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THE RESEARCH INSTITUTE

Duchenne and Becker Muscular Dystrophy: Clinical Features Genetics, and Diagnostics

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Columbus, Ohio

Outline

- The dystrophinopathies: Duchenne and Becker muscular dystrophy
- Clinical features
- Lessons from mutational analysis
  - Genotypically and phenotypically catalogued patients
  - Newborn screening
- Emerging therapeutic approaches

Duchenne de Boulogne

École nationale supérieure des beaux-arts

Paralysie musculaire pseudohypertrophique ou paralysie myosclérosique

Duchenne:

- *De l'électrisation localisée et son application à la pathologie et à la thérapeutique*. (1861)
- *Récherches sur la paralysie musculaire pseudohypertrophique, ou paralysie myosclérosique*. Arch Gén Méd. (1868)

Edward Meryon (England):

- "granular and fatty degeneration of the voluntary muscles" in eight boys (1852)

Gaetono Conte (Italy):

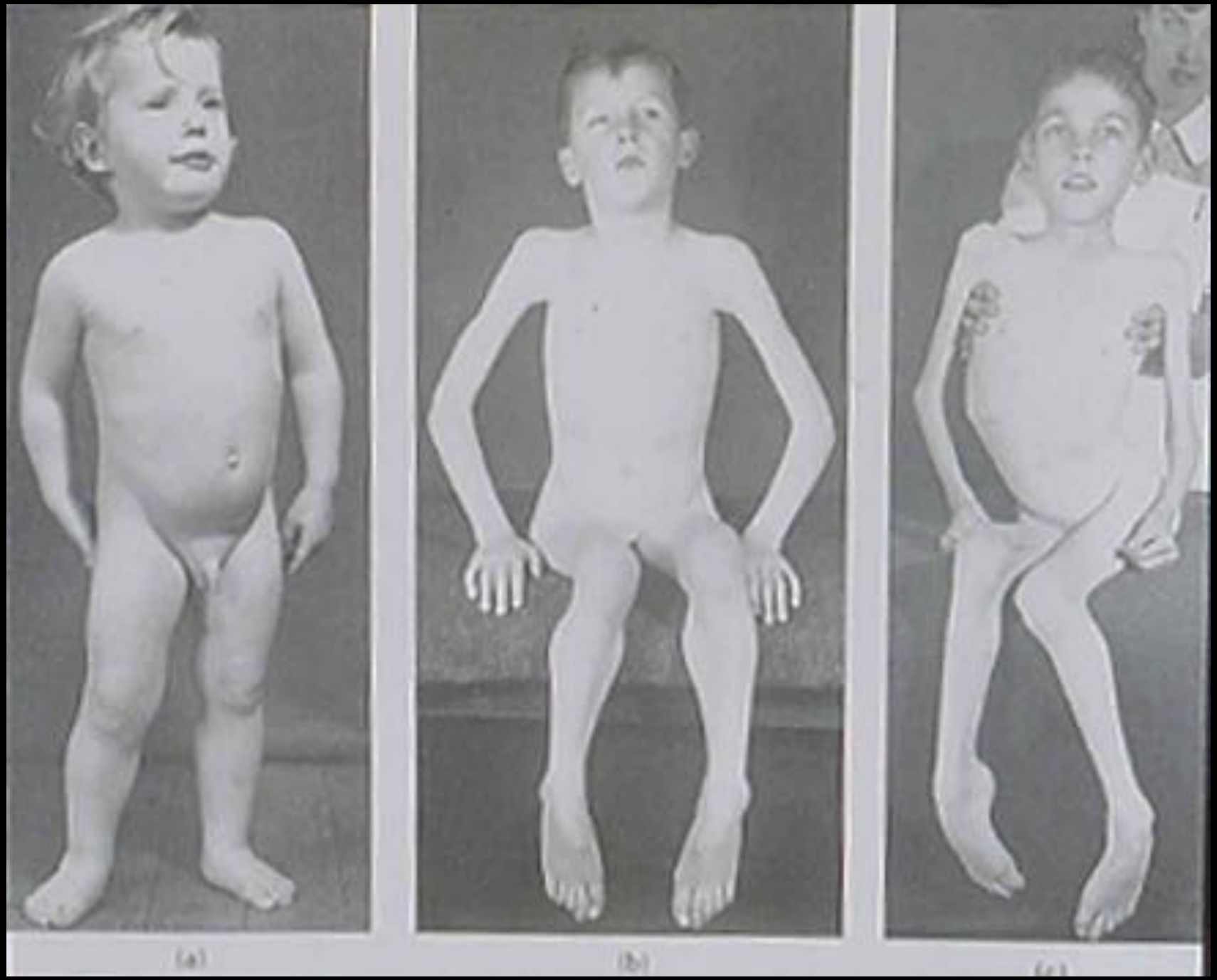
- affected brothers (1836)

Gowers

Duchenne Muscular Dystrophy

- Progressive skeletal muscle degeneration
  - Onset age 3-5:
    - Pelvic girdle weakness (difficulty arising/climbing stairs)
    - Gait abnormalities (toe walking)
    - Serum CK 50-100X normal

- Commonly cited incidence of 1:3500 live male births
- MDA estimates up to 12,000 boys with DMD registered in clinics

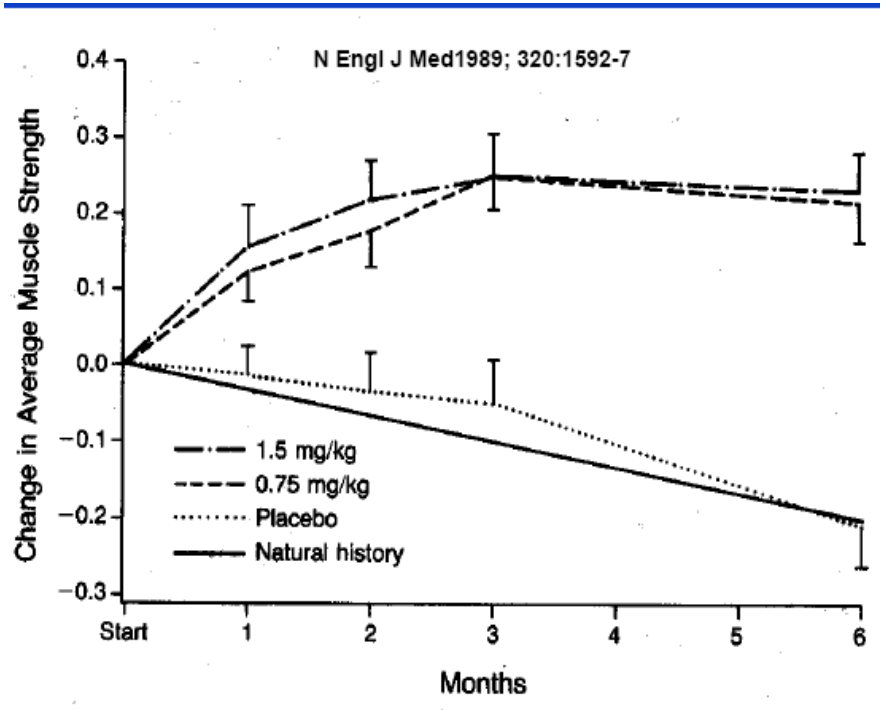


- Loss of ambulation by age 12 years (range 7-12)
- Mean age at death around 19 years
  - Dilated cardiomyopathy
  - Ventilatory insufficiency

Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy

Mendell JR, Moxely RT, Griggs RC, et al

N Engl J Med. 1989 Jun 15;320(24):1592-7.



- Improve muscle strength
  - Prolongs ambulation by 2-3 years
  - Prevents scoliosis
- Limitations
  - No change in functional grades
  - Many cannot tolerate side effects

- Loss of ambulation by age 12 years (range 7-12)
- Mean age at death around 19 years
  - Dilated cardiomyopathy
  - Ventilatory insufficiency
- Glucocorticoid corticosteroids
  - Prednisone 0.75 mg/kg/day
  - Deflazacort 0.9 mg/kg/day
  - AAN Practice Parameters; Cochrane review
  - Prolonged ambulation (up to 1-3 years)
  - Significant side effects
- Supportive Care
  - Nocturnal ventilatory support
  - Spinal surgery in appropriate candidates

Dystrophinopathies: Clinical diagnosis

- DMD:
  - Onset age 3-5
  - Pelvic girdle weakness
  - Tight heel cords
  - CK 50-100X normal
  - Loss of ambulation by age 12
- BMD:
  - Classic definition: loss of ambulation > age 12
  - Alternatively:
    - "intermediate muscular dystrophy" for loss of ambulation ages 12 through15
    - BMD for loss of ambulation >age 15
  - Limb-girdle syndromes in adulthood
  - Myalgias
  - Isolated cardiomyopathy

Dystrophin Mutations

- Dystrophin gene (Xp21.1) is huge:
  - 2.4 million base pairs
  - 79 exons and 8 promoters
- Large deletions (≥ 1 exon) account for ~65% of DMD/ BMD patients
- ~5% have duplications
- ~15% of boys have nonsense mutations
- Remainder are frameshifting insertions/deletions, splice site mutations, missense mutations

Distribution of mutations in an unselected cohort

(Dent *et al*; AJMG, 2005 Apr 30;134(3):295-8)

Mutation Type	DMD	BMD	Carrier	Total
≥1 exon deletion	32	13		45 (66%)
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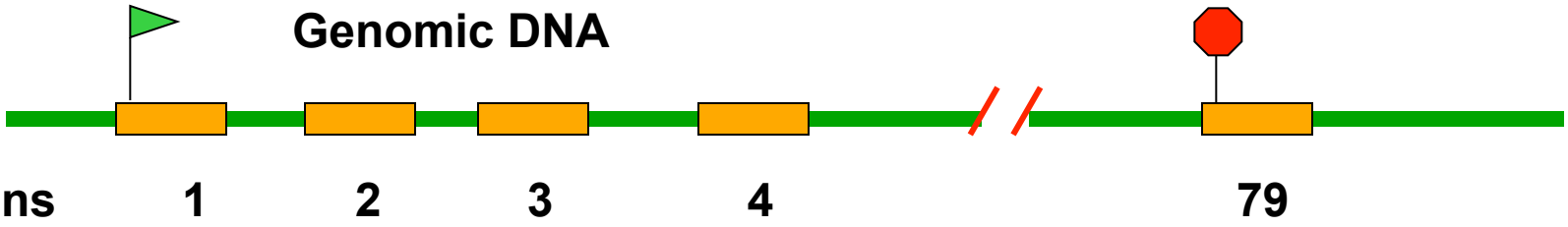
Total	45	21	2	68

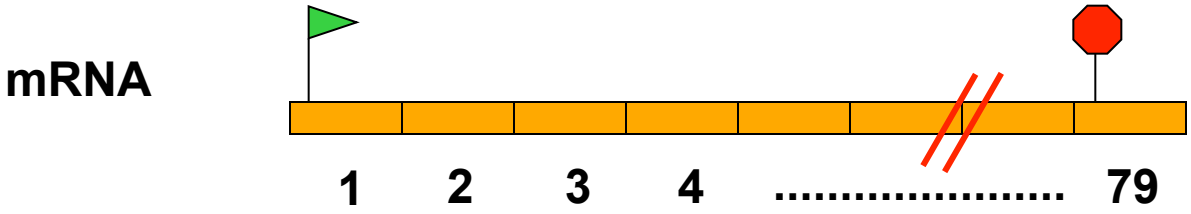
Currently available methodology can detect 93%-96% of dystrophinopathy mutations from blood samples.

(Yan *et al*, Hum Mutat 2004; 23:203-204).

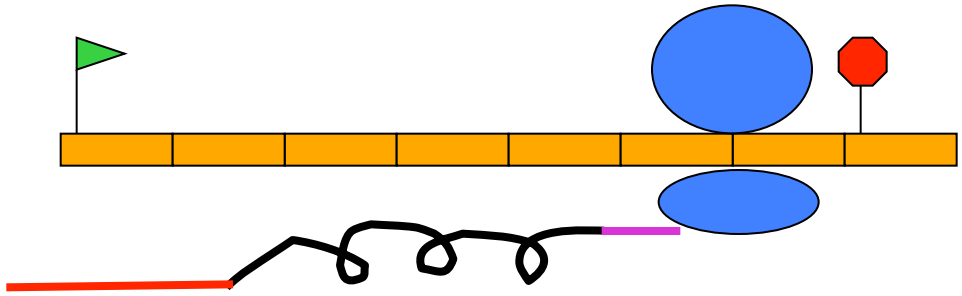
Dystrophin mutations: Duchenne vs Becker

- Size of deletion does not correlate well with phenotype
- in-frame deletions are more likely to result in translation of a protein with partial function
  - (i.e., out-of-frame deletions are DMD ~90% of the time)





Protein synthesis



Reading Frame

THE BIG RED DOG RAN AND SAT

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Reading Frame

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Reading Frame

- THE BIG RED DOG RAN AND SAT
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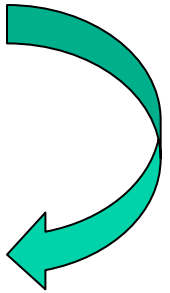

Reading Frame

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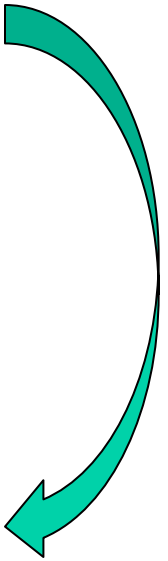
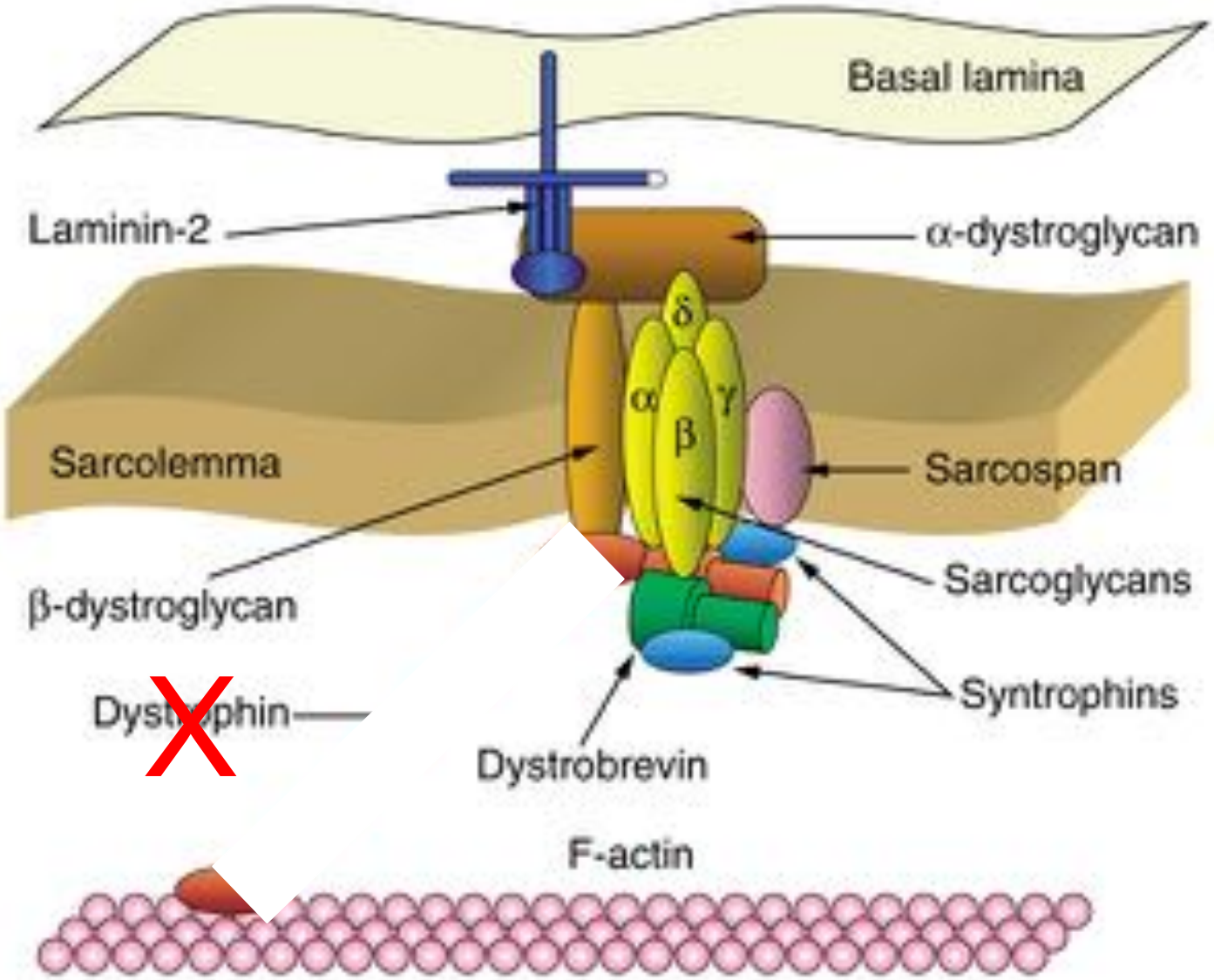
- ~~THE BIR EDD OGR ANA NDS AT~~
- THE DOG RAN AND SAT

