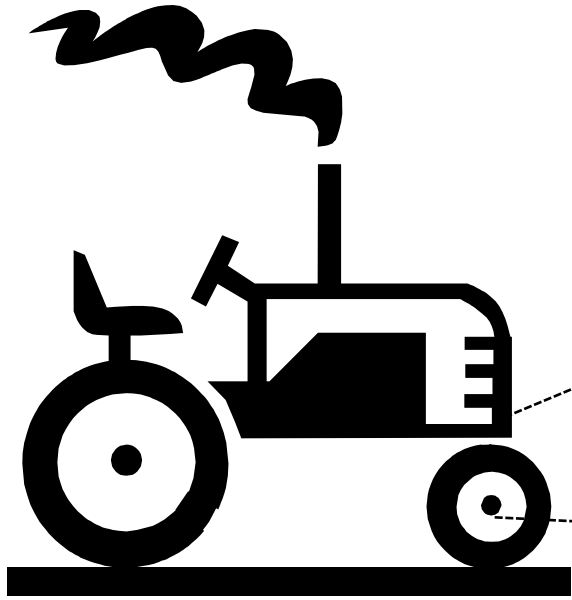


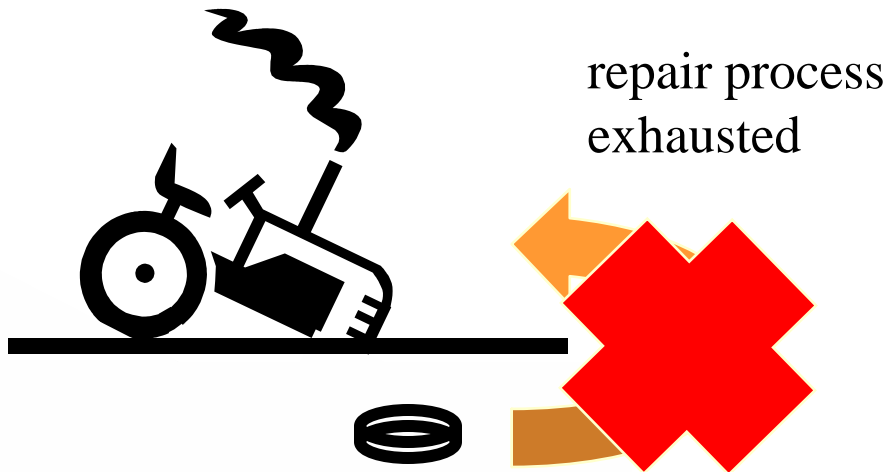
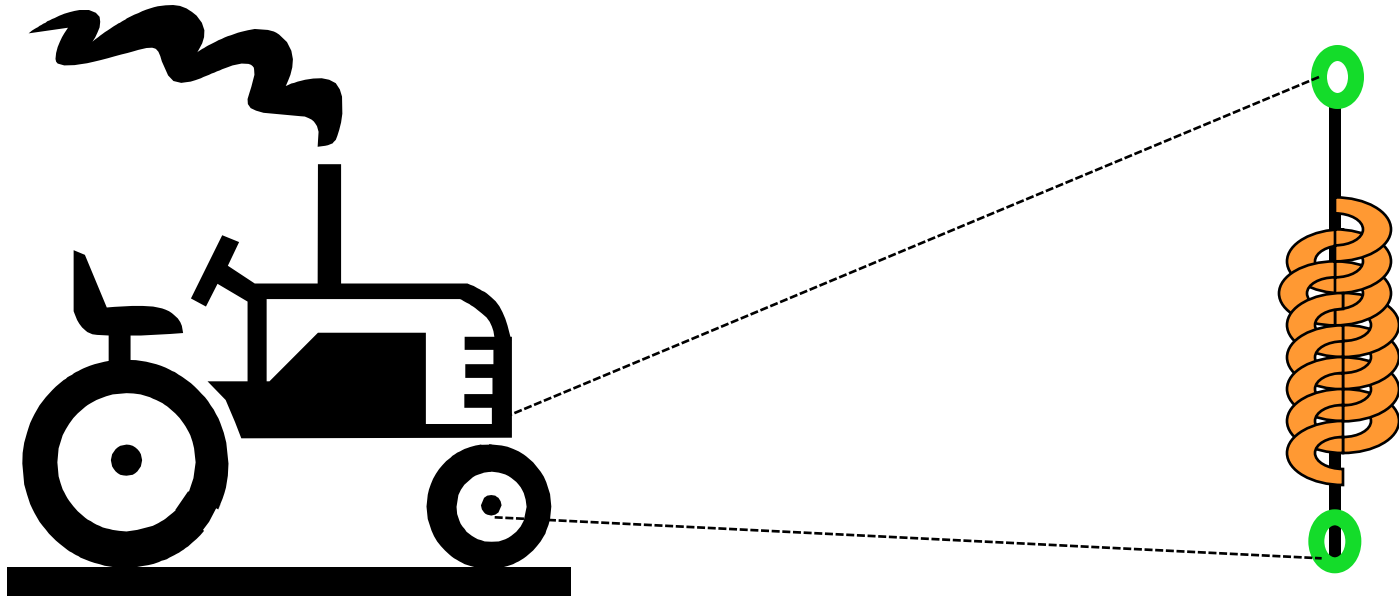








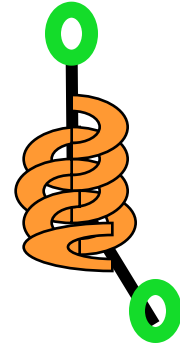
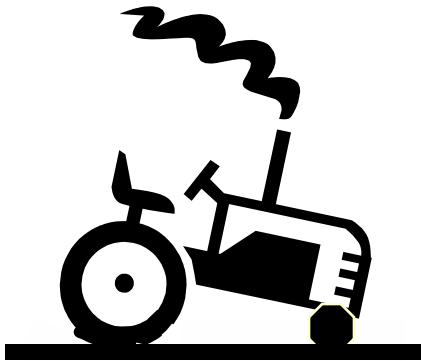
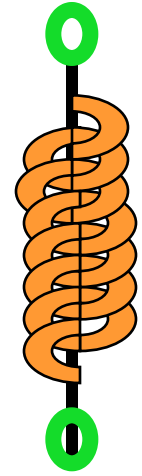
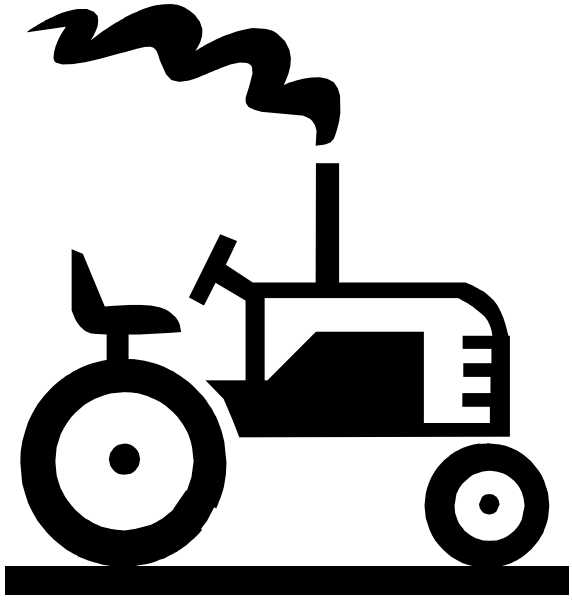


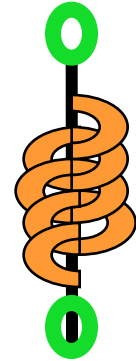
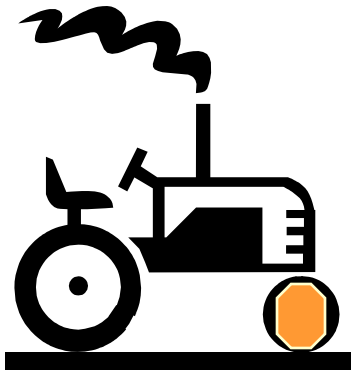
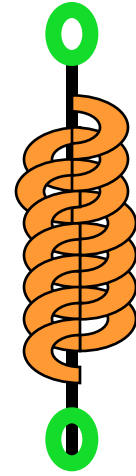
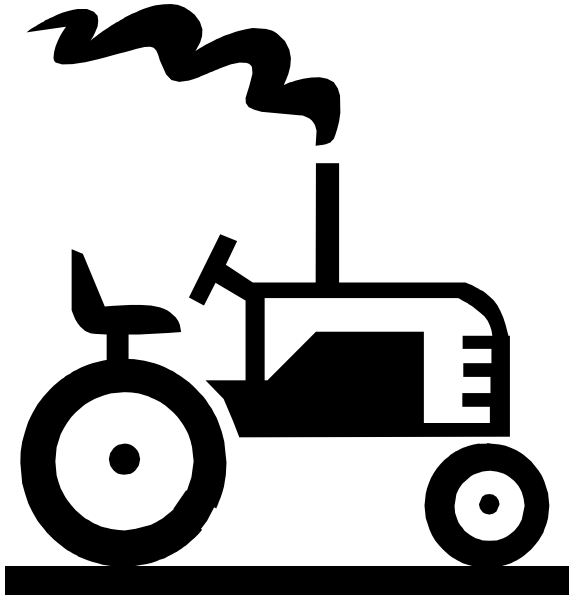