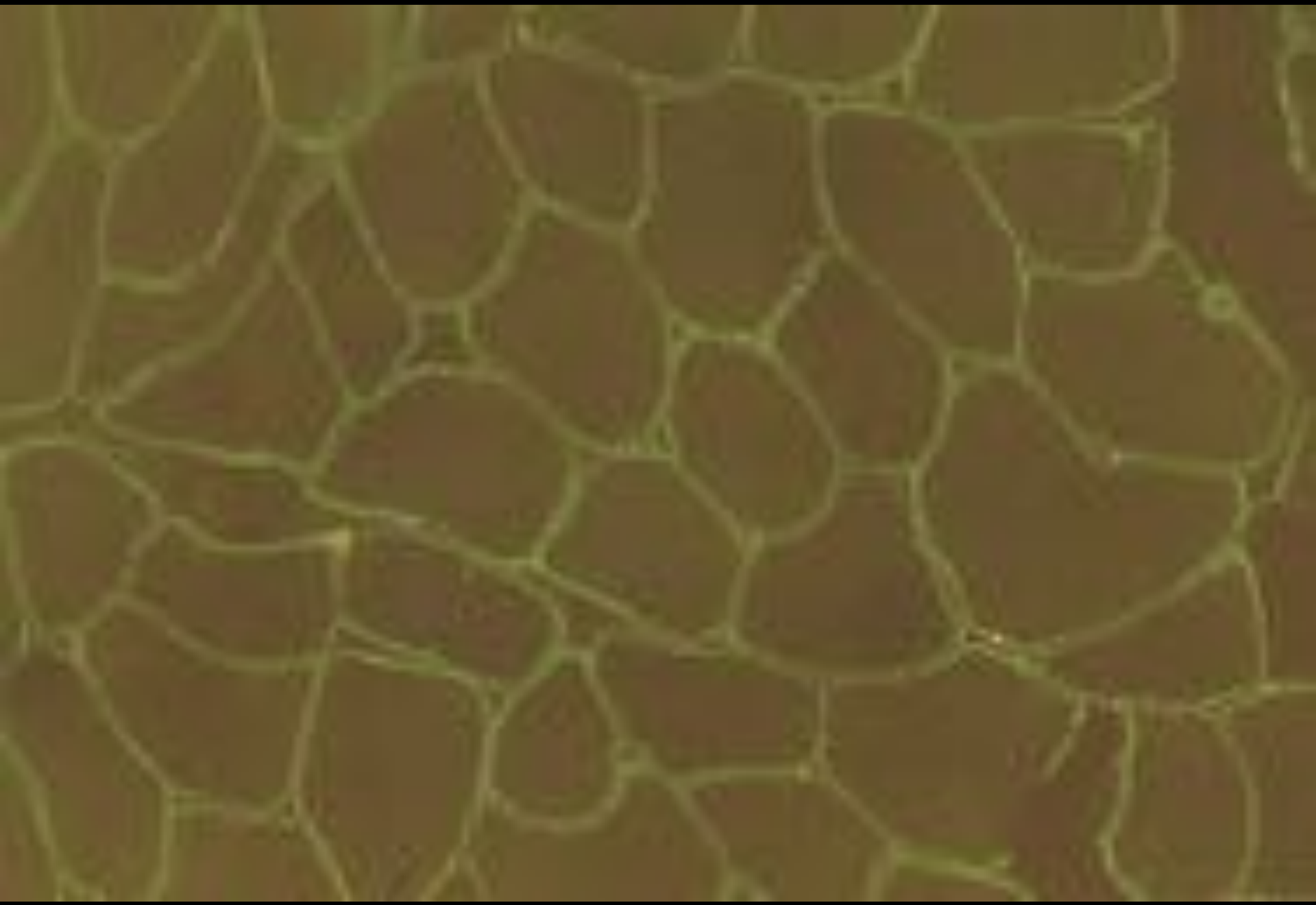


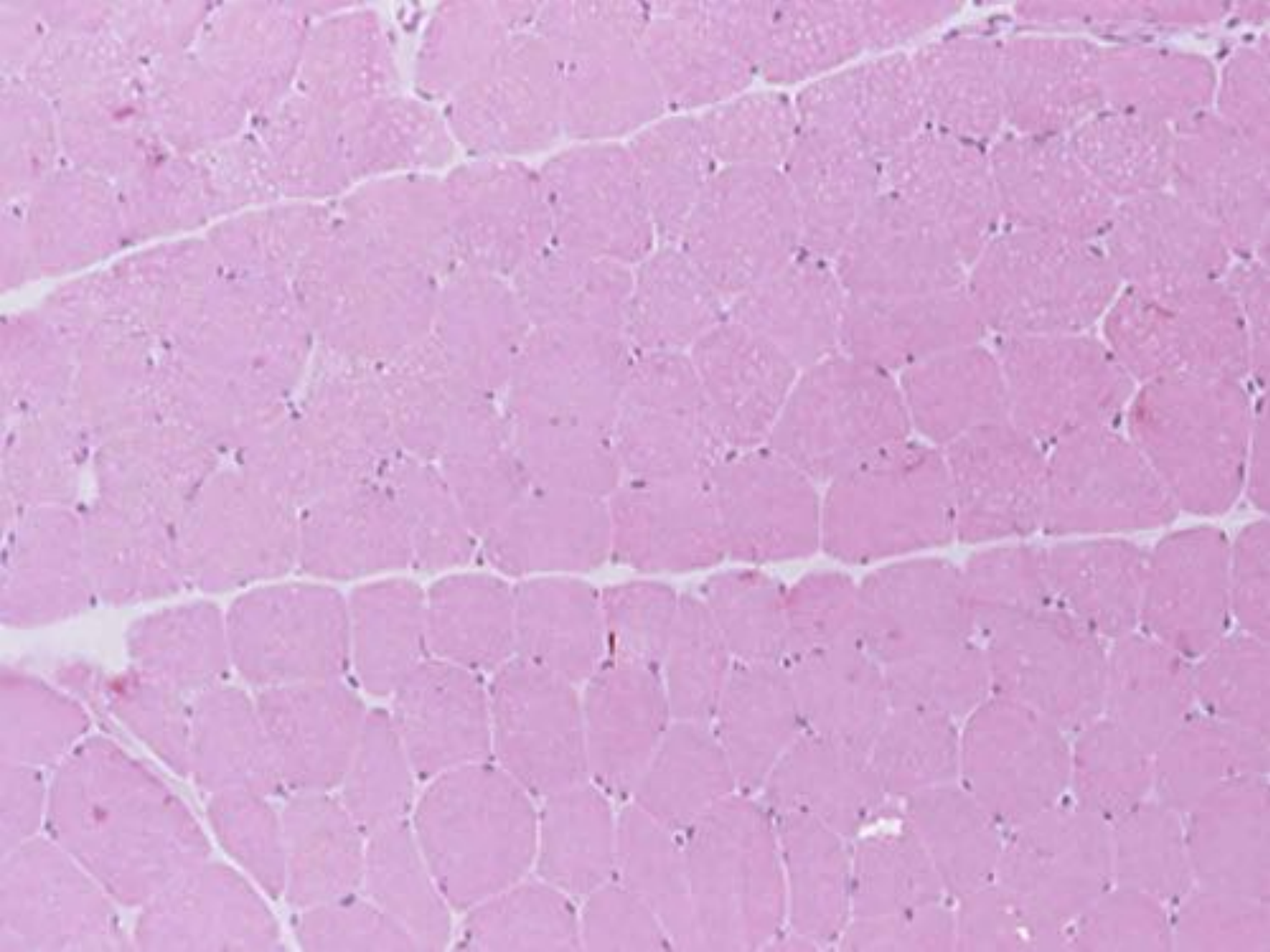
Reading Frame

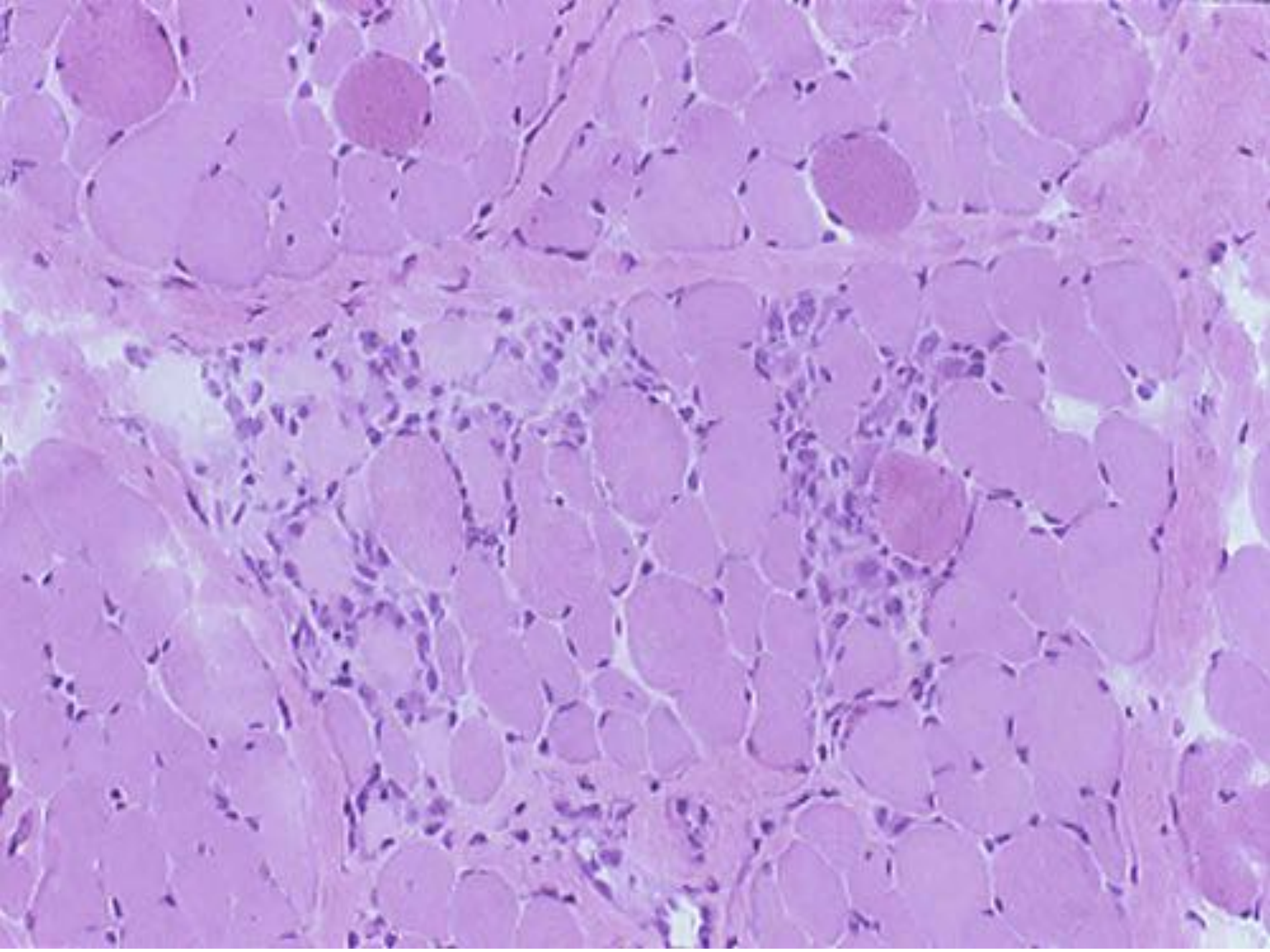
- THE BIG RED DOG RAN AND SAT
- THE BIR EDD OGR ANA NDS AT
  = Duchenne Muscular Dystrophy
- THE DOG RAN AND SAT
  = Becker Muscular Dystrophy