BMD Mutations (reading frame intact but spectrum of severity)



- | | |
|---|---------|
| THE OLD DOG RAN AND RAN AND RAN AND ATE THE FAT CAT END | Normal |
| THE OLD DOG AND RAN AND RAN AND ATE THE FAT CAT END | BMD++++ |
| THE OLD DOG AND RAN AND ATE THE FAT CAT END | BMD+++ |
| THE OLD DOG AND ATE THE FAT CAT END | BMD+ |
| THE OLD ATE THE FAT CAT END | DMD |





Mild / Asymptomatic BMD cases

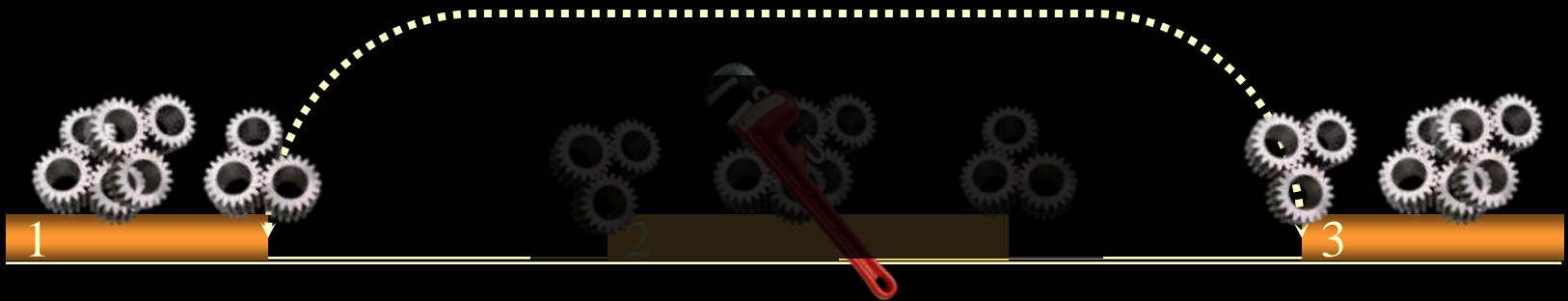
| <i>Exon Deletion</i> | <i>Comments</i> | <i>Reference</i> |
|----------------------|---|-------------------|
| <i>3-9</i> | <i>Playing competitive badminton at age 62 years.</i> | <i>Heald 94</i> |
| <i>9-22</i> | <i>High CK, myalgia but well developed musculature and no evidence of muscle weakness</i> | <i>Gospe 89</i> |
| <i>17-48</i> | <i>Source of the dystrophin mini-gene used in gene replacement studies</i> | <i>England,91</i> |
| <i>35-44</i> | <i>Cramping after soccer or mountain climbing</i> | <i>Koenig 89</i> |
| <i>41-44</i> | <i>Elevated CK, otherwise asymptomatic</i> | <i>Comi 94</i> |
| <i>45-53</i> | <i>Diagnosed age 60</i> | <i>Bosone</i> |
| <i>50-53</i> | <i>Elevated CK, otherwise asymptomatic</i> | <i>Comi 94</i> |

Concept of exon skipping

Alter processing to remove disease associated / causing exon(s)



Concept of exon skipping



Antisense oligomers

- Exquisitely specific spanners!!



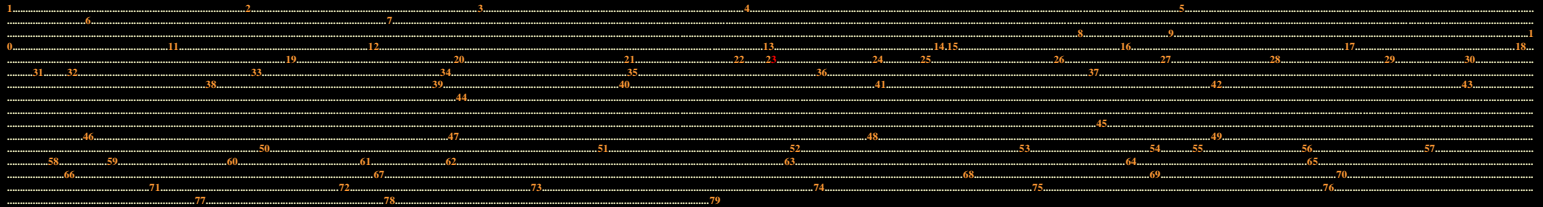
a g a c a g u c a c u



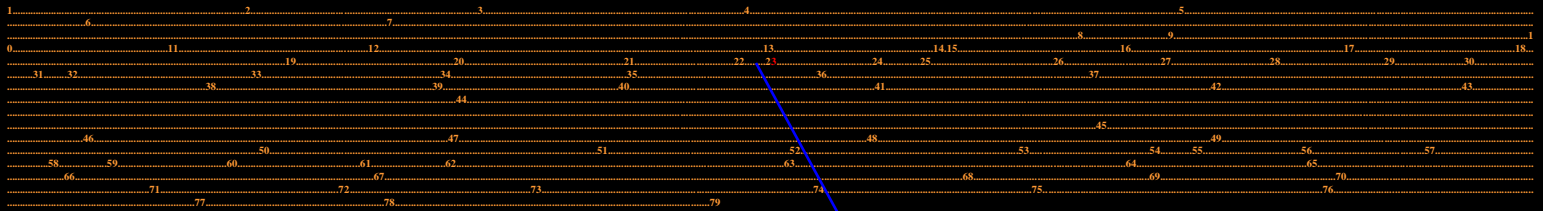
accuugcuauaucugucagugaucgaugcaugcacgu

Exon skipping targets the mdx defective exon

DNA



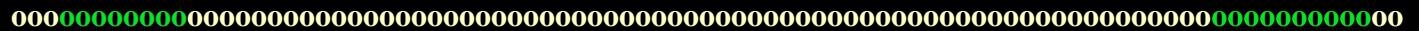
premRNA



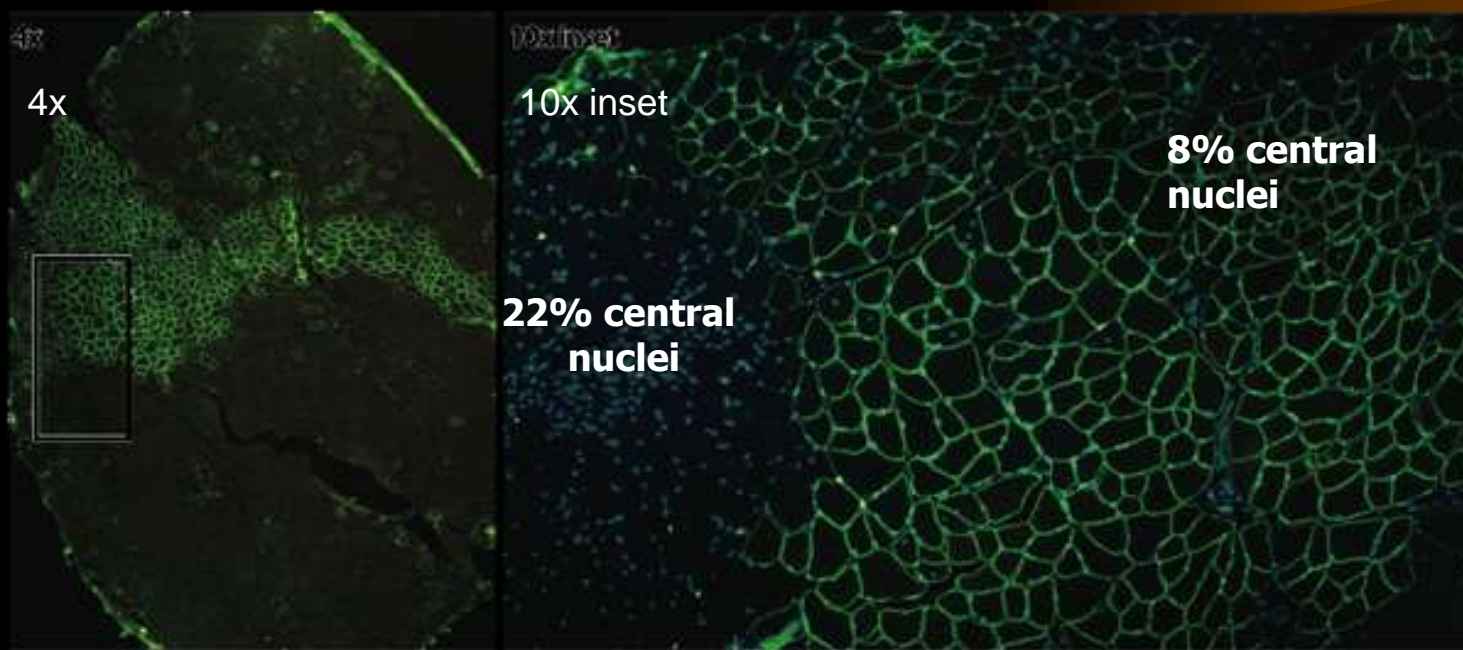
mRNA



Protein

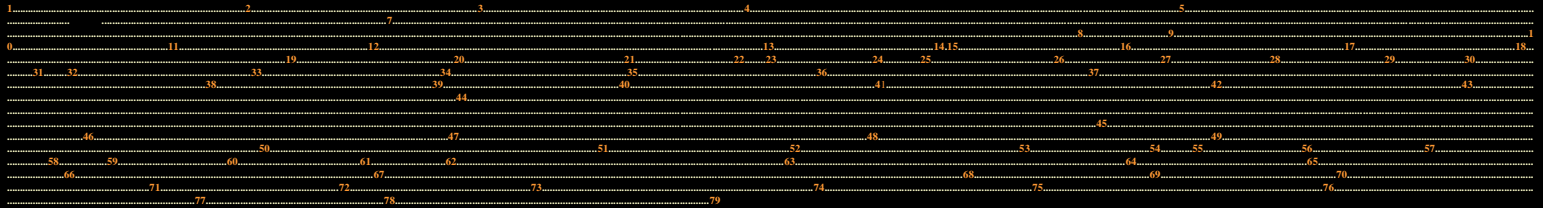


Dystrophin expression : 6 weeks after injection of morpholino AO into TA of 11-day old mdx mouse

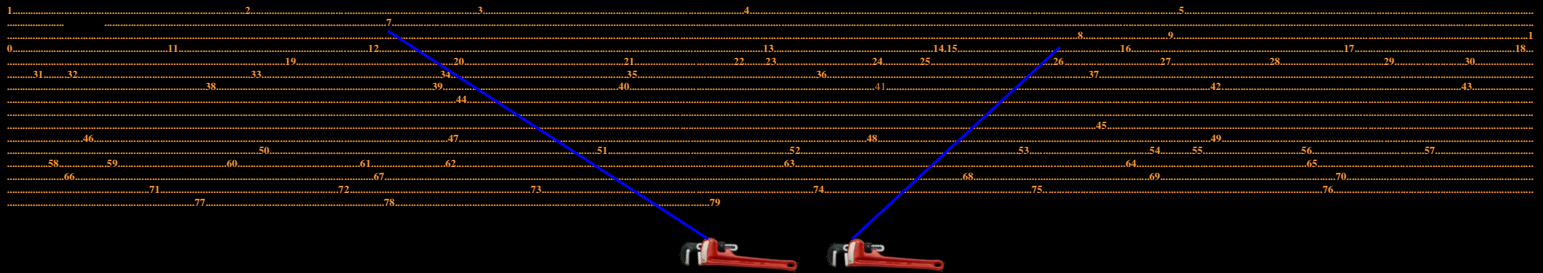


Targeting two exons

DNA



premRNA



mRNA

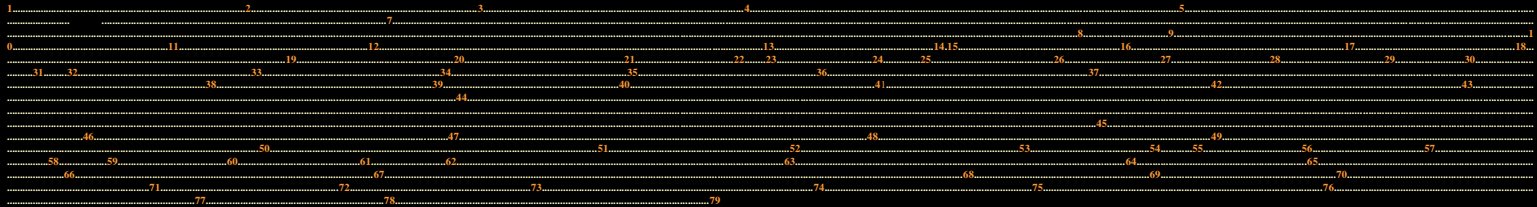
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Protein

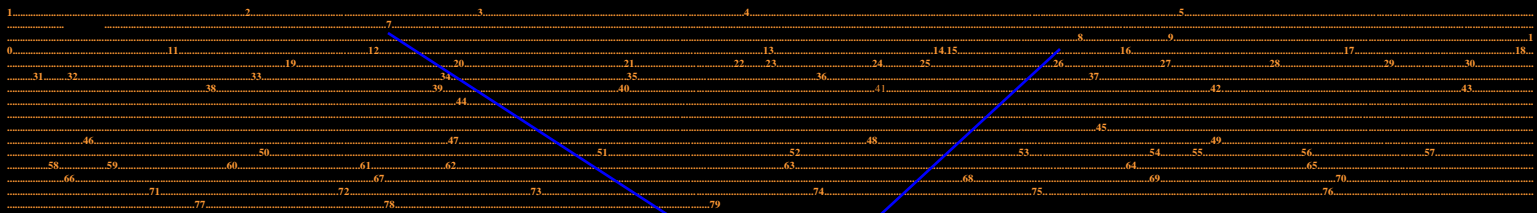
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Targeting two exons