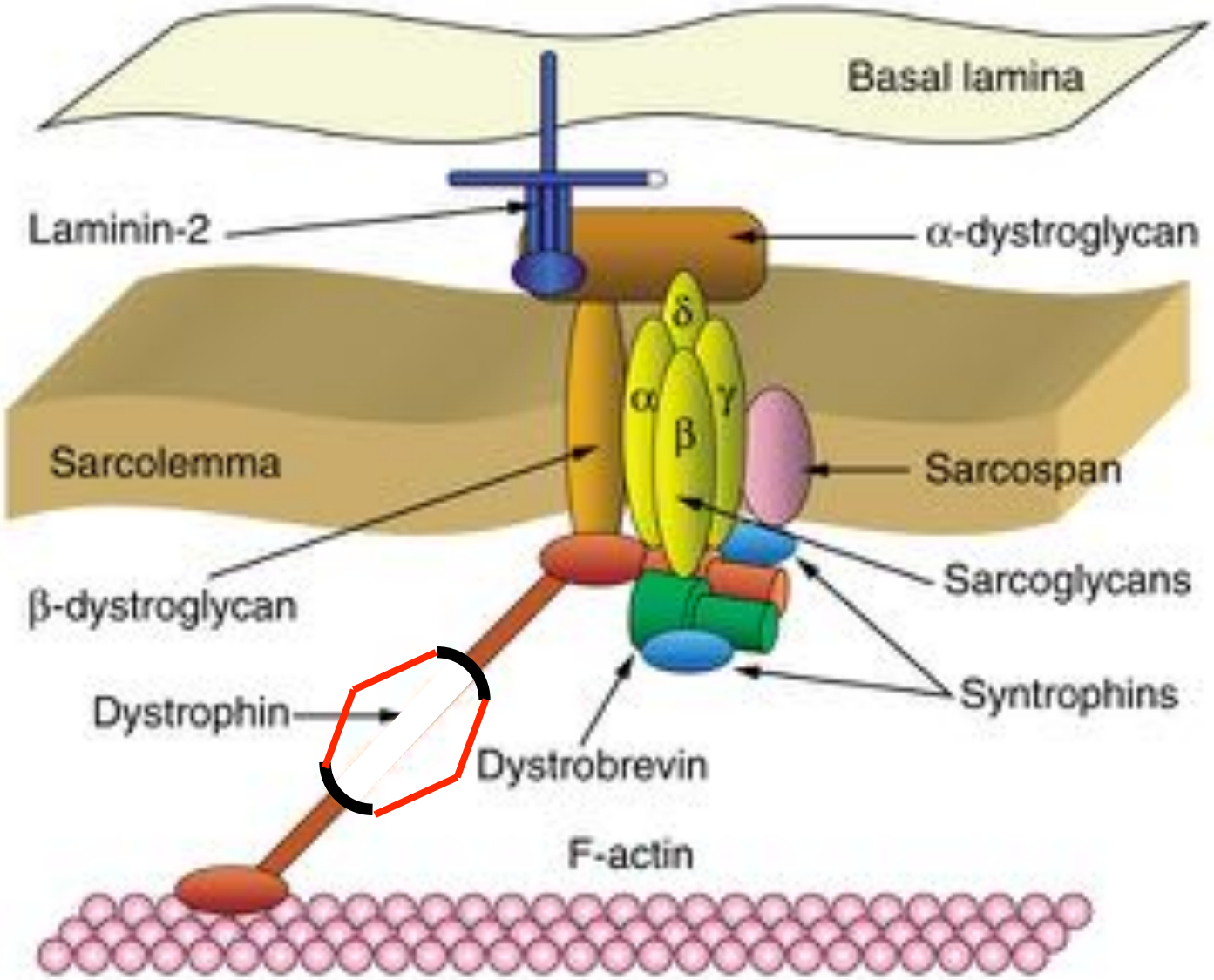


Roberts, *Genome Biology*, 2001

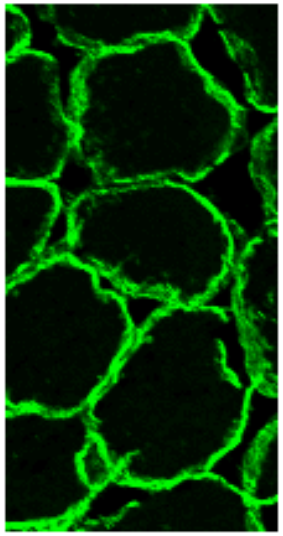
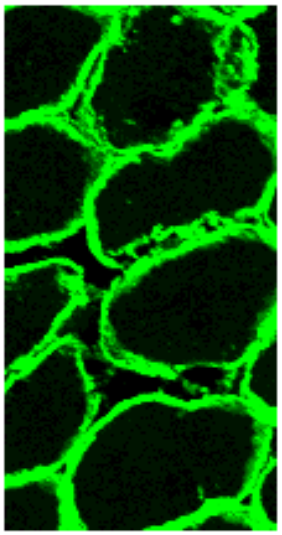
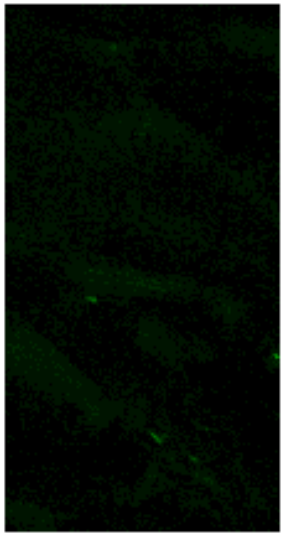
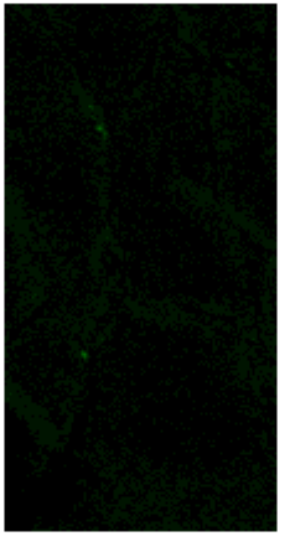


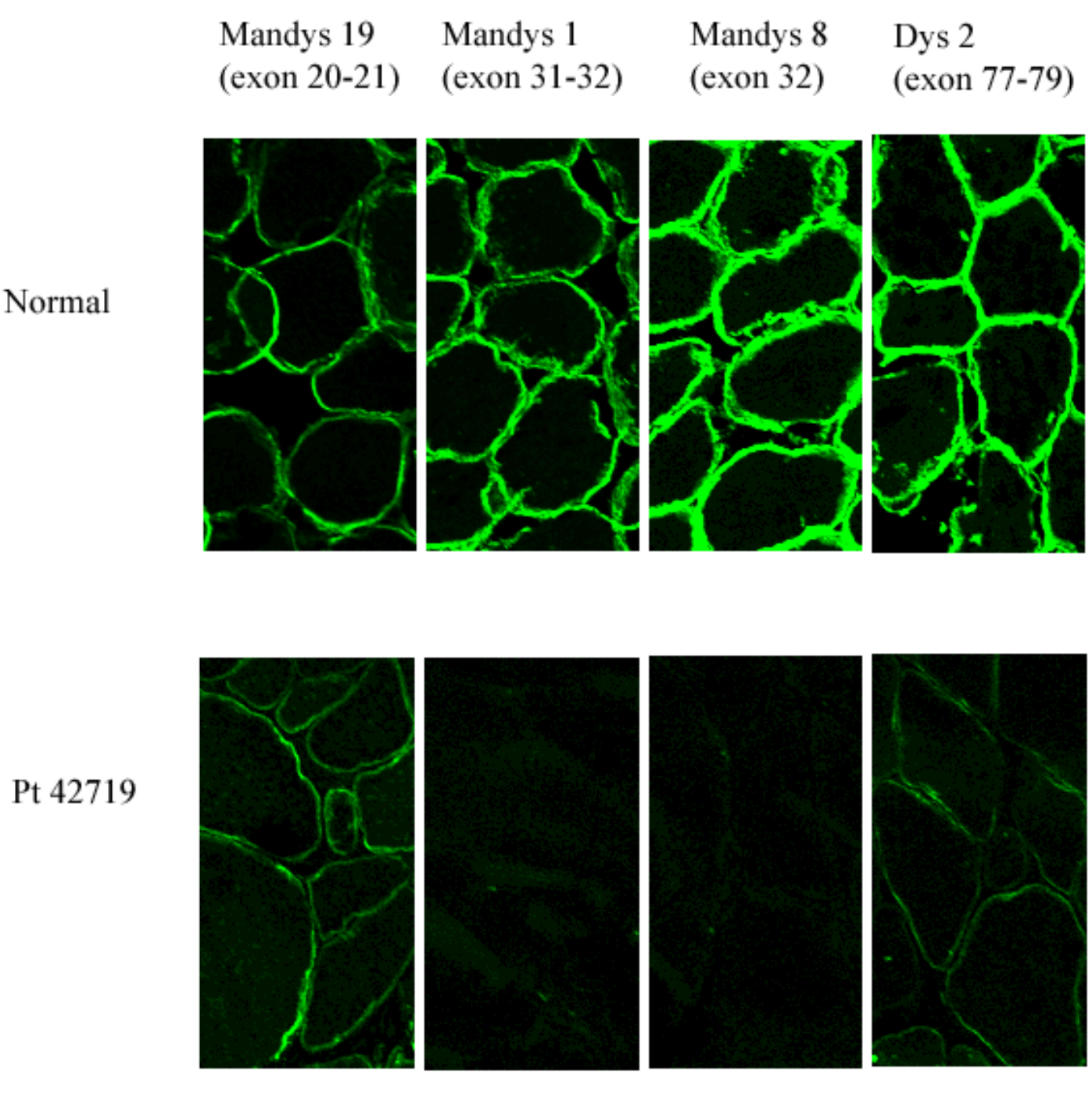


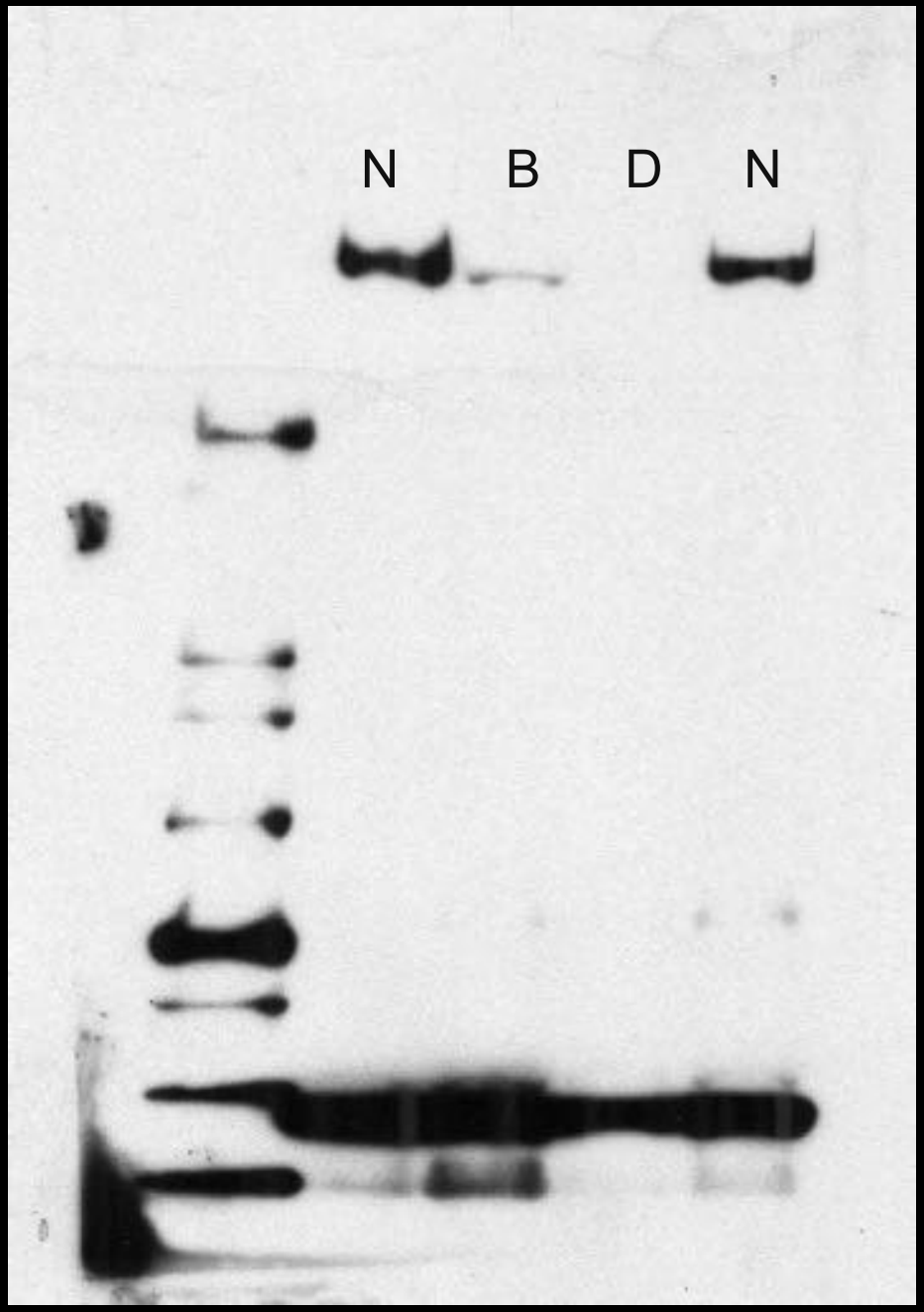




Roberts, *Genome Biology*, 2001

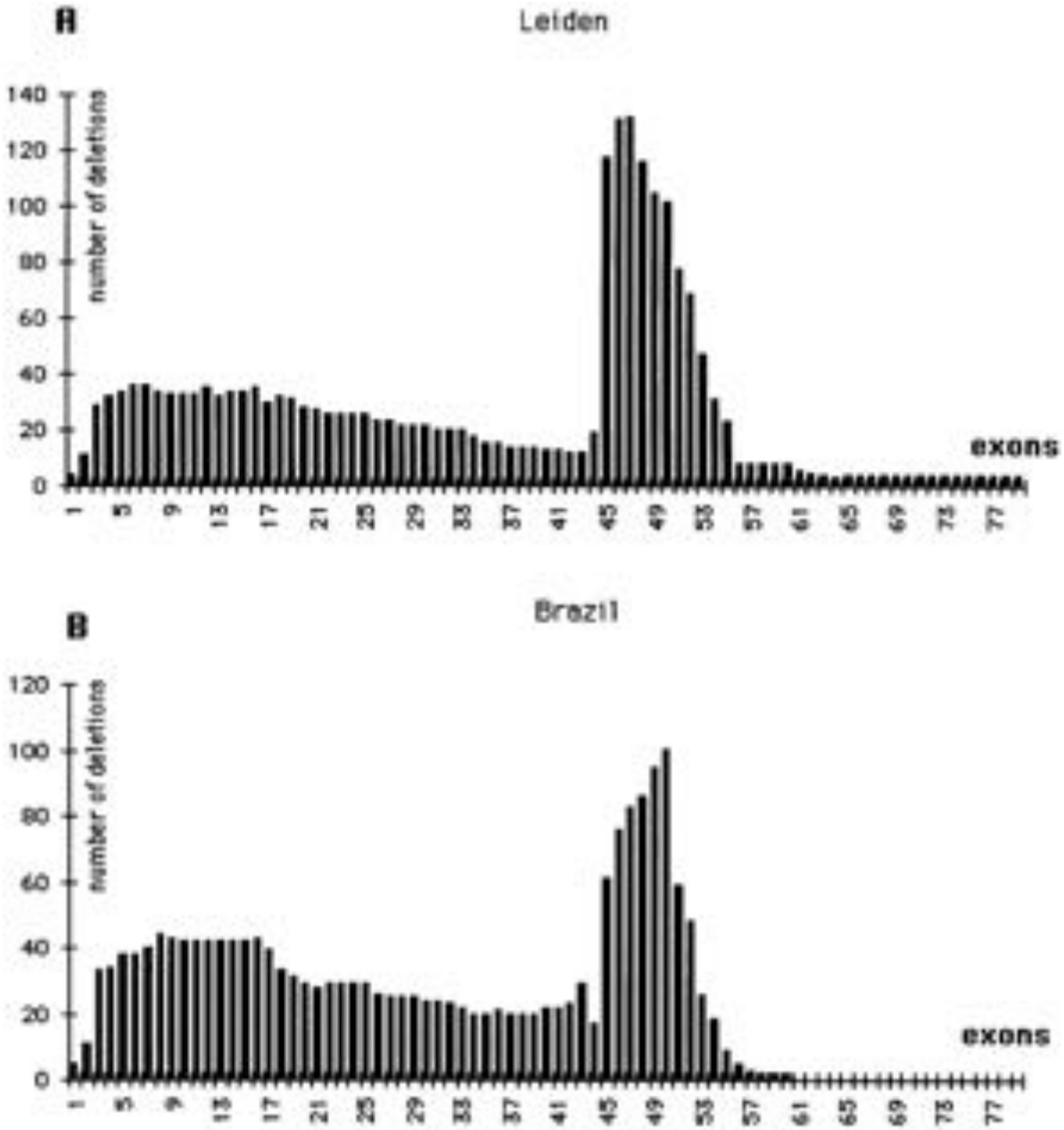
	Mandys 1 (exon 31-32)	Mandys 8 (exon 32)
Normal		
Pt 42719		


Mandys 19
(exon 20-21)
Mandys 1
(exon 31-32)
Mandys 8
(exon 32)
Dys 2
(exon 77-79)
Normal
Pt 42719