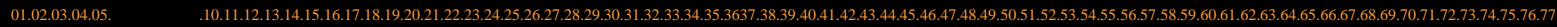
DNA



premRNA



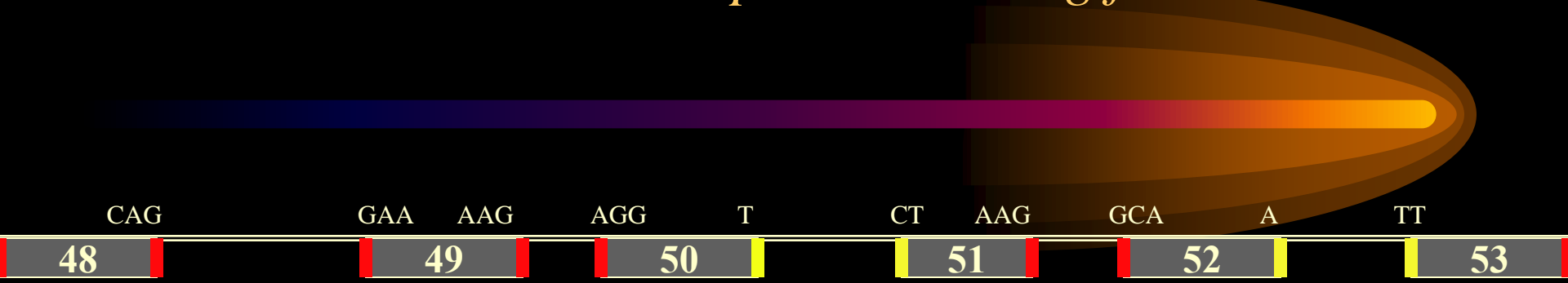
mRNA



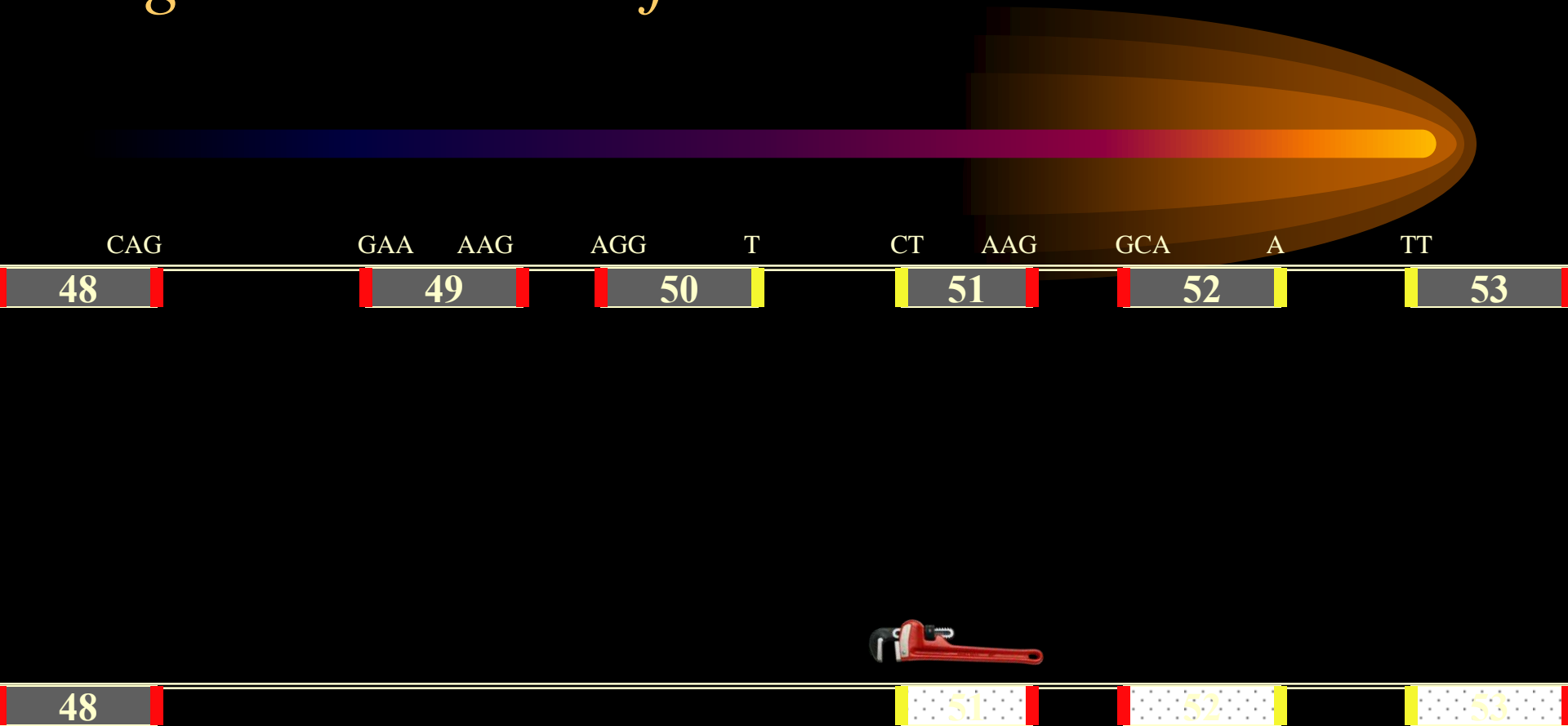
Protein



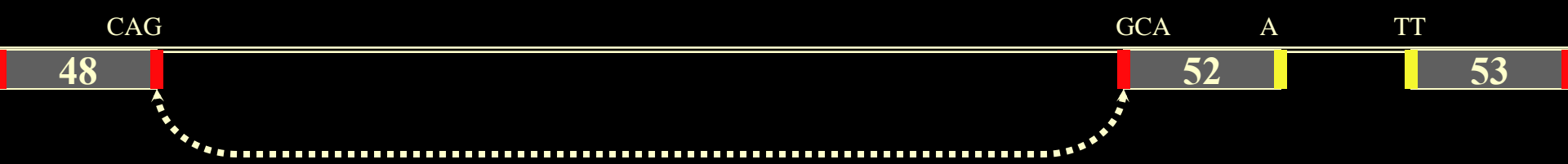
Exon 49 & 50 deletion disrupts the reading frame > DMD



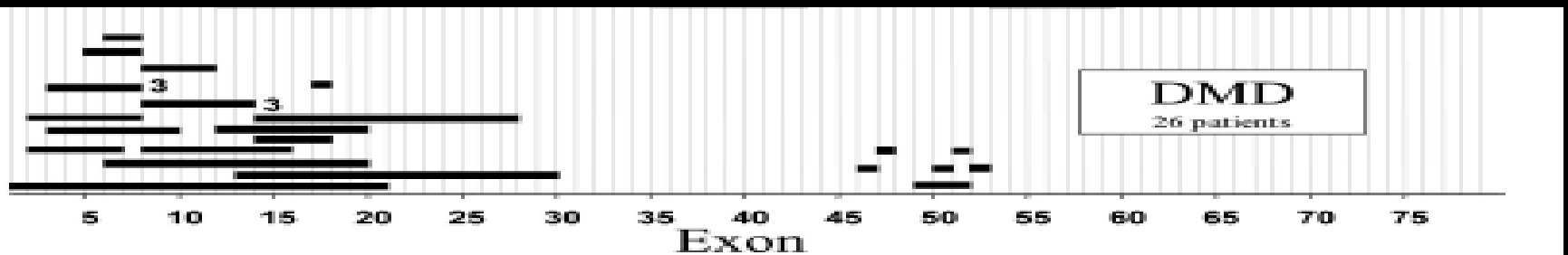
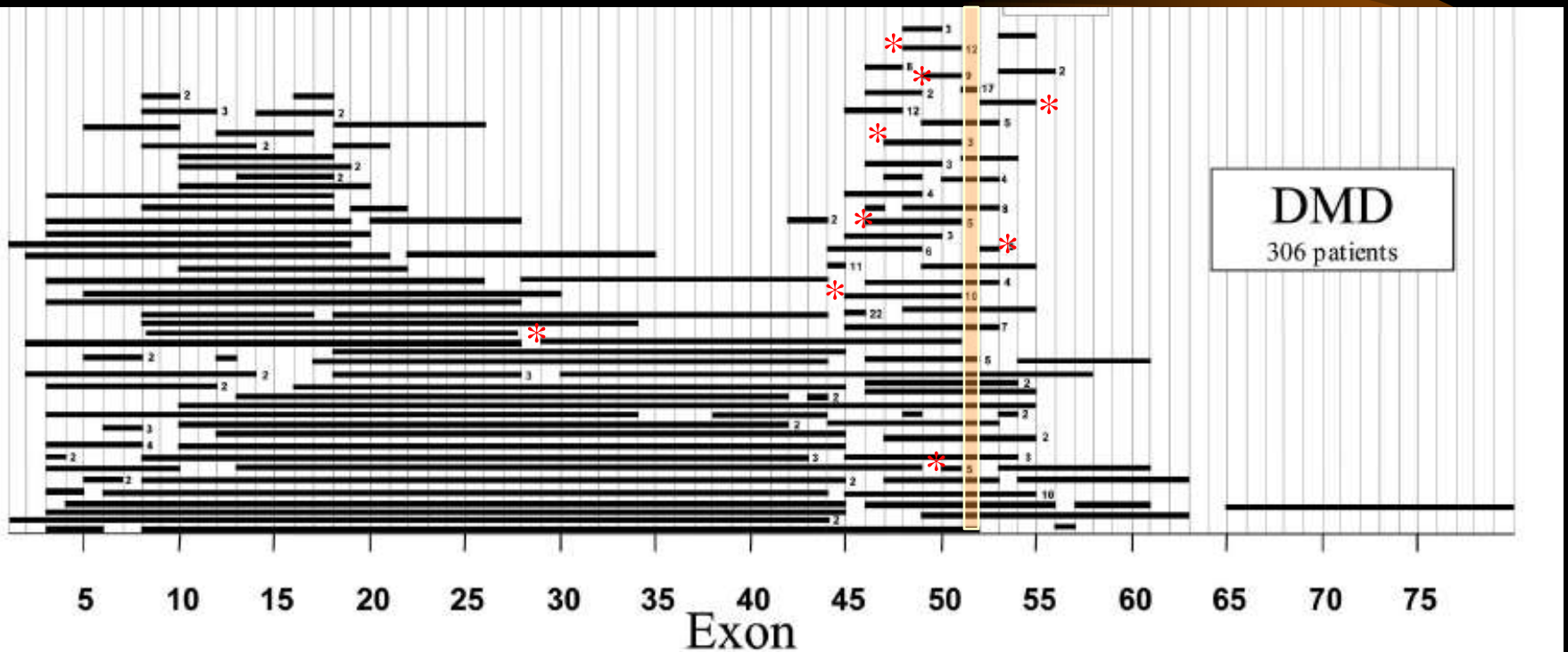
Targeted excision of Exon 51



Exon 51 skipping restores reading frame



Individual responses (ex 51 skipping)



Different “spanners”

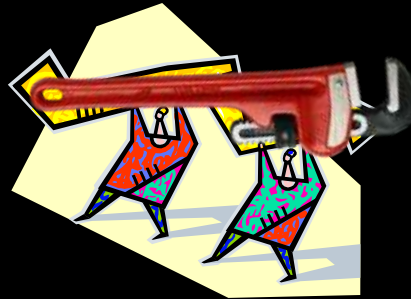
- 2OMeAOs



- PMOs (morpholinos)



- CPP-PMOs
(PPMO)