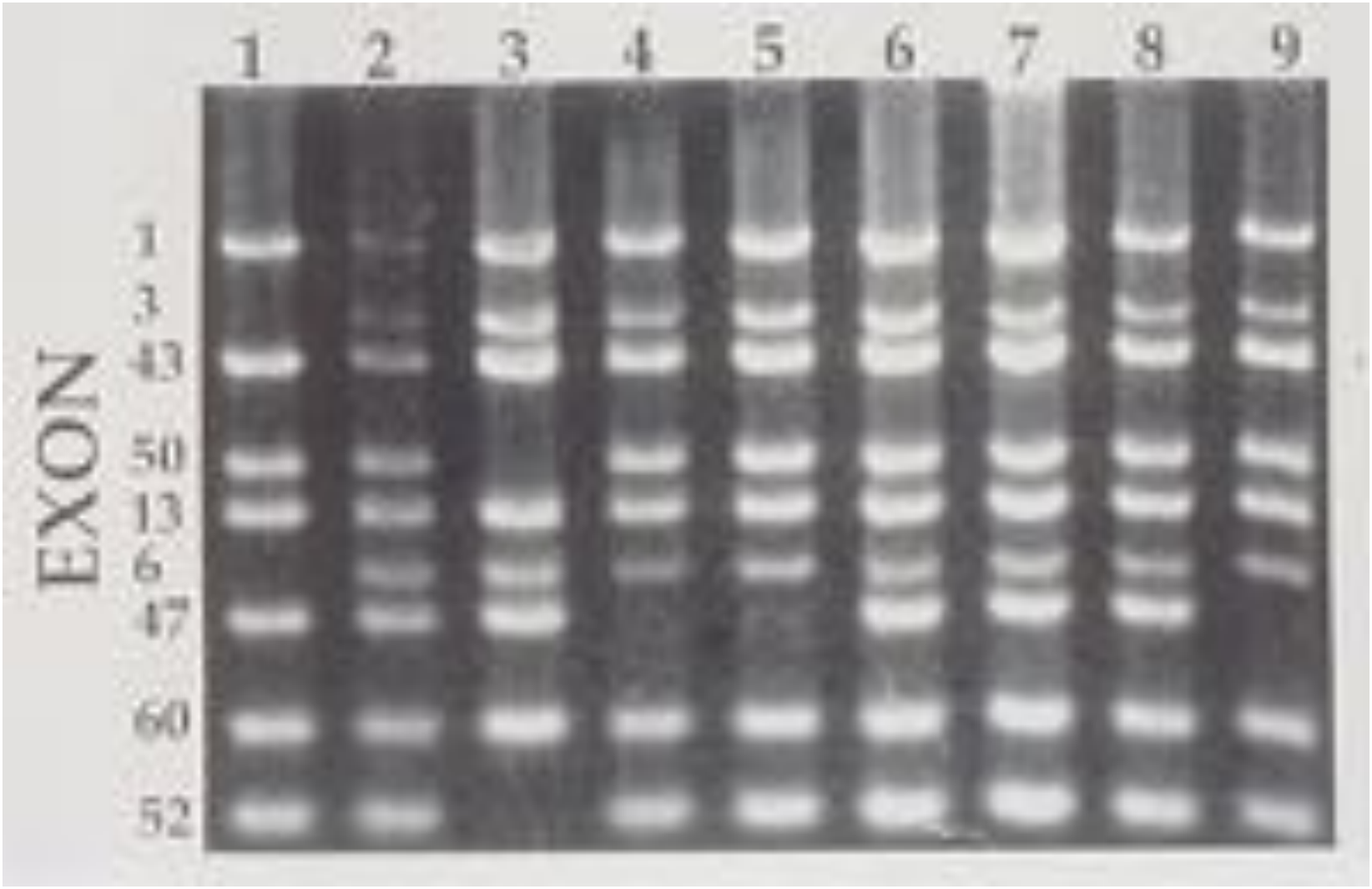


Dystrophin (427 kd)

GAPDH



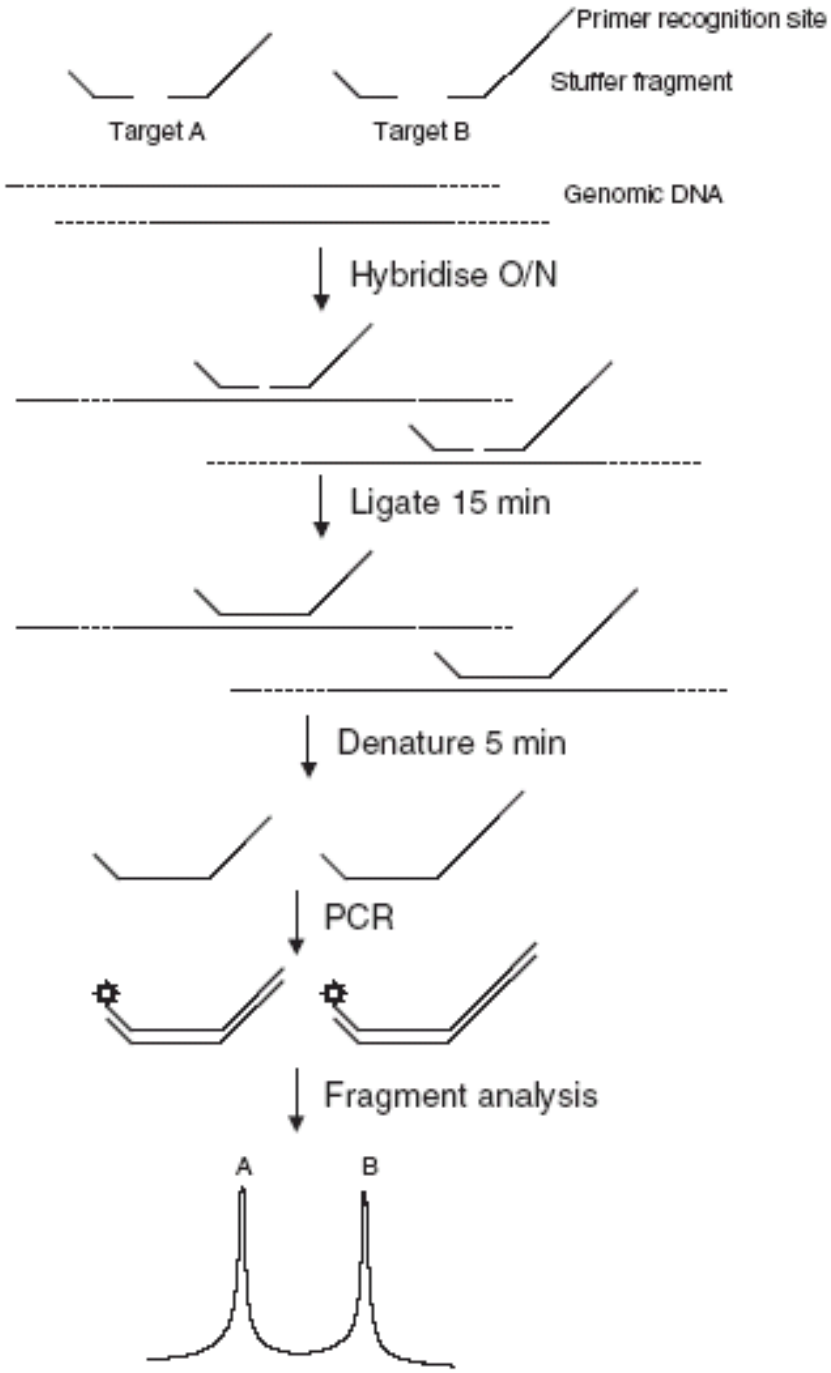
Rosenberg, *Neuromuscular Disorders*, 1998



Amplification of 25 exons detects ~98% of exonic deletions

Quantitative techniques detect duplications, and carriers of deletions/duplications

- ~~Multiplex PCR~~
- Southern blotting
- Quantitative PCR (radiolabeled/fluorophore/ dye concentration)
- Multiplex Amplifiable Probe Hybridization (MAPH)
- Multiplex Ligation-dependent Probe Amplification (MLPA)
- Comparative Genomic Hybridization (CGH)



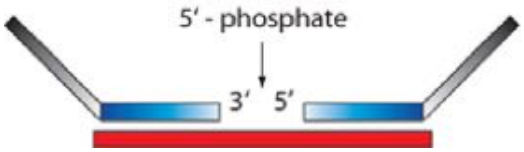
Multiplex Ligation-Dependent Probe Amplification

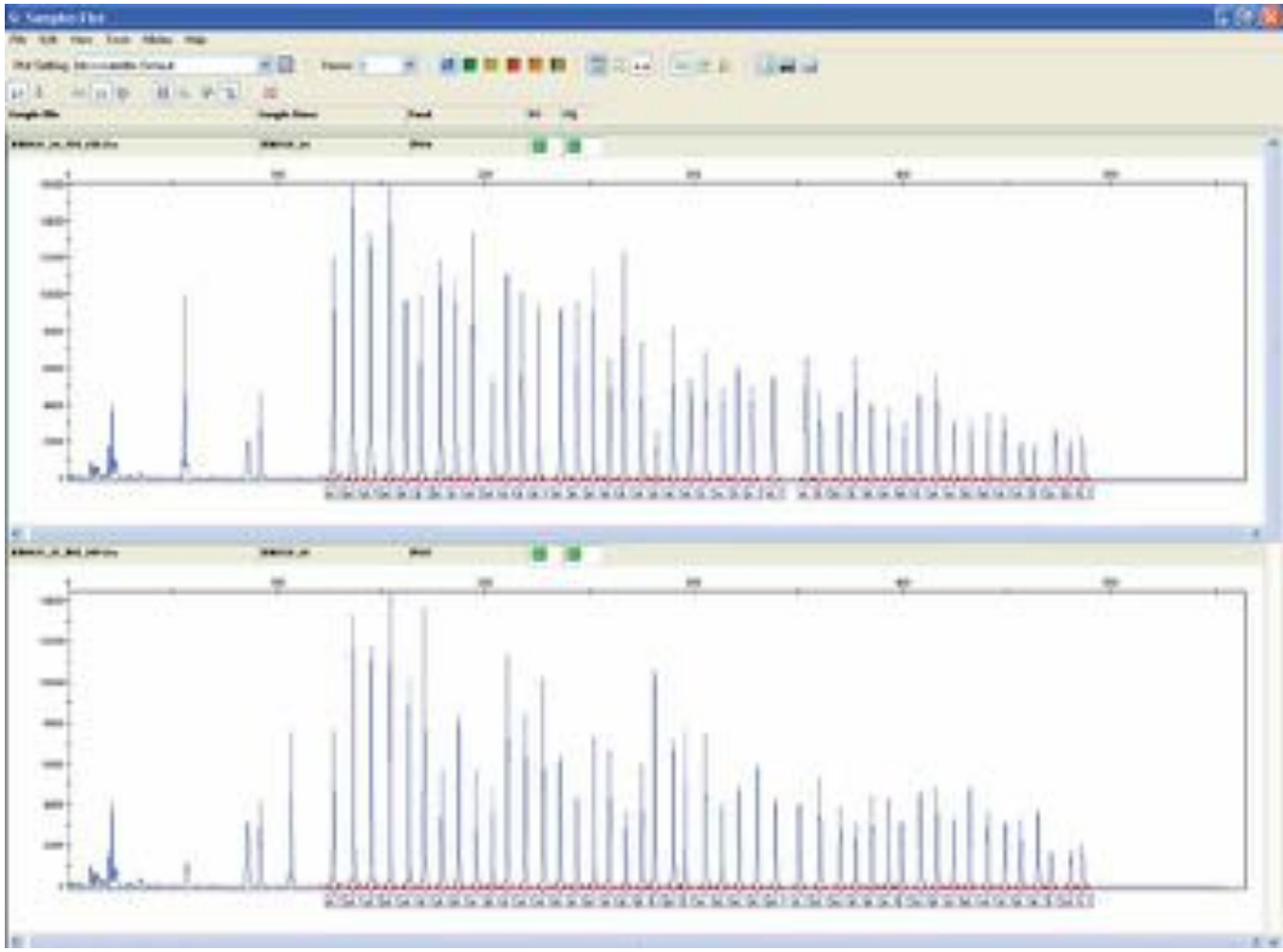
(MLPA)