- AAV Expression cassettes



Optimizing targeting spanners



cacggcuu

cguccuug

cacggcuu

gucguacgu

gccaacgua

uuuucugcagGAACGUCCGGUGCCGAAACGGUGCAGCAUGCACUGC AUGCGguugcaucg

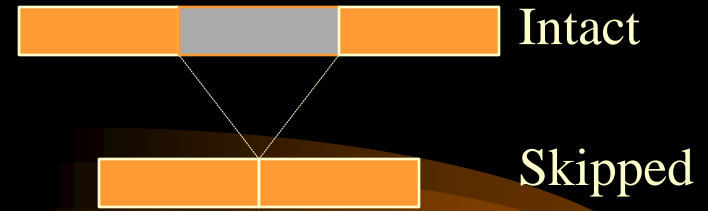


gccacggc

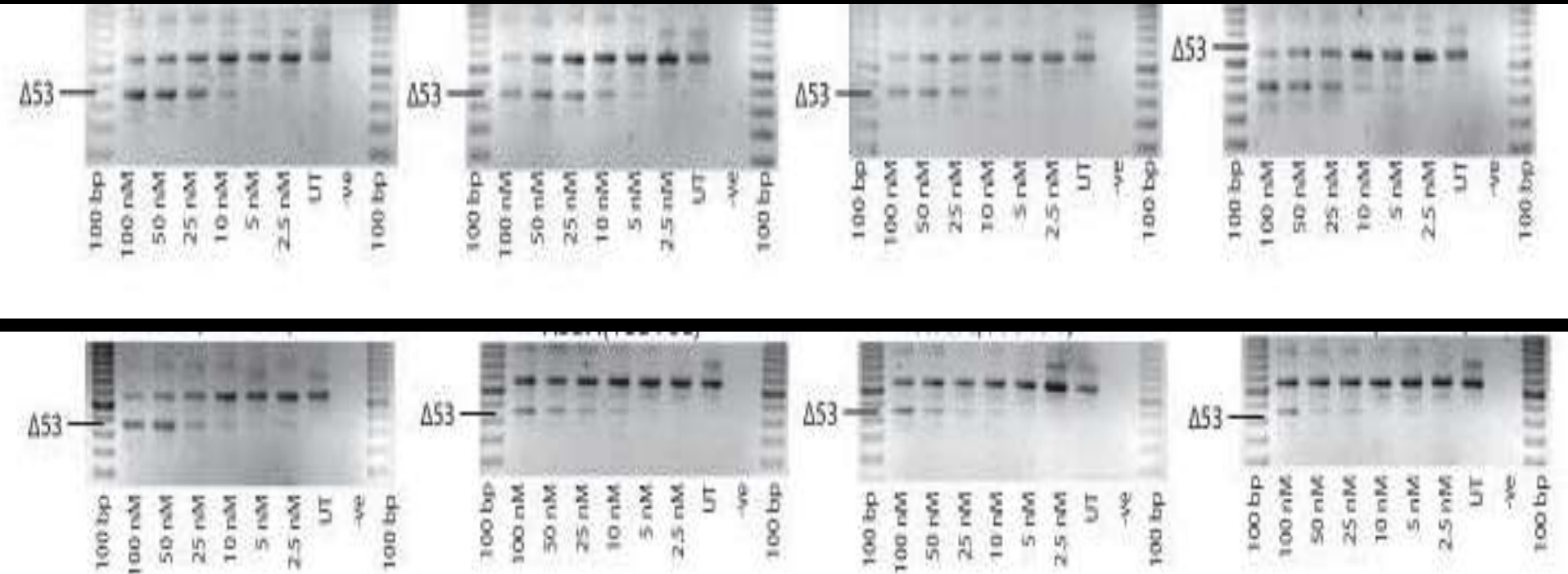


ggcuuugcca

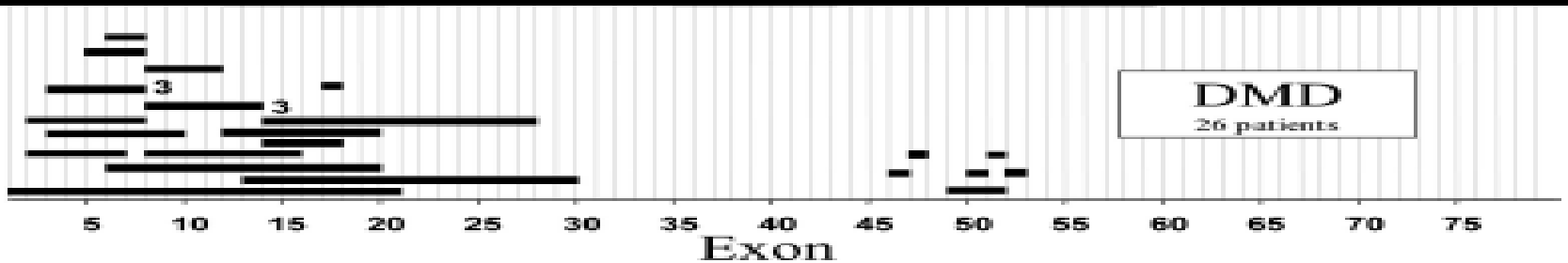
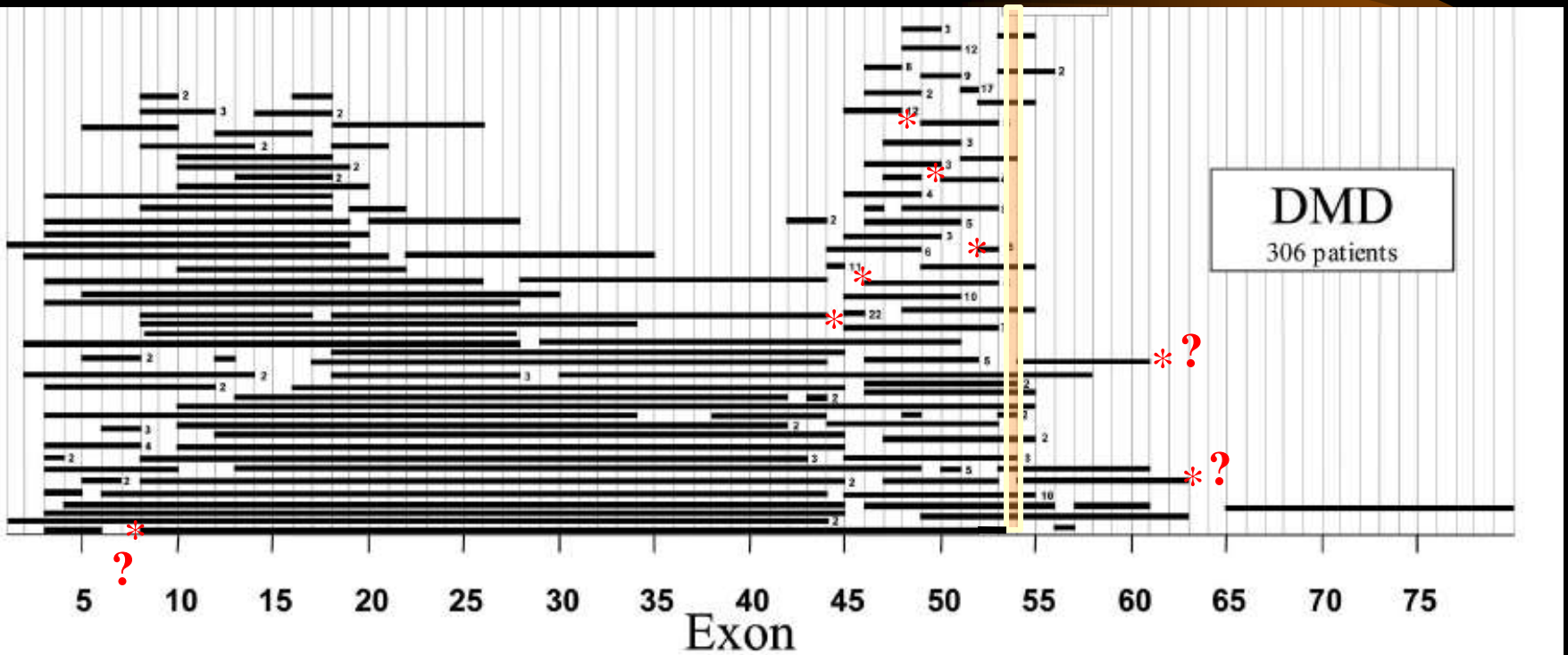
Spanner refinement:



- Exon 53 skipping
 - Varying annealing coordinates and length



Individual responses (ex 53 skipping)

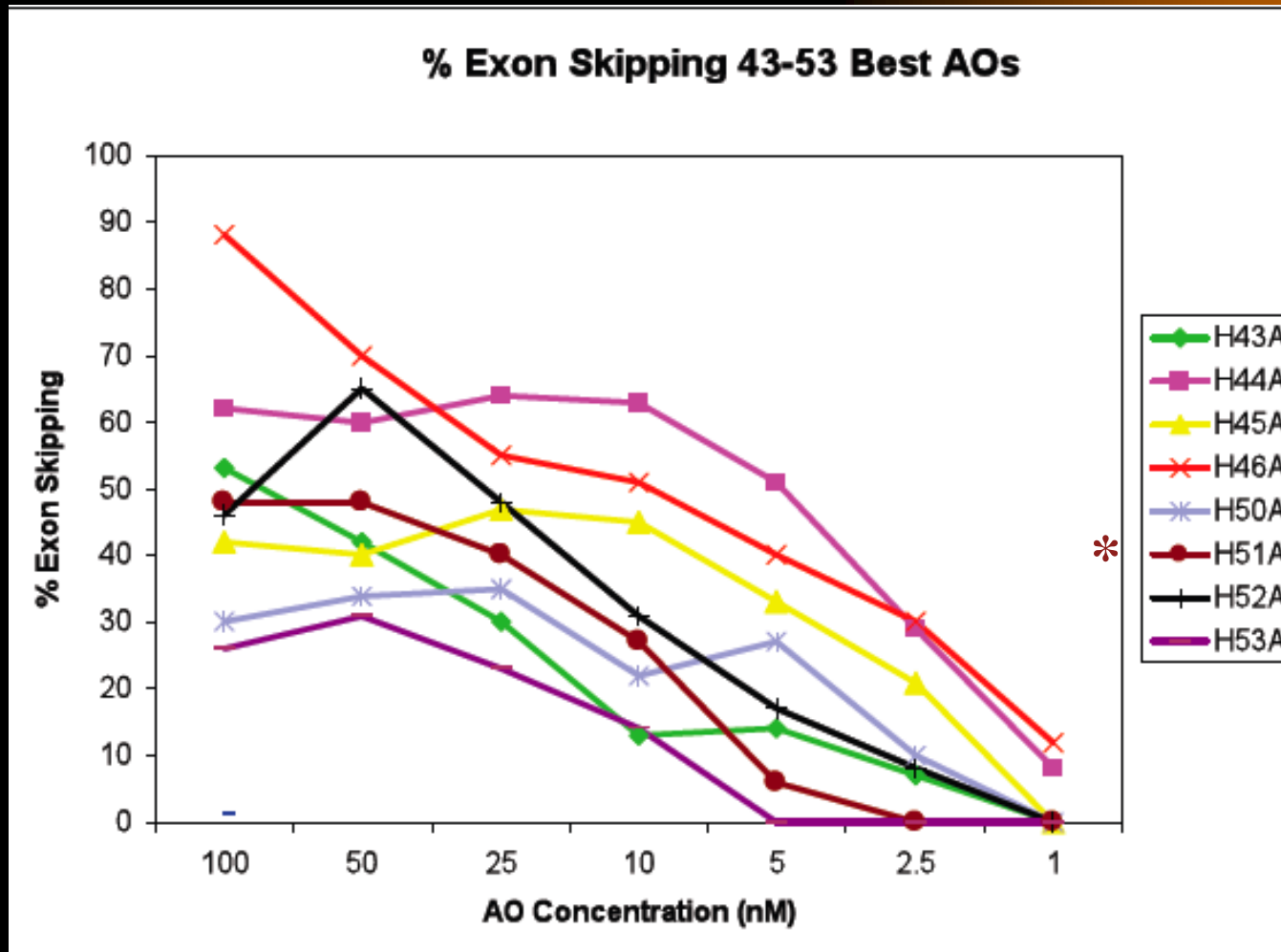


Individual Responses



- Different dystrophin isoforms induced
- Extent of pathology before treatment
- Genetic background
- Deletion break-points?
- DNA Polymorphisms (exonic and intronic)

Clinical trials: efficiency or frequency?



Safety testing

- Spanner designed to target dystrophin pre-mRNA
- Cross binding to another transcript???
 - designed to target one part of one dystrophin exon/intron
 - over 230,000 different exons identified to date
 - all exons represent ~2% of transcriptome



cacggcuu

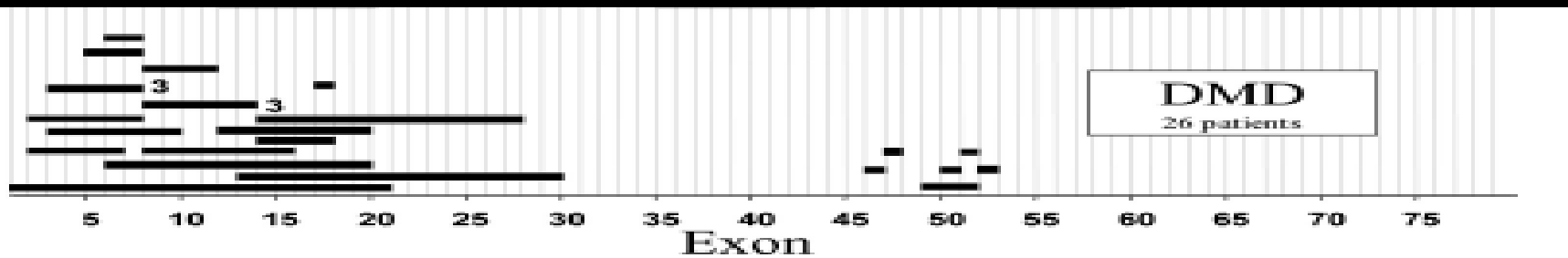
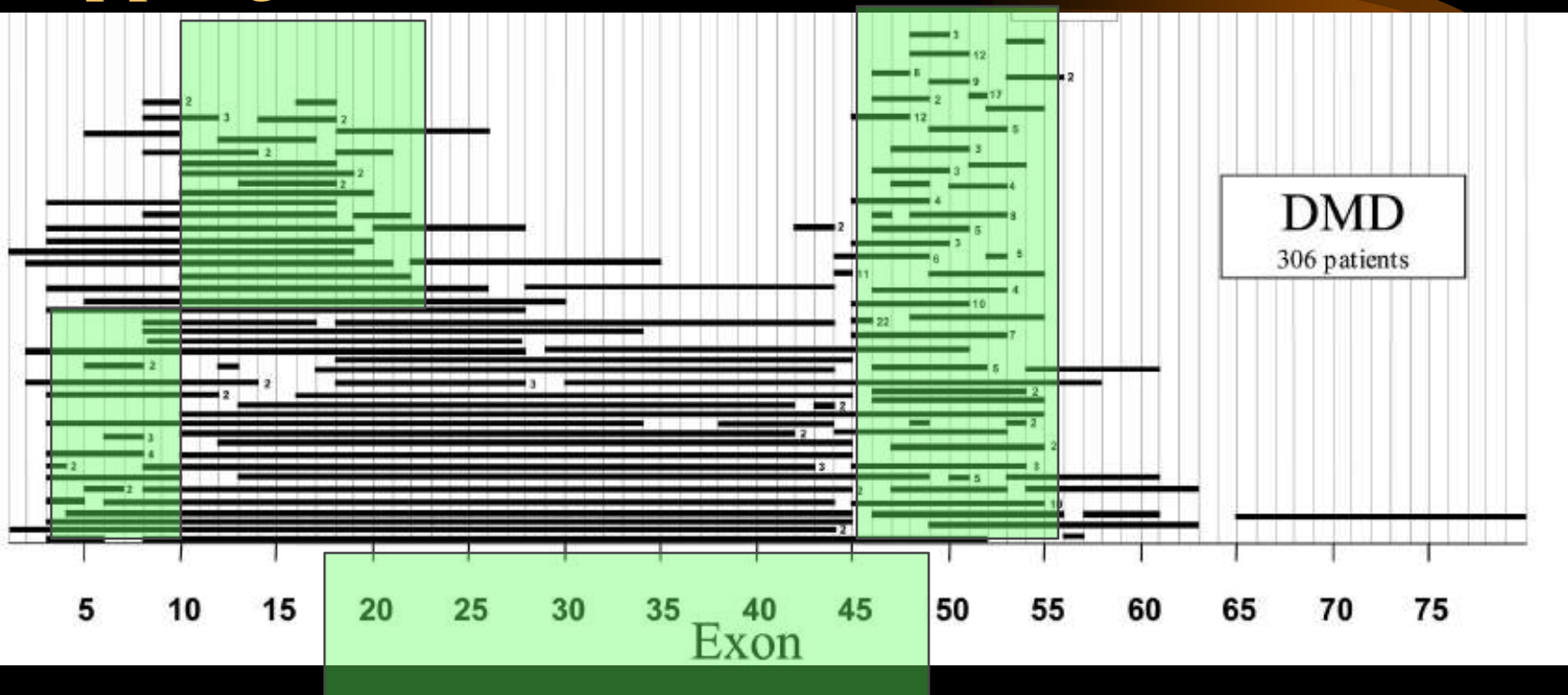
CGCGCAUGUGCGAAACGAGCUG