Sellner & Taylor, Hum Mutat. 2004 May;23(5):413-9

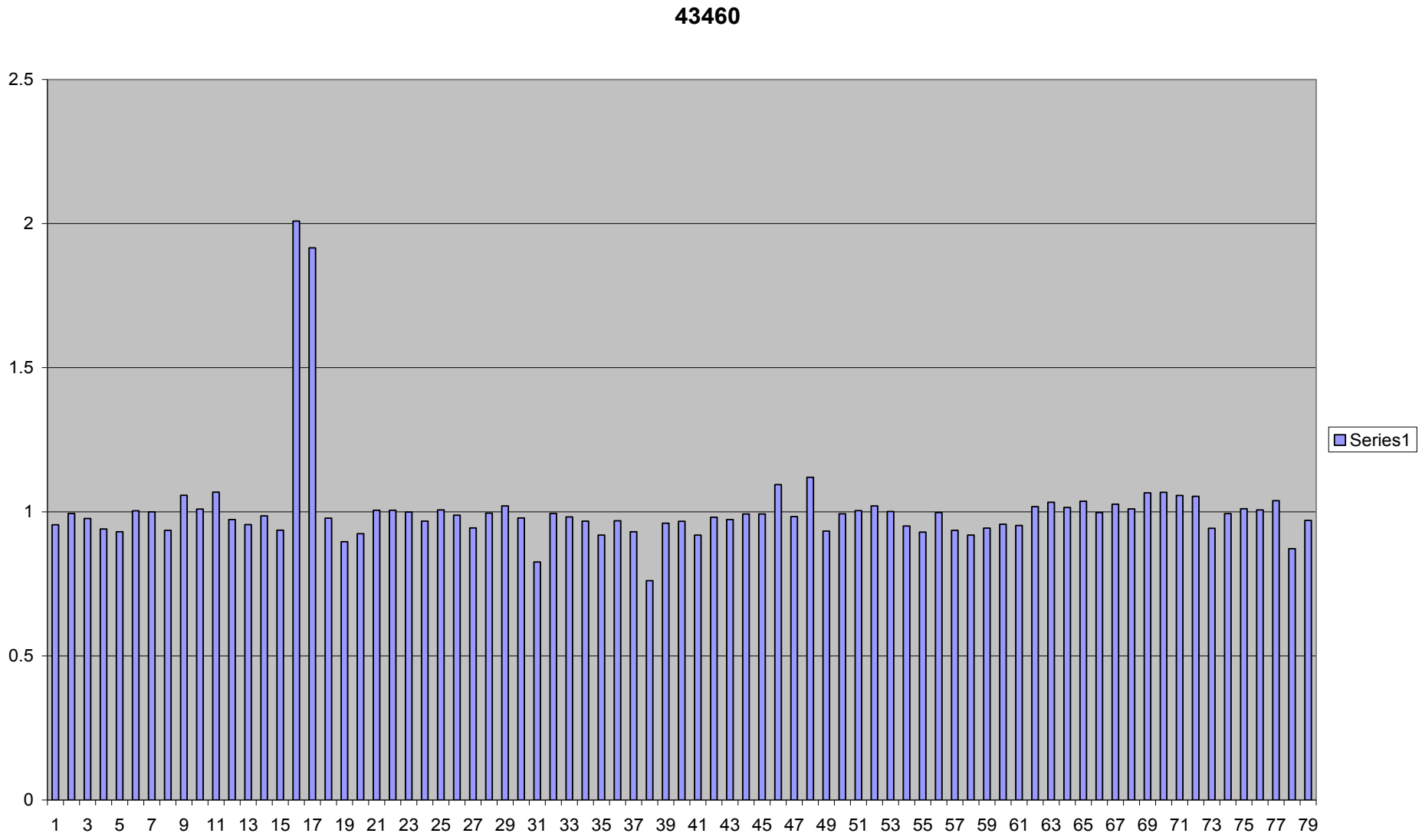
MLPA as first step

MLPA Probes: total length 115-400 bp

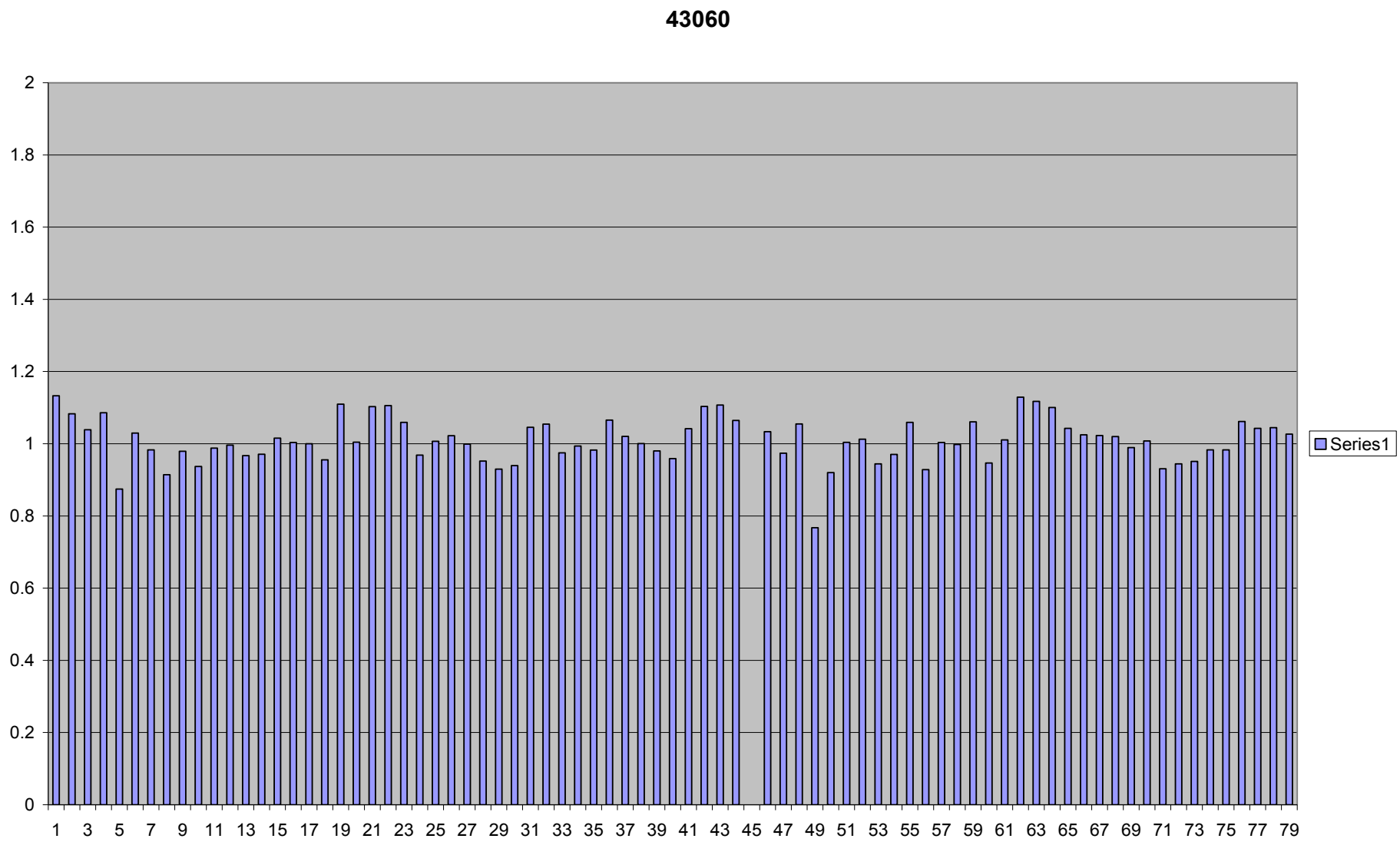




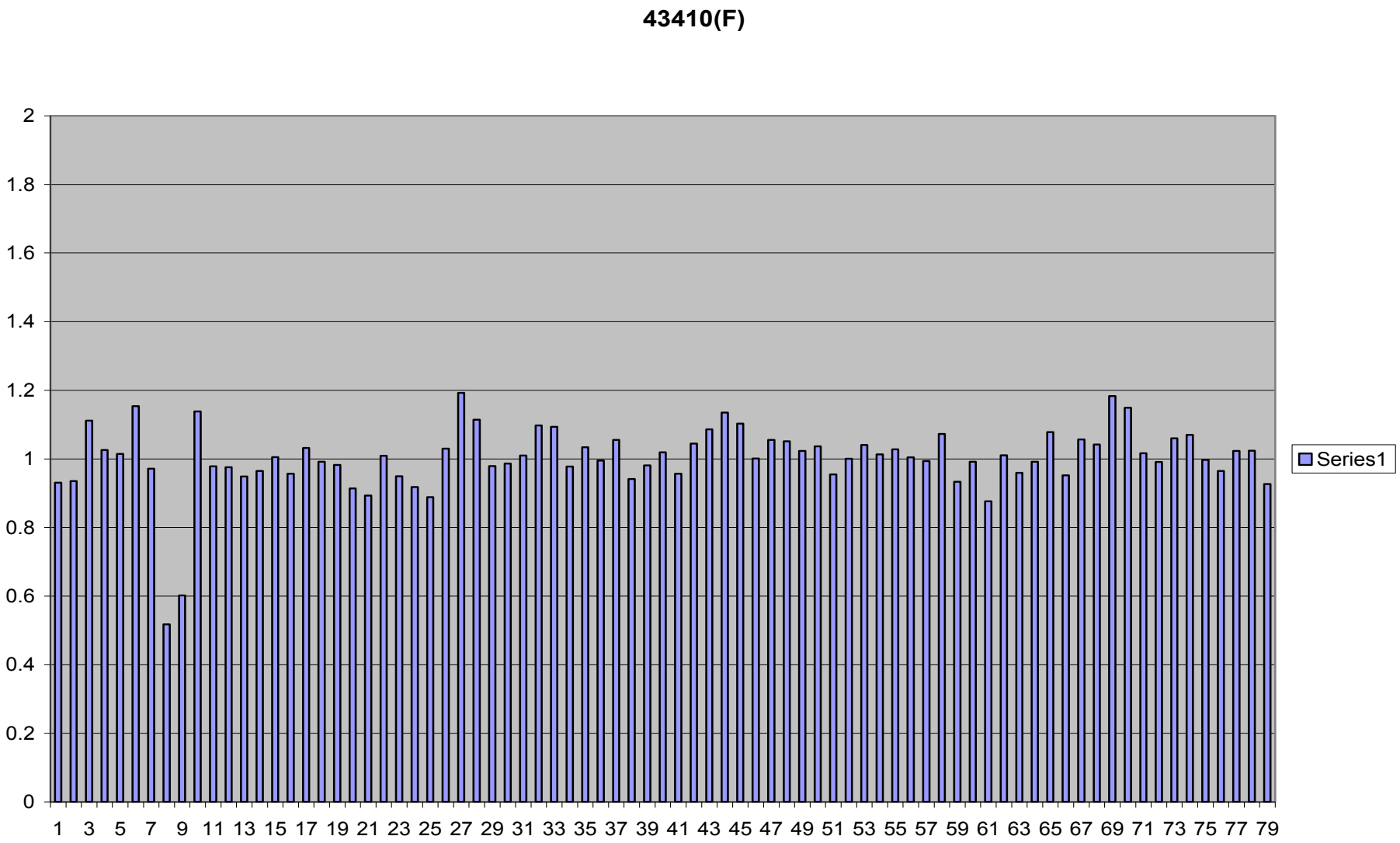
Duplication exons 18-19



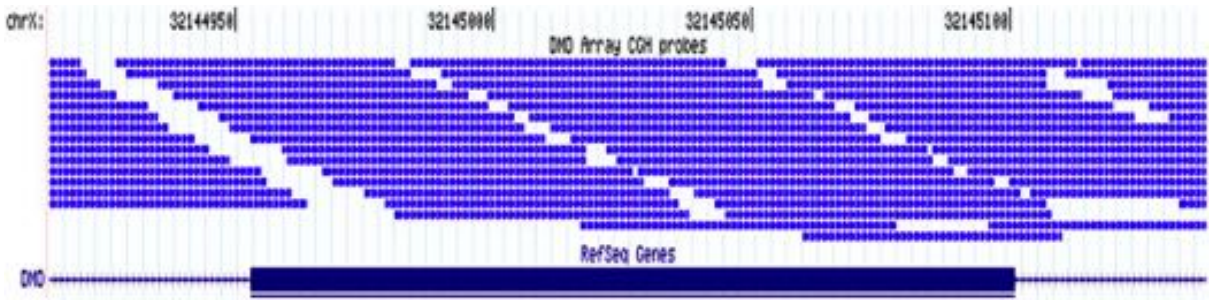
Deletion exon 45



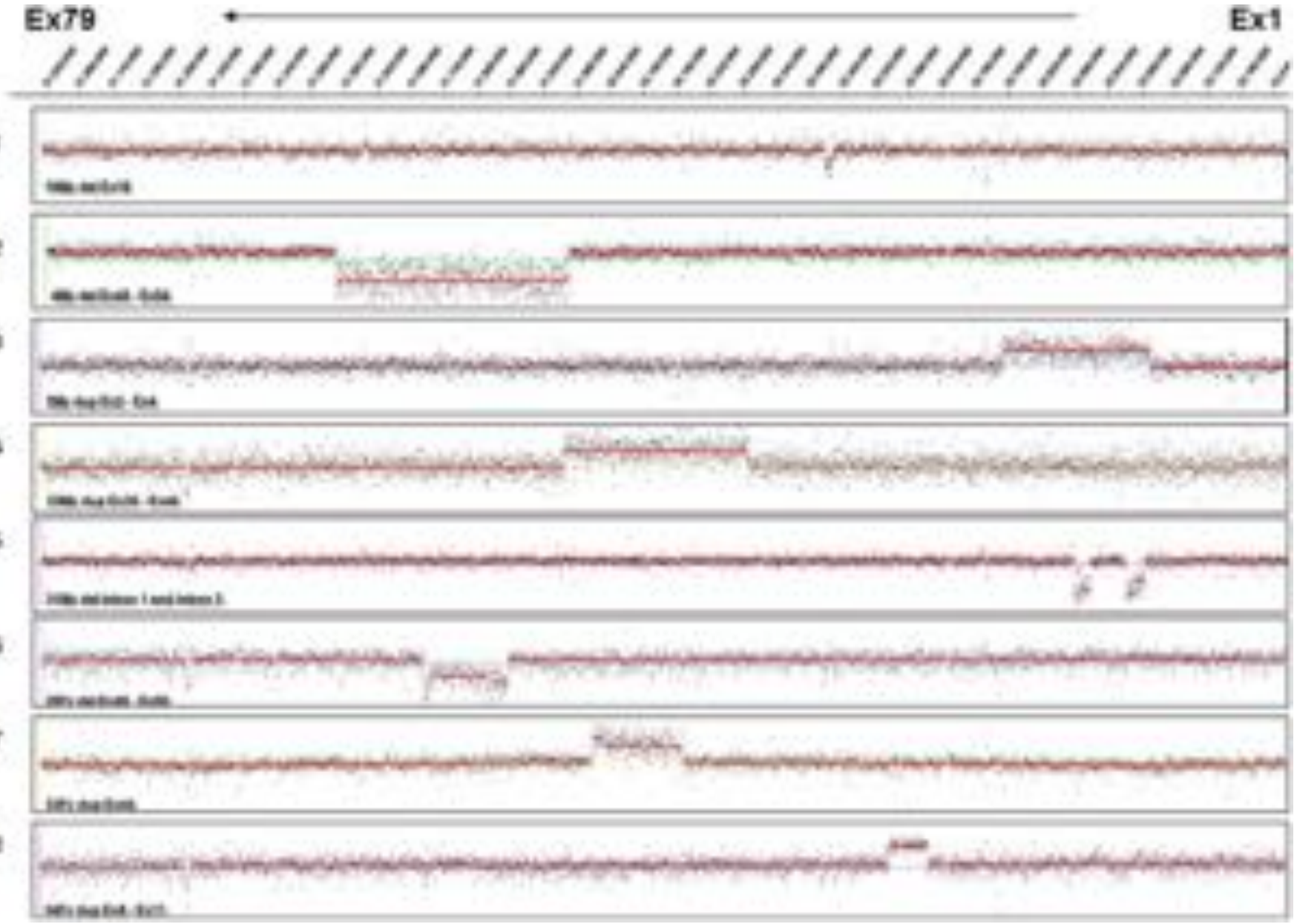
Deletion exons 8-9



Array-CGH
(comparative genomic hybridization)



Hegde et al, Human Mutation 29(9),1091-1099, 2008



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RESEARCH ARTICLE

Human Mutation

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Mutational Spectrum of DMD Mutations in Dystrophinopathy Patients: Application of Modern Diagnostic Techniques to a Large Cohort

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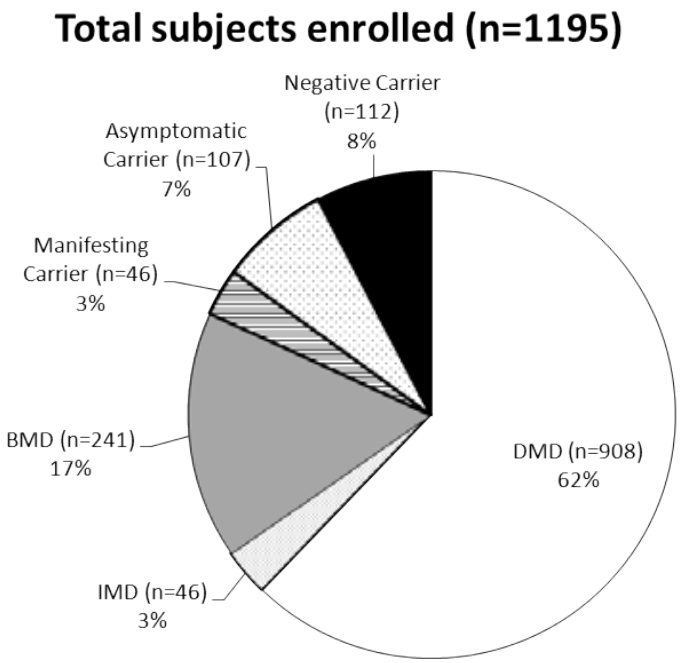
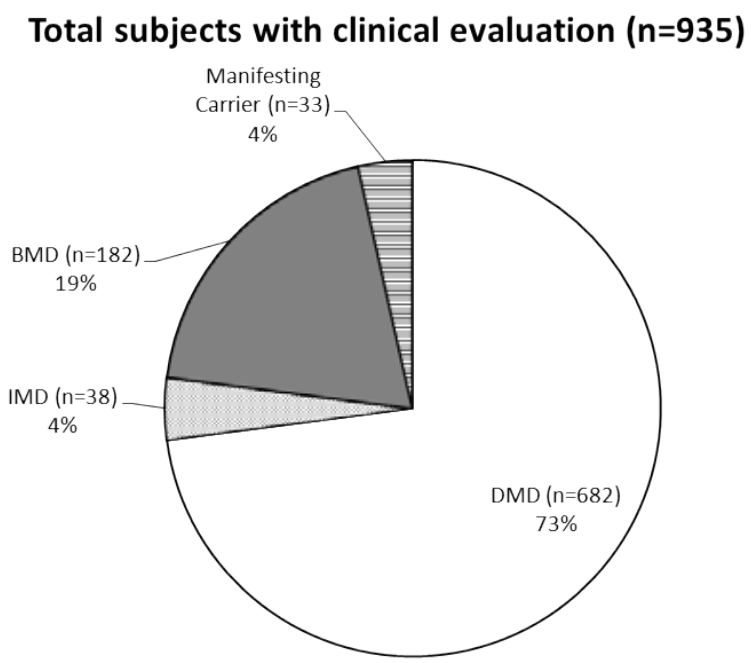
1,111 mutation-positive patients

The United Dystrophinopathy Project: a patient catalogue and clinical research resource

A

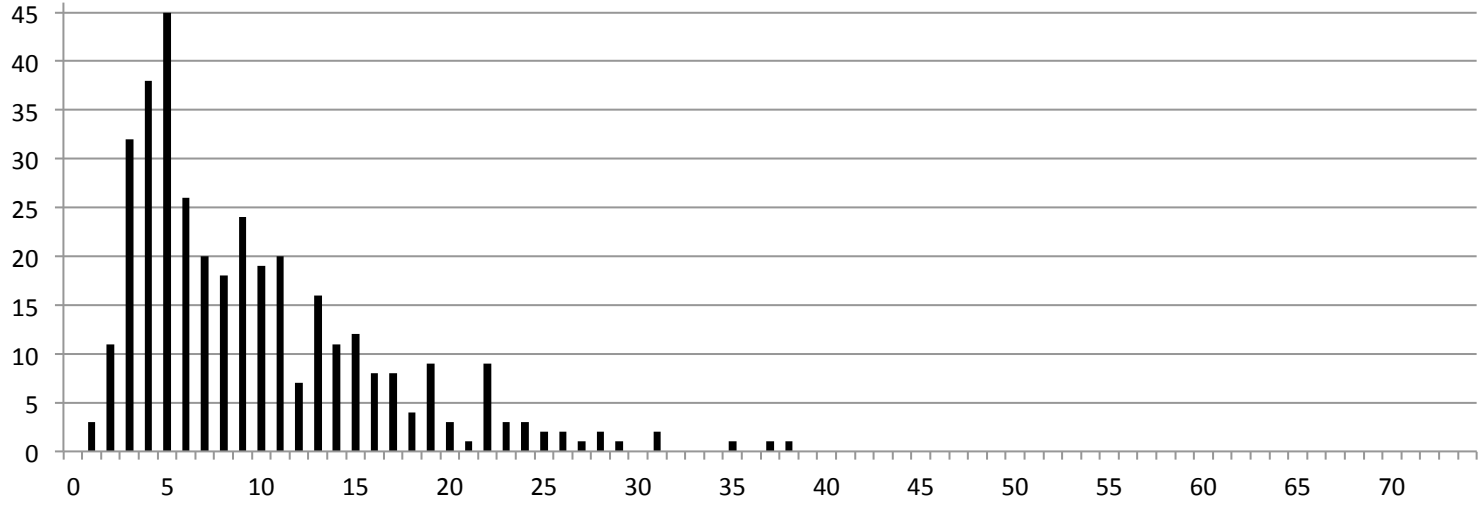


B

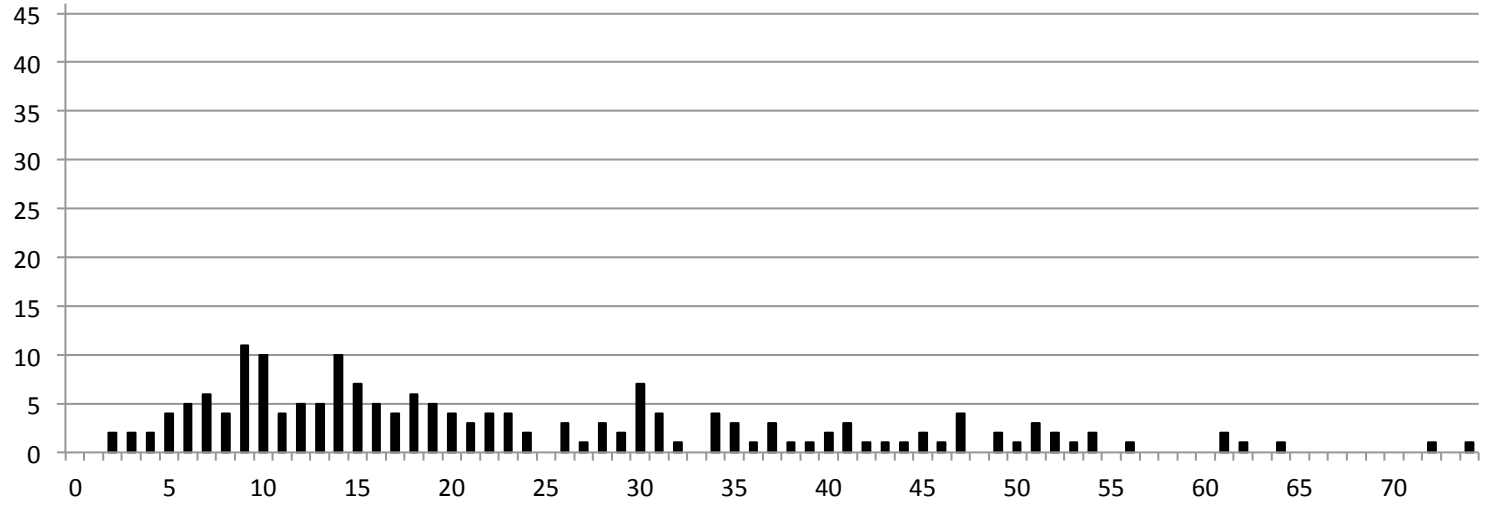


Age at enrollment

DMD



BMD



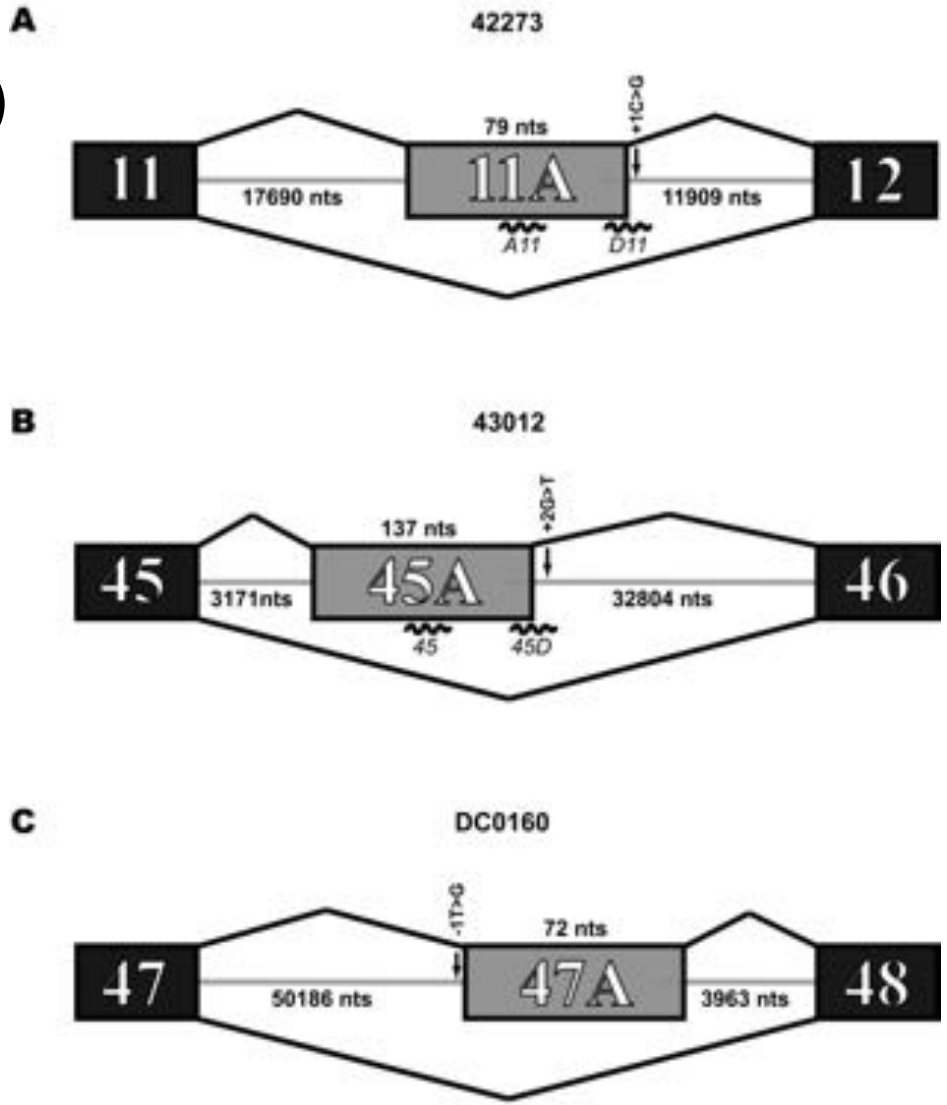
MUTATION CLASS	DMD	IMD	BMD	Unknown (B/DMD)	Manifesting Carrier[b]	Carrier (all phenotypes) [a]	Total	%
DELETION	283	15	55	107	3	14	**477**	42.9%
in	*30*	*2*	*36*	*17*	*1*	*2*	***88***	
out	*243*	*13*	*18*	*88*	*1*	*12*	***375***	
other	*10*	*0*	*1*	*2*	*1*	*0*	***14***	
STOP	176	4	30	46	4	34	**294**	26.5%
UGA	*60*	*1*	*13*	*20*	*3*	*15*	***112***	
UAG	*71*	*0*	*11*	*13*	*0*	*4*	***99***	
UAA	*45*	*3*	*6*	*13*	*1*	*15*	***83***	
SUBEXONIC	70	0	10	32	1	14	**127**	11.4%
FS Ins	*22*	*0*	*1*	*7*	*1*	*6*	***37***	
FS	*46*	*0*	*4*	*23*	*0*	*8*	***81***	
FS Ins/Del	*1*	*0*	*2*	*2*	*0*	*0*	***5***	
in-frame deletion	*1*	*0*	*3*	*0*	*0*	*0*	***4***	
DUPLICATION	87	7	10	8	5	5	**122**	11.0%
SPLICE	22	3	7	18	2	12	**64**	5.8%
MISSENSE	2	1	6	6	0	0	**15**	1.4%
PSEUDOEXON	0	2	2	0	0	2	**6**	0.5%
POTENTIAL	2	0	0	3	0	1	**6**	0.5%
OTHER	0	0	0	0	0	0	**0**	0.0%
TOTAL MUTATIONS	642	32	120	220	15	82	**1111**	100.0%

Flanigan, et al, Hum Mutat. 2009 Dec;30(12):1657-66

Mutations may not be detectable by genomic DNA analysis

Pseudoexon mutations (e.g.)

- Deep intronic point mutations
- Create splice donor or acceptor sites
- Intronic DNA included as a “pseudoexon” in mRNA
- Undetectable from blood



The reading frame rule in DMD/BMD

Table 2. The Value of Mutational Reading Frame in Predicting a Phenotype of Duchenne Muscular Dystrophy

	DMD	I/BMD		
Exonic deletions only				
Truncating (out-of-frame) mutations	254	32	88.8%	*Positive predictive value*
Non-truncating (in-frame) mutations	30	38	55.9%	*Negative predictive value*
Sensitivity	89.4%			
Specificity	—	54.3%		
All mutations[a]				
Truncating mutations	519	79	86.8%	*Positive predictive value*
Non-truncating mutations	37	63	63.0%	*Negative predictive value*
Sensitivity	93.3%			
Specificity	—	44.4%		

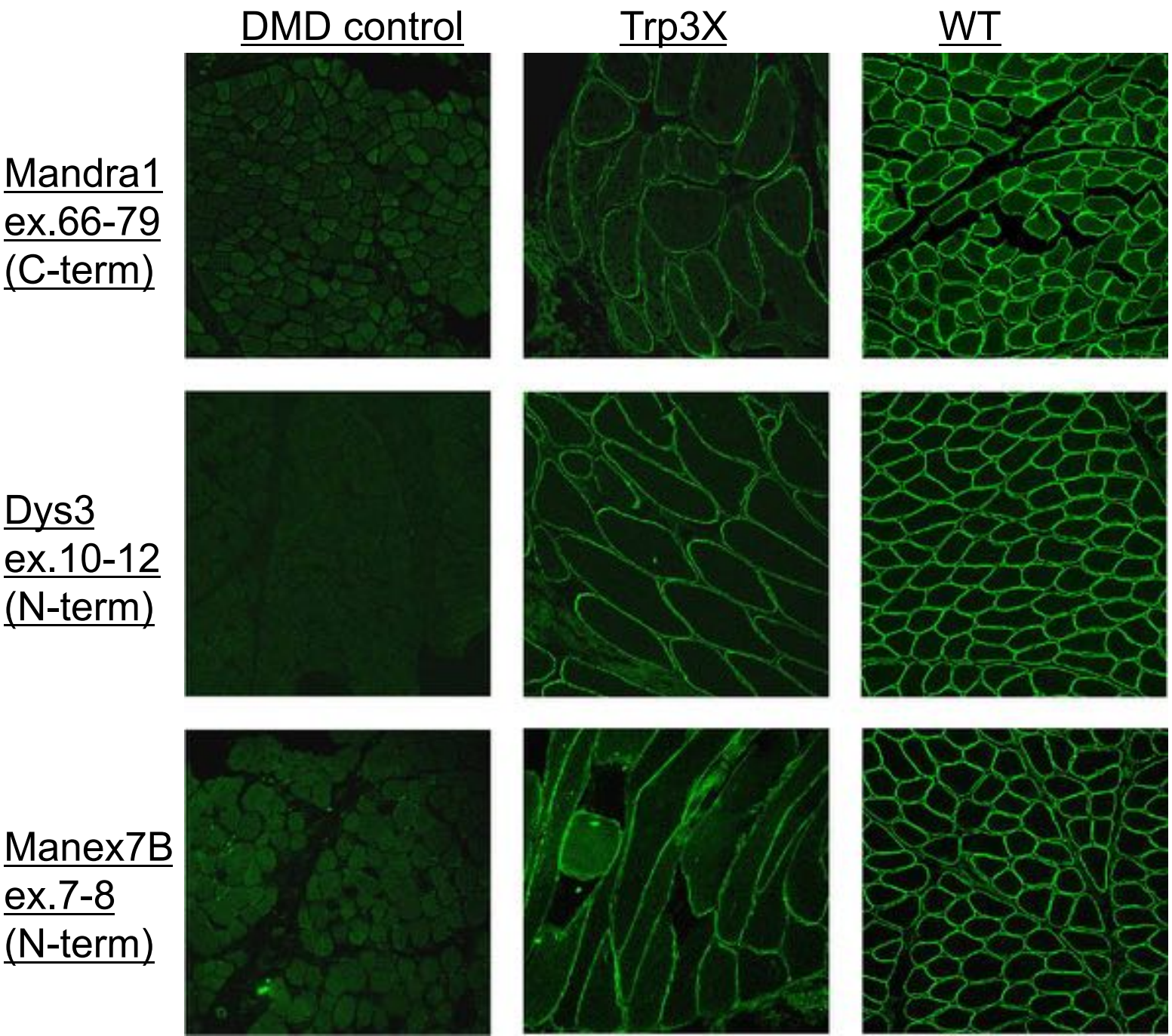
45-55% of BMD patients have out-of-frame mutations

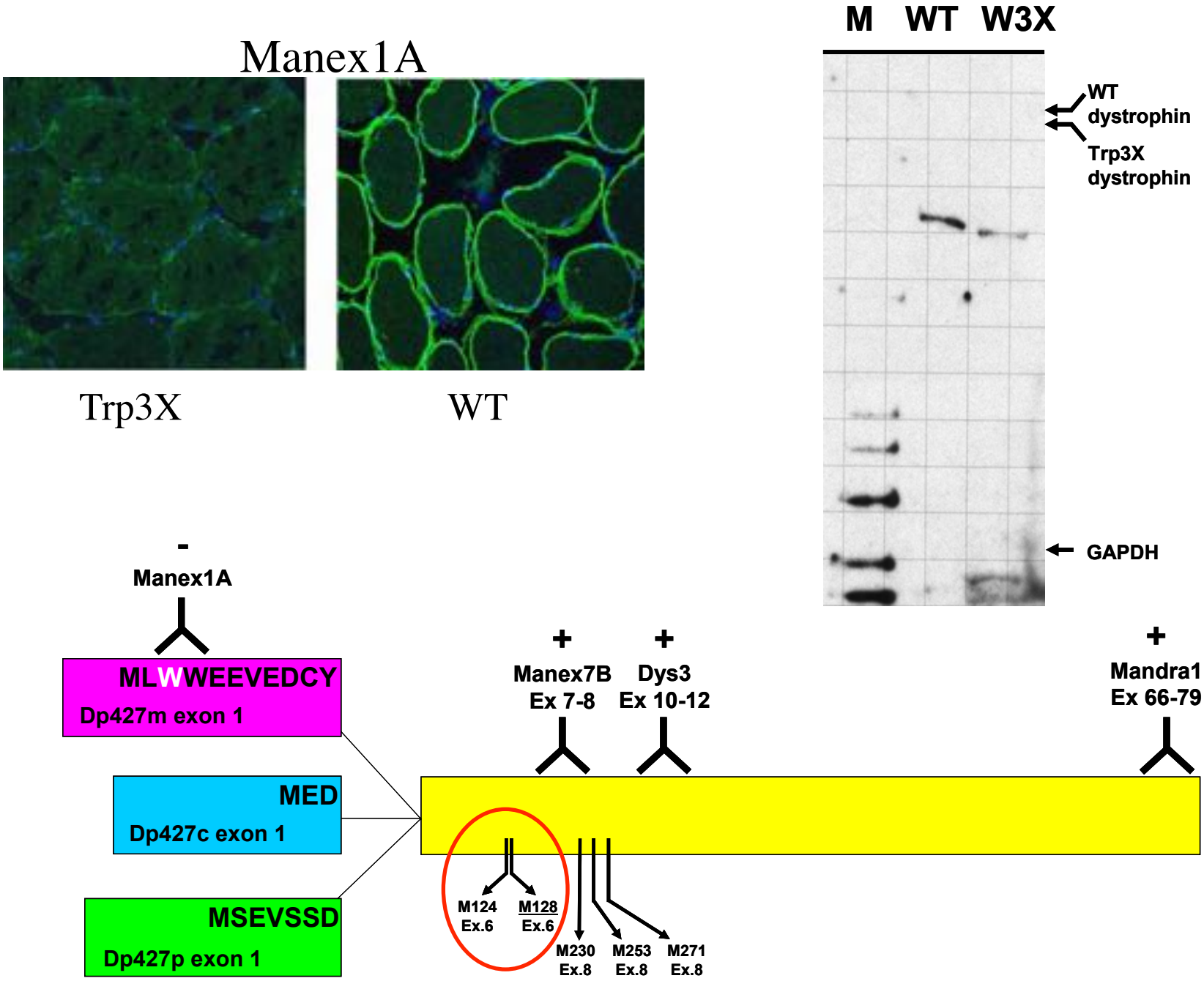
Nonsense mutations do not always predict DMD

- Mutations 5’ in the gene may result in altered translational initiation
  - Founder allele: Trp3X (exon 1)

Pt. 42790
c.9G>A; Trp3X (*DMD*)

- 65 year old man
  - onset of proximal weakness at age 20
  - wheelchair age 62.
- The proband's brother (examined at age 58)
  - denied symptoms
  - only minimal-to-mild pelvic girdle weakness upon examination.
- Five other historically unrelated families: all had proband boys (<age 10) with no symptoms, but were diagnosed due to elevated serum CPK.