Unrelated gene X

Milder / Asymptomatic BMD cases

| <i>Exon Deletion</i> | <i>Comments</i> | <i>Reference</i> |
|----------------------|---|-------------------|
| <i>3-9</i> | <i>Playing competitive badminton at age 62 years.</i> | <i>Heald 94</i> |
| <i>9-22</i> | <i>High CK, myalgia but well developed musculature and no evidence of muscle weakness</i> | <i>Gospe 89</i> |
| <i>17-48</i> | <i>Source of the dystrophin mini-gene used in gene replacement studies</i> | <i>England,91</i> |
| <i>35-44</i> | <i>Cramping after soccer or mountain climbing</i> | <i>Koenig 89</i> |
| <i>41-44</i> | <i>Elevated CK, otherwise asymptomatic</i> | <i>Comi 94</i> |
| <i>45-53</i> | <i>Diagnosed age 60</i> | <i>Bosone</i> |
| <i>50-53</i> | <i>Elevated CK, otherwise asymptomatic</i> | <i>Comi 94</i> |

Redundant domains & Multi-exon skipping



Multi-exon skipping

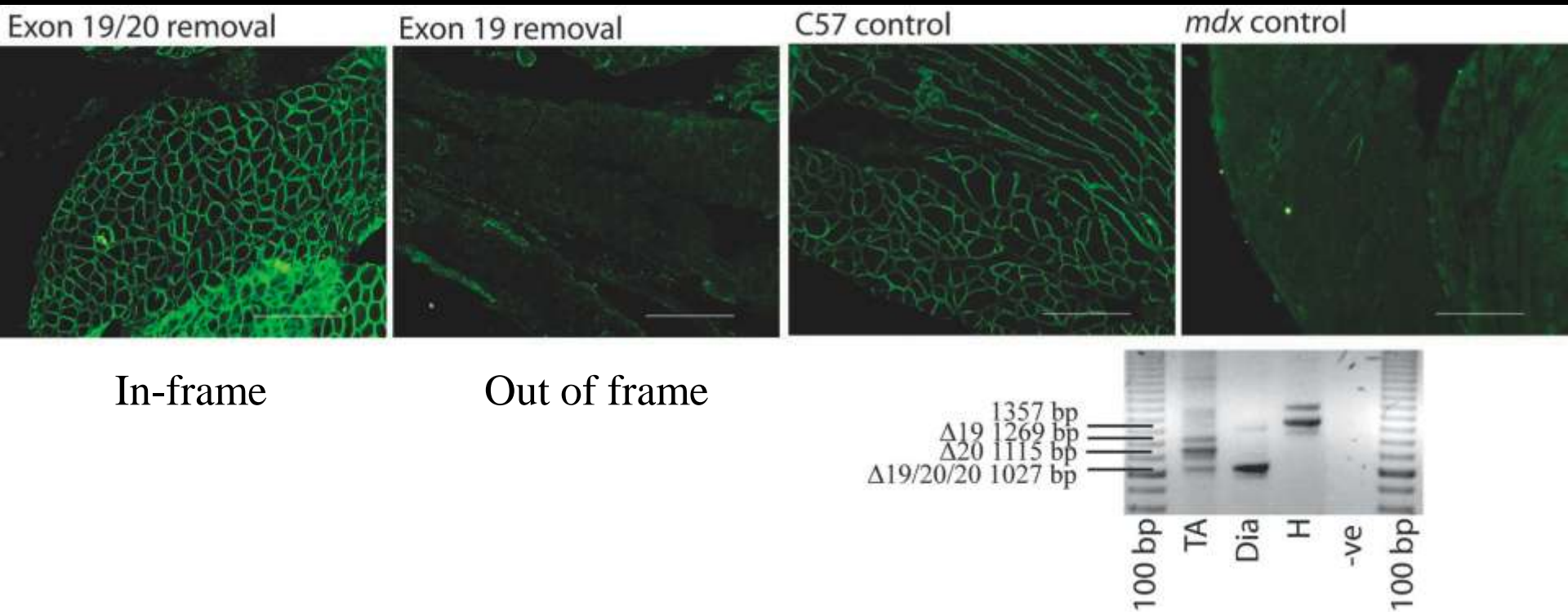


- Advantages
 - Induce dystrophin isoforms of known function
 - Address clustered mutations
 - Fewer oligomer preparations needed
- Limitations
 - Efficiency
 - Not as simple as combining best AOs
 - Dystrophin gene variation (polymorphisms/disease)
 - Increased potential for off- target effects
 - Cost

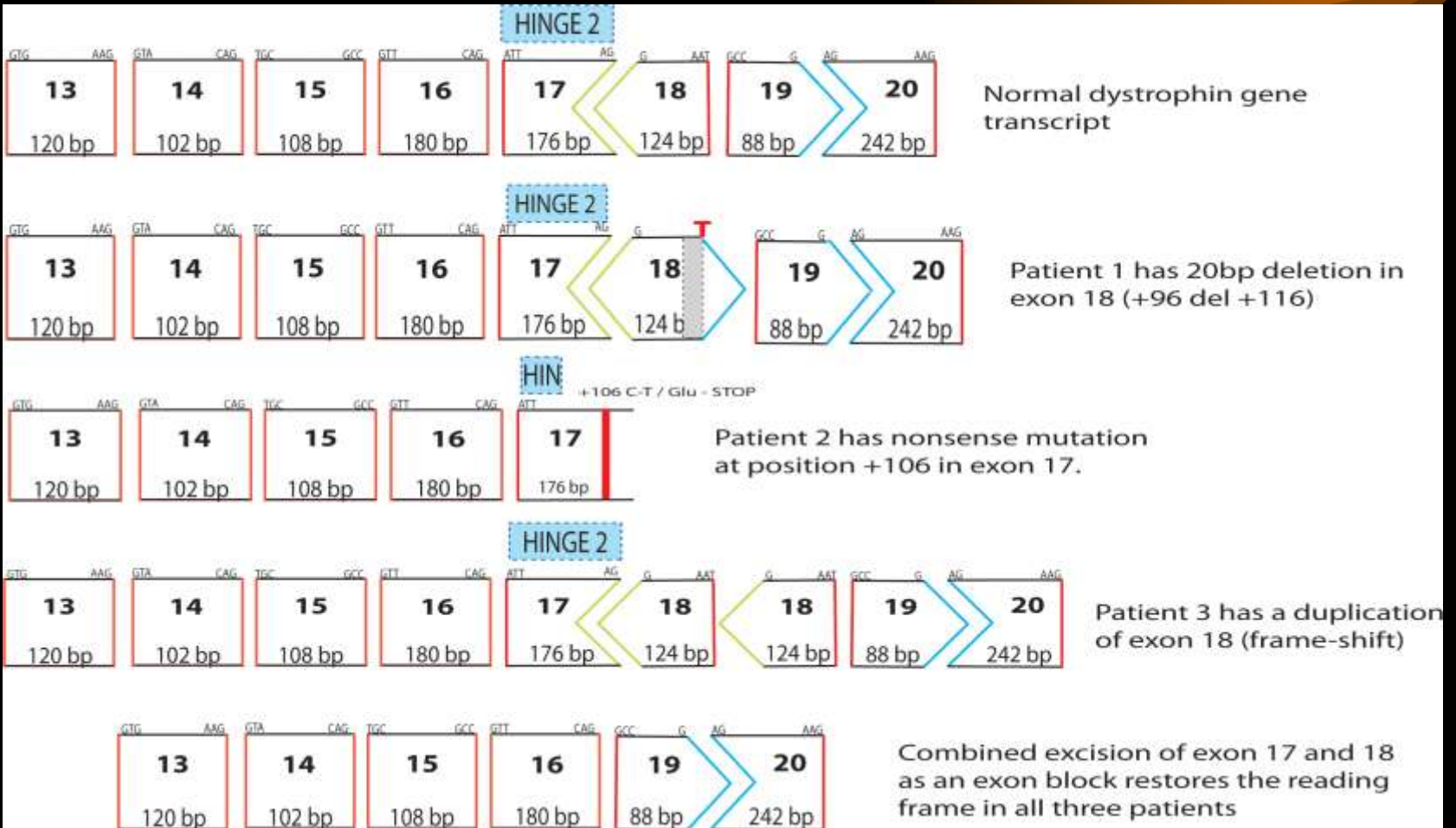
Mapping functional dystrophin domains

Induction of BMD-like Mice

- Mapping dystrophin isoforms based upon exon boundaries



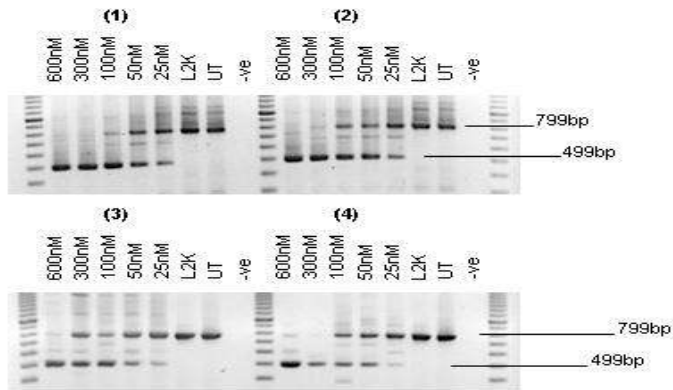
Multi-exon skipping (exon 17 + 18)