DMD control
Trp3X
WT
Mandra1 ex.66-79 (C-term)
Dys3 ex.10-12 (N-term)
Manex7B ex.7-8 (N-term)

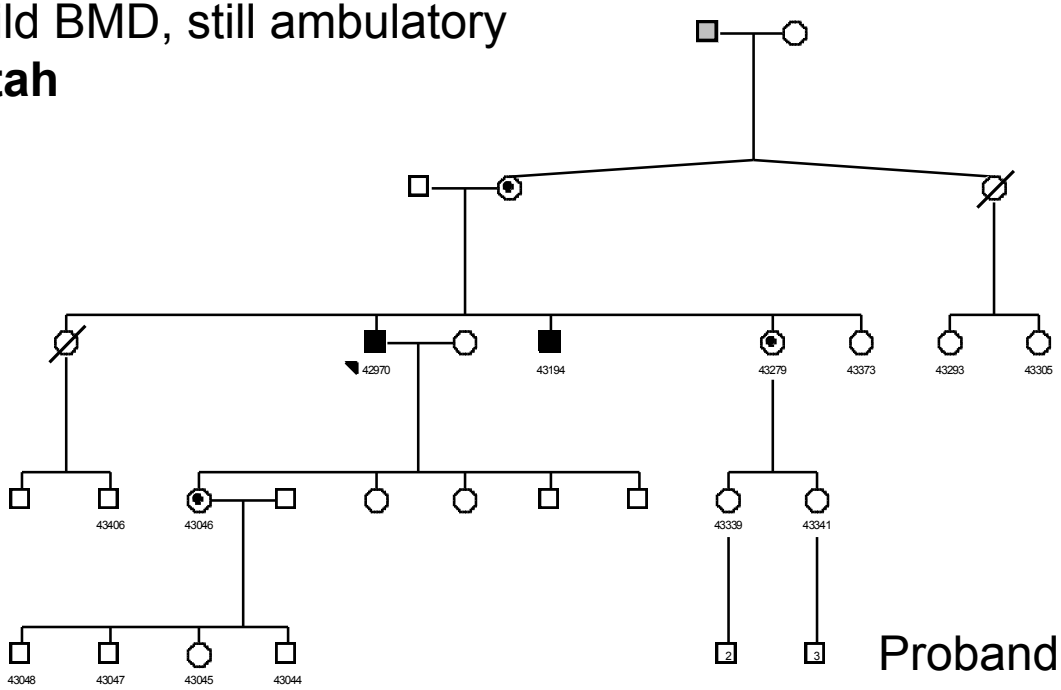


Gurvich *et al*, 2009; Hum Mutat 30:633-40

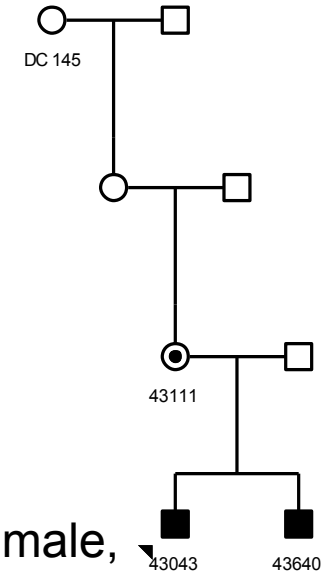
p.Trp3X BMD Pedigrees

Proband = 62 yr. old male, mild BMD, still ambulatory
Utah

Family 1



Family 2



Proband = 3.5 yr. old male, incidental to elevated CK levels, Younger brother CK = 5080 iu/L
Michigan

→ Concordant resequencing haplotypes across the DMD gene


WA
OR
CA
ID
NV
MT
WY
UT
AZ
CO
NM
ND
SD
NE
KS
OK
TX
MN
IA
MO
AR
LA
WI
IL
MS
MI
IN
KY
TN
AL
OH
WV
GA
FL
SC
NC
VA
PA
NY
MD
DE
NJ
CT
RI
MA
VT
NH
ME
AK
HI
Family 1
Family 2
Family 3
Family 4
Family 5
Family 6

Trp3X is a founder allele in the *DMD* gene

- Associated with childhood hyperCKemia, with or without myoglobinuria
- Compatible with no significant weakness at age 70
- No effect on reproductive fitness

Neuromuscular Disorders 19 (2009) 743–748

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

DMD Trp3X nonsense mutation associated with a founder effect in North American families with mild Becker muscular dystrophy

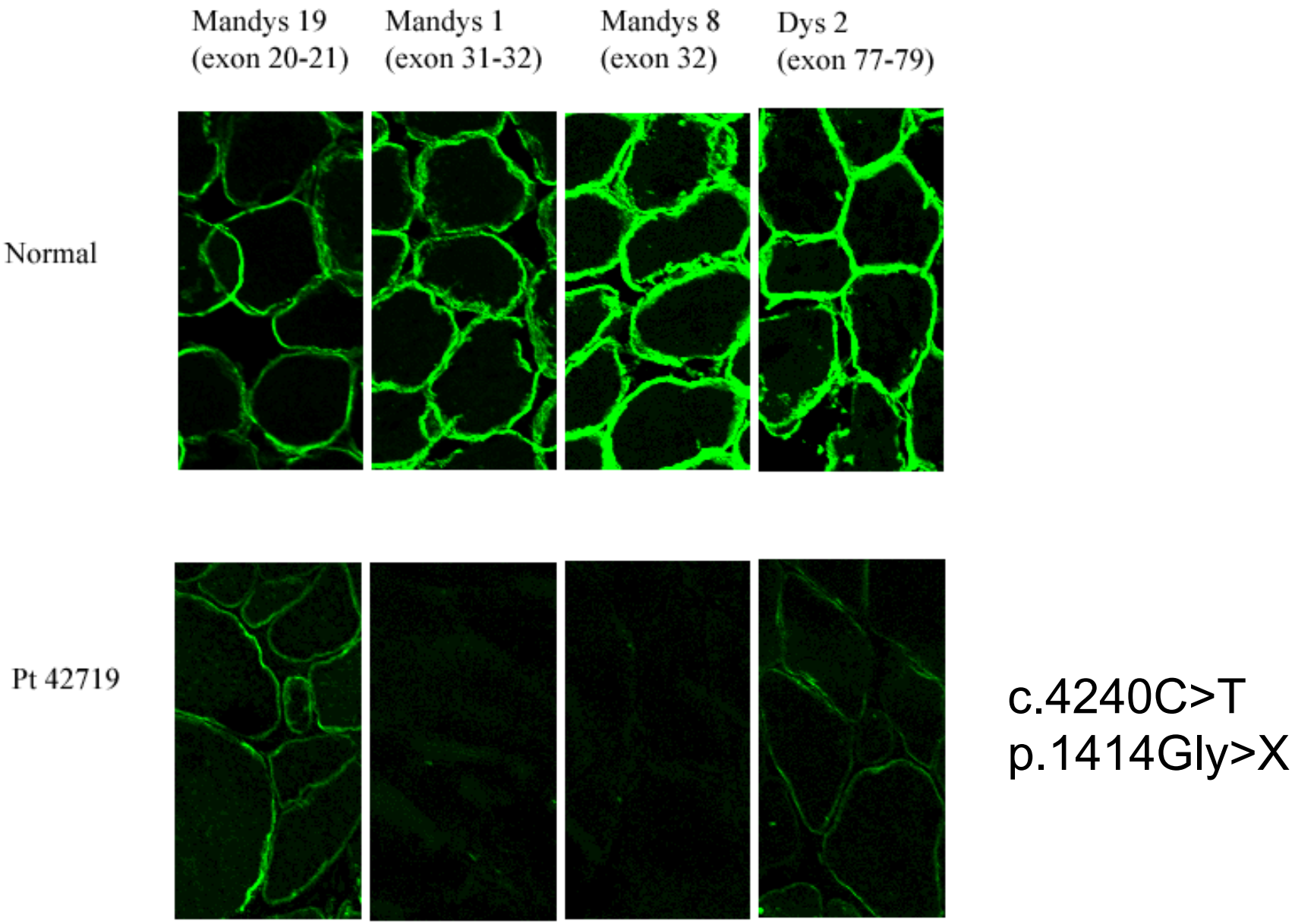
Kevin M. Flanigan [a,b,c,d], Diane M. Dunn [a], Andrew von Niederhausern [a], Michael T. Howard [a], Jerry Mendell [e], Anne Connolly [f], Carol Saunders [g], Ann Modrcin [g], Majed Dasouki [h], Giacomo P. Comi [i], Roberto Del Bo [i], Angela Pickart [j], Richard Jacobson [j], Richard Finkel [k], Livija Medne [k], Robert B. Weiss [a,*]

Nonsense mutations do not always predict DMD

- Mutations 5’ in the gene may result in altered translational initiation
  - Founder allele: Trp3X (exon 1)

- Mutations predicted as nonsense mutations may instead affect exon splice regulatory signals
  - This results in exclusion of exons
  - The remaining mRNA may be in-frame