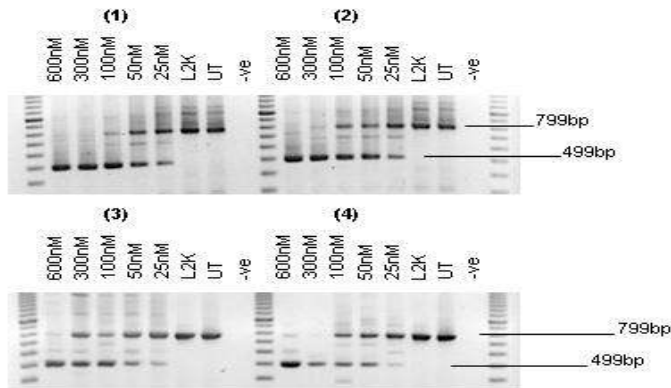
Exon 17 + 18 Skipping

(a) Normal Primary Human Myoblasts

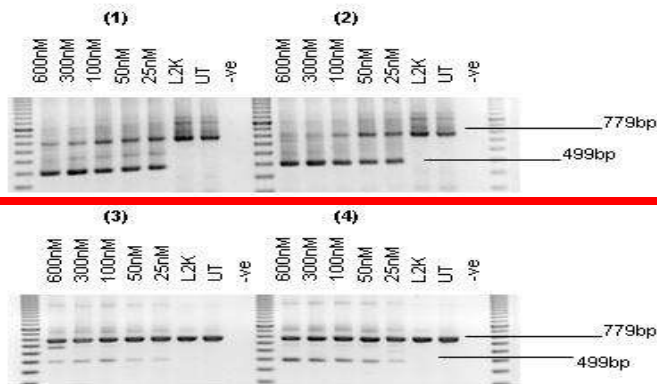


Exon 17 + 18 Skipping

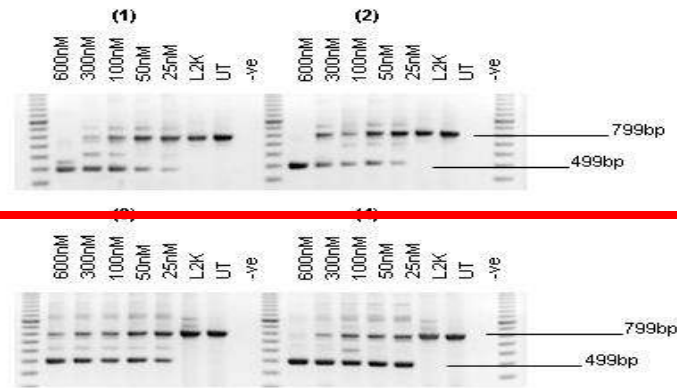
(a) Normal Primary Human Myoblasts



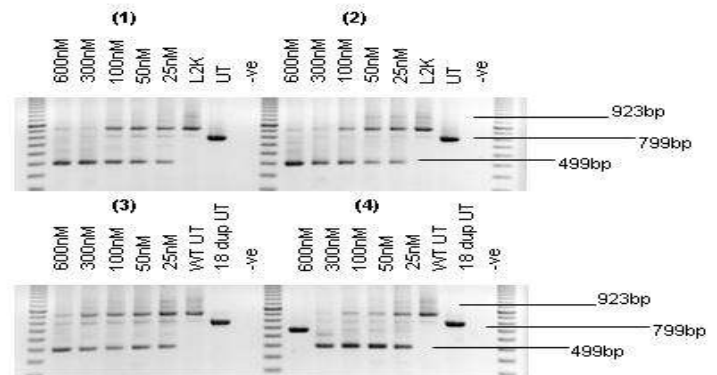
(b) Exon 18 20bp micro-deletion



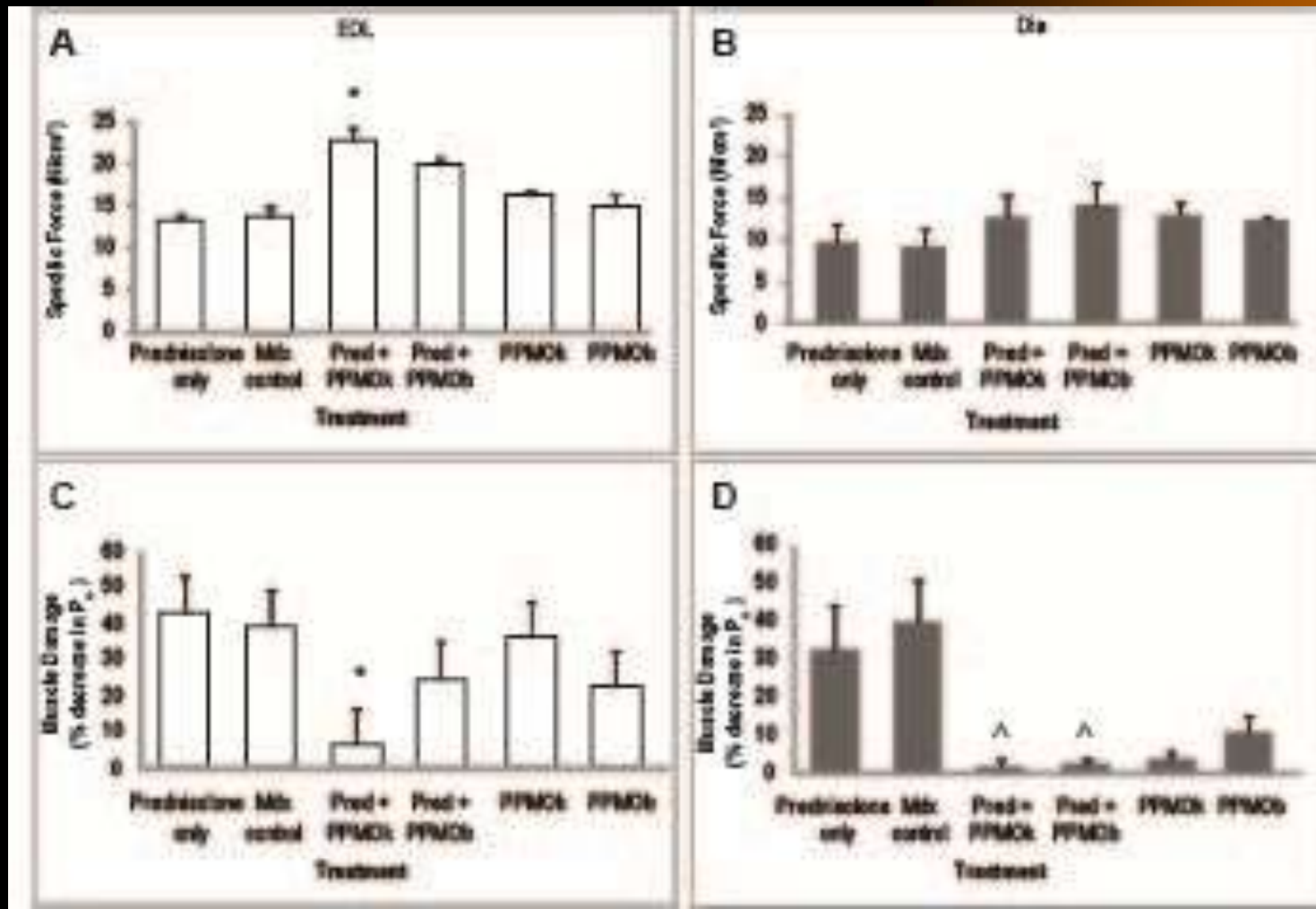
(c) Exon 17 NSM



(d) Exon 18 Duplication



Steroids and exon skipping



A Very Personalized Genetic Therapy

- PMOs: administered under close clinical supervision
 - Individual responses will determine delivery regimen
- Regard PMOs as “molecular spanners” designed to specifically modify dystrophin splicing
- Must expedite skipping of other exons
- Clinical trials updates this afternoon.
 - New paradigms in clinical trial structure and drug supply

How to go forward I

Extension study

Extension study.

Planned extension study with the effective dose identified in study 28 currently not supported by AVI

None of the 19 children in study 28 have received the AVI-4658 since the end of the study, and this raises ethical issues

Clinical trial protocol and information for parents clearly stated that patient would be offered to go in an extension study if drug effective and safe.

"It would be appropriate and ethical that the subjects participating in the dose -ranging study be offered to continue weekly AVI-4658 administration for a total of 52 weeks if they agree to continue rigorous assessment of safety as would be performed in the pivotal study".

Discussion with AVI ongoing regarding the extension study, as AVI remains intentioned to perform it, although when and level of dystrophin for taking an extension study forward under review.

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9 week old dko mouse ($dys/utr^{-/-}$)



Treated Dko mouse (age matched littermate)