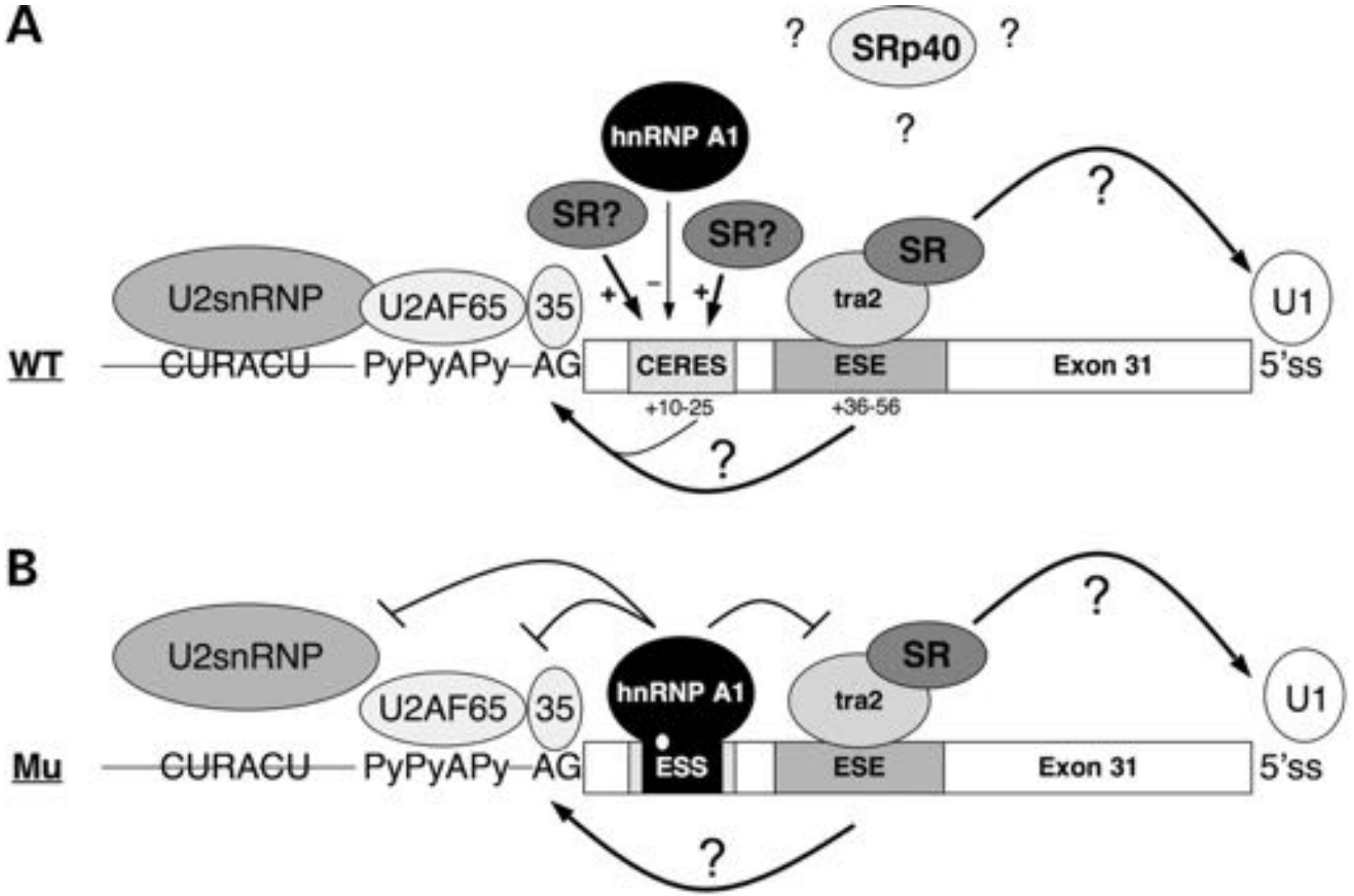
Nonsense-induced exon skipping



c.4240C>T
p.1414Gly>X

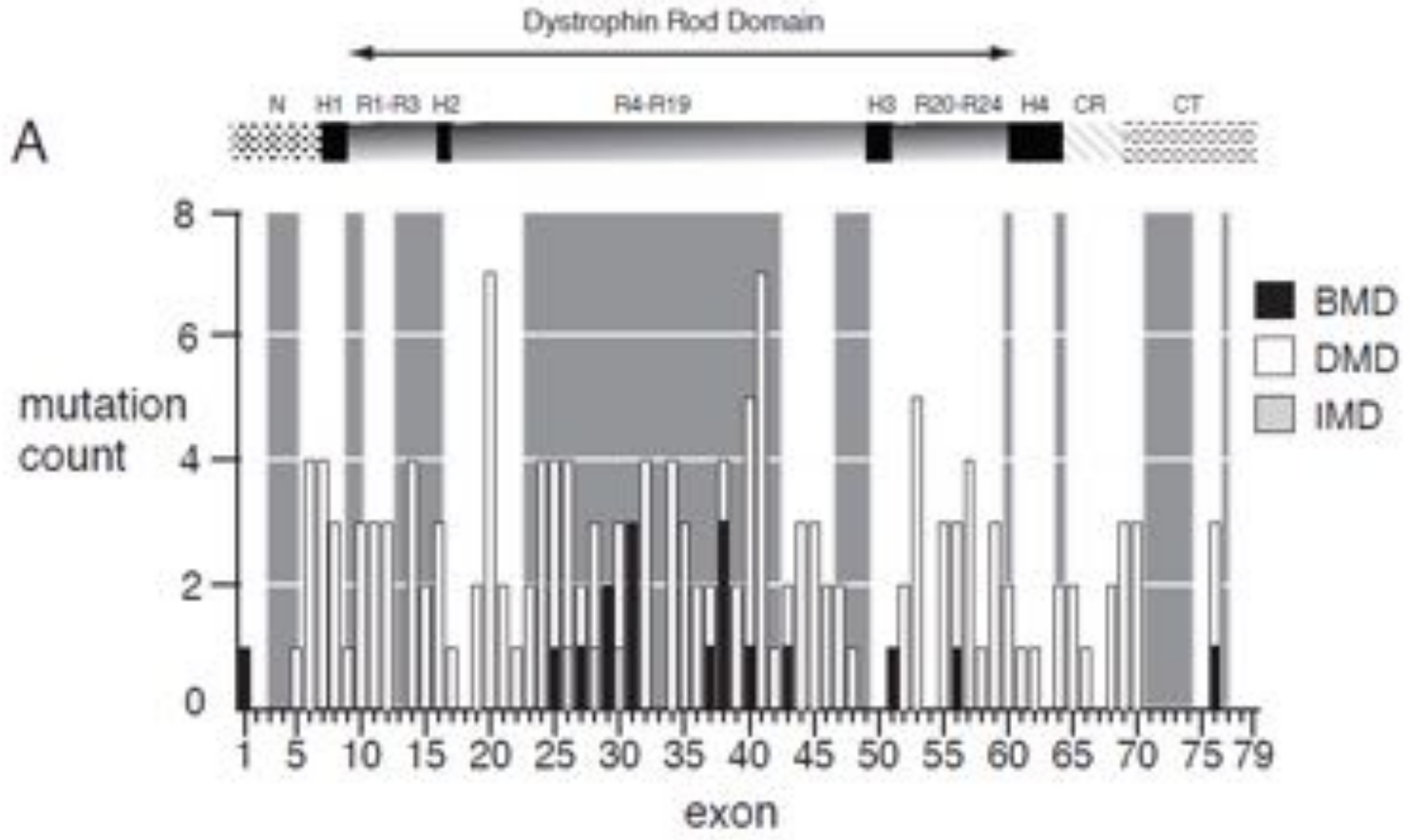
BMD-associated stop codon mutation in exon 31

c.4250T>A p.1417Leu>X



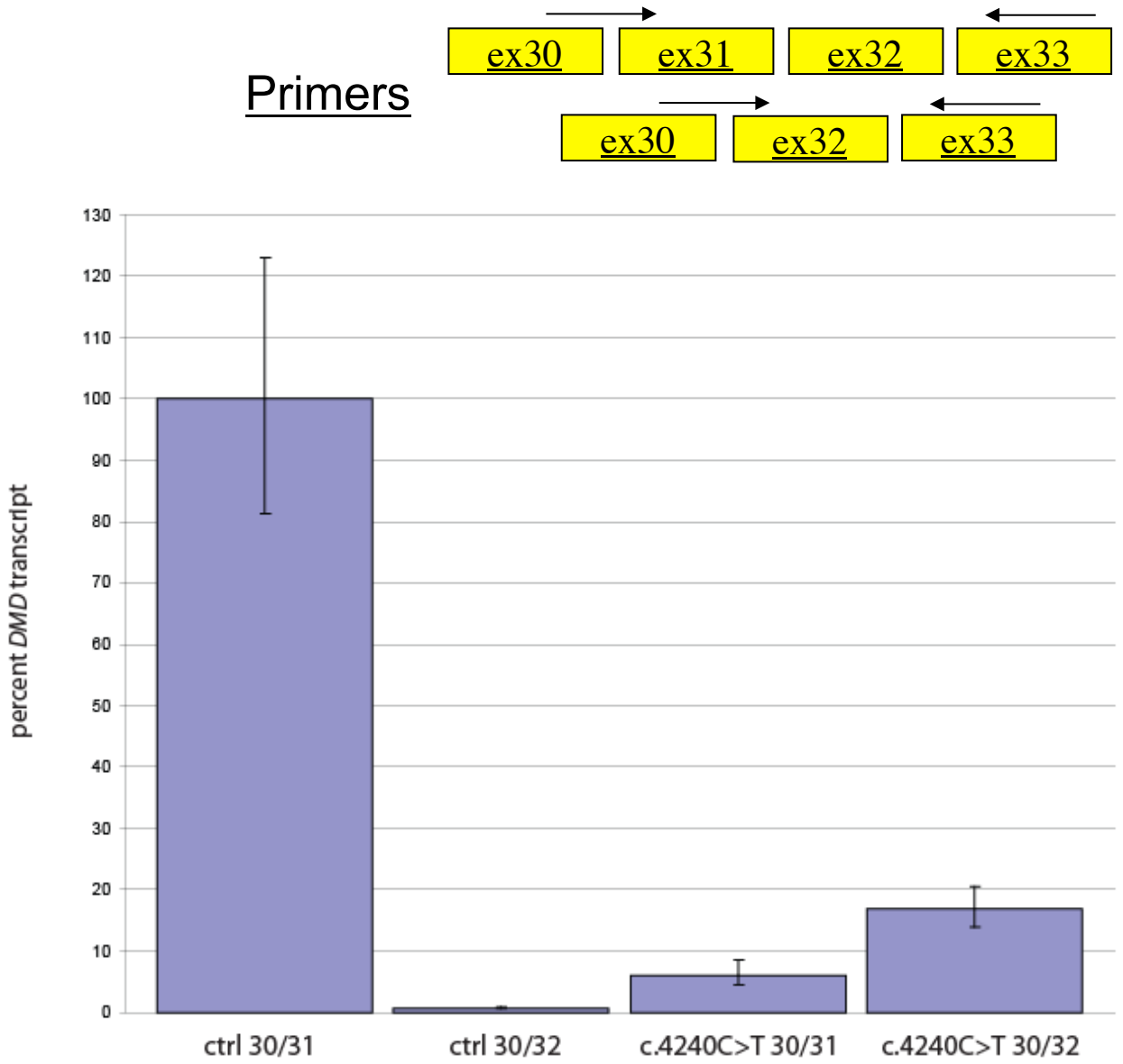
Disset, A. et al. Hum. Mol. Genet. 2006 15:999-1013

161 unique nonsense mutations



Flanigan, et al, Hum Mutat 32:299–308,

Quantitative RT-PCR of exon 31 (c. 4240C>T)



Dystrophinopathies: key points

- Point (non-exon deletion) mutations are nearly all “private” mutations
- Family history is most helpful (but not perfect) in guiding prognosis
- In the absence of family history, reading frame alone should be used cautiously in predicted prognosis
- A place for muscle biopsy remains in the clinical workup

Emerging therapeutic approaches

- Nonsense suppression
- Antisense oligomer-induced exon skipping
- Myostatin inhibition
- Viral gene transfer
  - Gene replacement (microdystrophin, *SGCA*)
  - Expression of other genes (Galgt2)
- Recombinant protein approaches
  - NF-kB modulation
  - Biglycan

Acknowledgements

- University of Utah
  - Robert Weiss, PhD
  - Mike Howard, PhD
  - Olga Gurvich, PhD
  - Jay Maiti, MD, PhD
- Nationwide Children's
  - Jerry Mendell, MD
  - Adeline Vulin-Chaffiol, PhD
  - Nicolas Wein, PhD
  - Linda Lowes
  - Lindsay Alfano
  - Wendy King
  - Jack Kaminoh
  - Laura Taylor
- Collaborators
  - Alan Pestronk, MD
  - Julaine Florence, PhD
  - Anne Connolly, MD
  - Richard Finkel, MD
  - Carsten Bonneman, MD
  - Livija Medne, MS
  - Brenda Wong, MD
  - Kathy Mathews, MD
  - John Day, MD
  - Craig McDonald, MD

What was thought before the cloning of the Dystrophin gene ?

1) Many hypotheses about how DMD worked. A) The vascular hypothesis B) The neural hypothesis C) The membrane theory (delta lesion in muscle plasma membrane)

2) It was known to be an X-linked recessive disorder. This allowed the first mapping.

3) Many people said positional cloning of DMD would not work. But geneticists thought it would.

4) Many biochemical experiments where done and showed all kinds of differences For instances as the membrane theory was popular and many cell types where investigated. Fibroblasts, blood cells etc. Indeed abnormalities were reported in many of these cell types latter where found not to have dystrophin. Relevance ?

5) Cloning of DMD is pre-PCR, pre-BACs and large genomic maps. It involved fun stuff like phage and cosmid walking, partial restriction digest and making the phage and cosmid libraries yourself (no kits). Finding single copy probes, lots of radioactivity and best of all southern blots.

Construction of a genetic linkage map in man using restriction fragment length polymorphism.
Botstein, D., R.L. White, M. Skolnick, and R.W. Davis. 1980.
Am. J. Hum. Genet. 32: 314. 1980

Cloning of a representative genomic library of the human X chromosome after sorting by flow cytometry. Nature
Davies KE, Young BD, Elles RG, Hill ME, Williamson R.
Nature. 1981 Oct 1;293(5831):374-6.

Human X chromosomes were physically separated using a fluorescence-activated cell sorter. A library of genomic fragments was constructed.

Linkage relationship of a cloned DNA sequence on the short arm of the X chromosome to Duchenne muscular dystrophy
Murray JM, Davies KE, Harper PS, Meredith L, Mueller CR, Williamson R.
Nature. 1982 Nov 4;300(5887):69-71.

Established that DMD was on short arm of the X flanking markers indicated Xp21

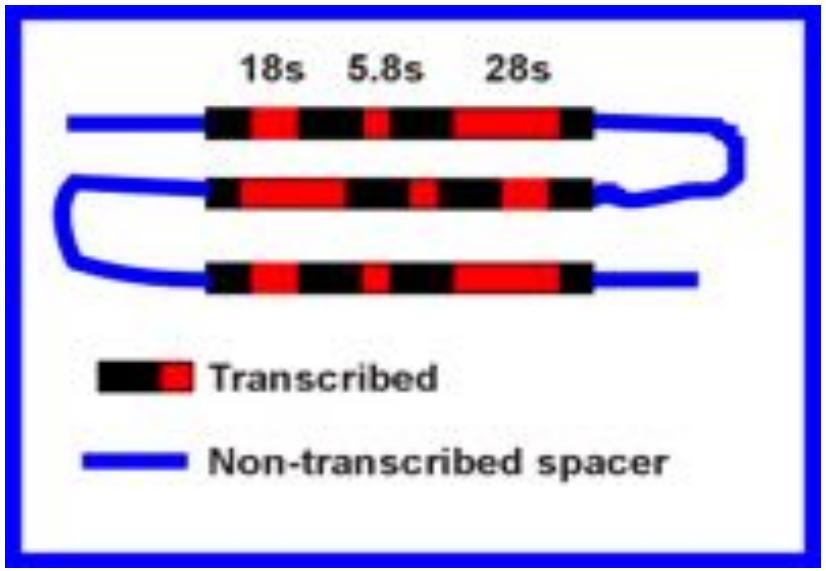
Two key patients for the cloning of DMD

Deletions

Bruce Bryer BB born in 1966 in Spokane WA and was an adopted child. At age three he was identified to have Chronic granulomatous disease (CGD), then at 9 DMD as well. Hans Ochs made cell lines from Bruce Bryer. Roberta Pagon determined that BB also suffered retinitis pigmentosa. Thus three X linked diseases. Pagon speculated that a deletion existed. Thus cytogenetics was performed and a deletion identified this was a large deletion but only just visible with the techniques of the day.

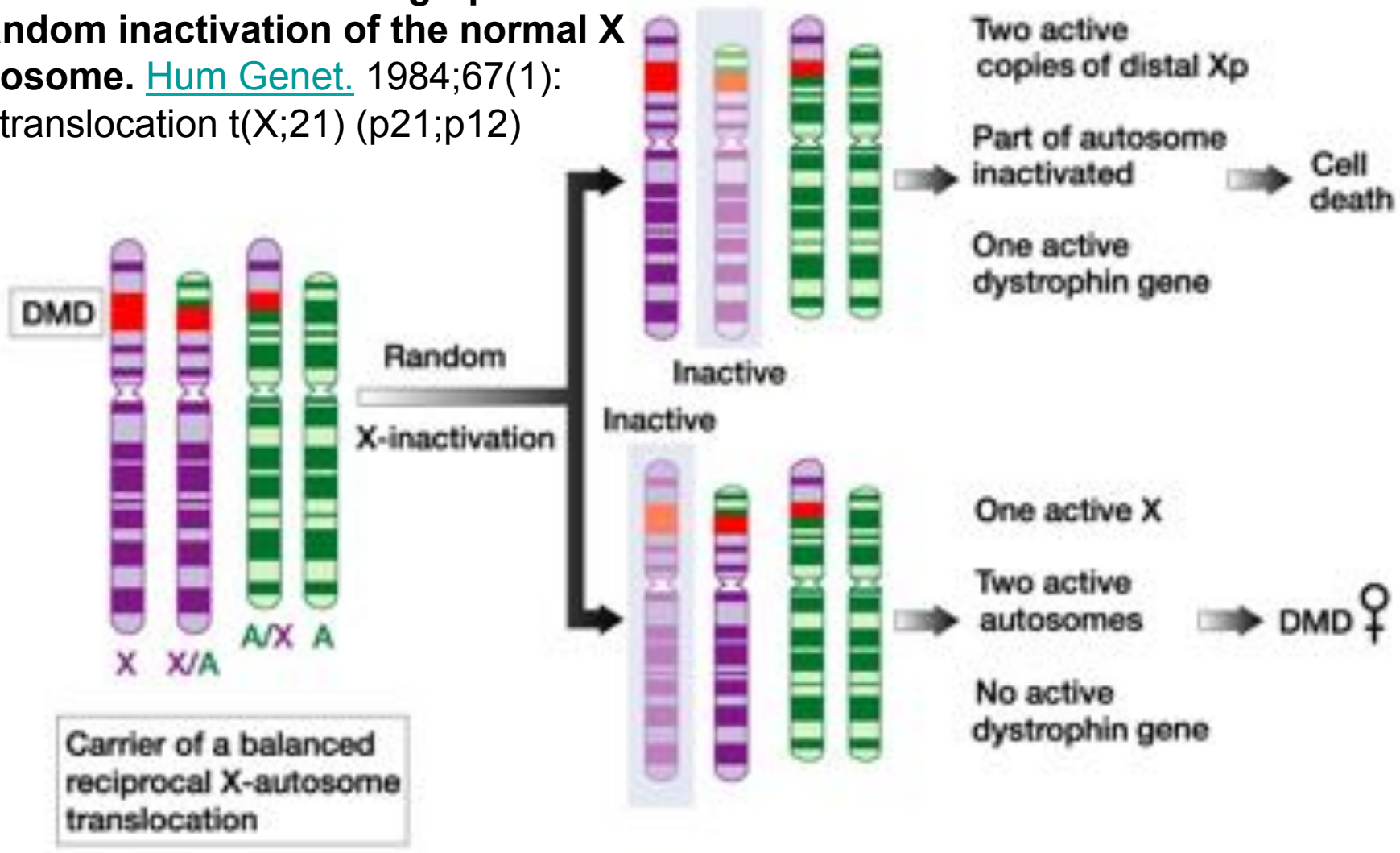
Translocations

Seven females with DMD all with an X-autosome balanced translocation. The X-chromosome break was in Xp21 in all cases. One occurred in a Belgium girl Anne and was t(X;21) (p21;p12) breaking the ribosomal gene cluster on chromosome 21.



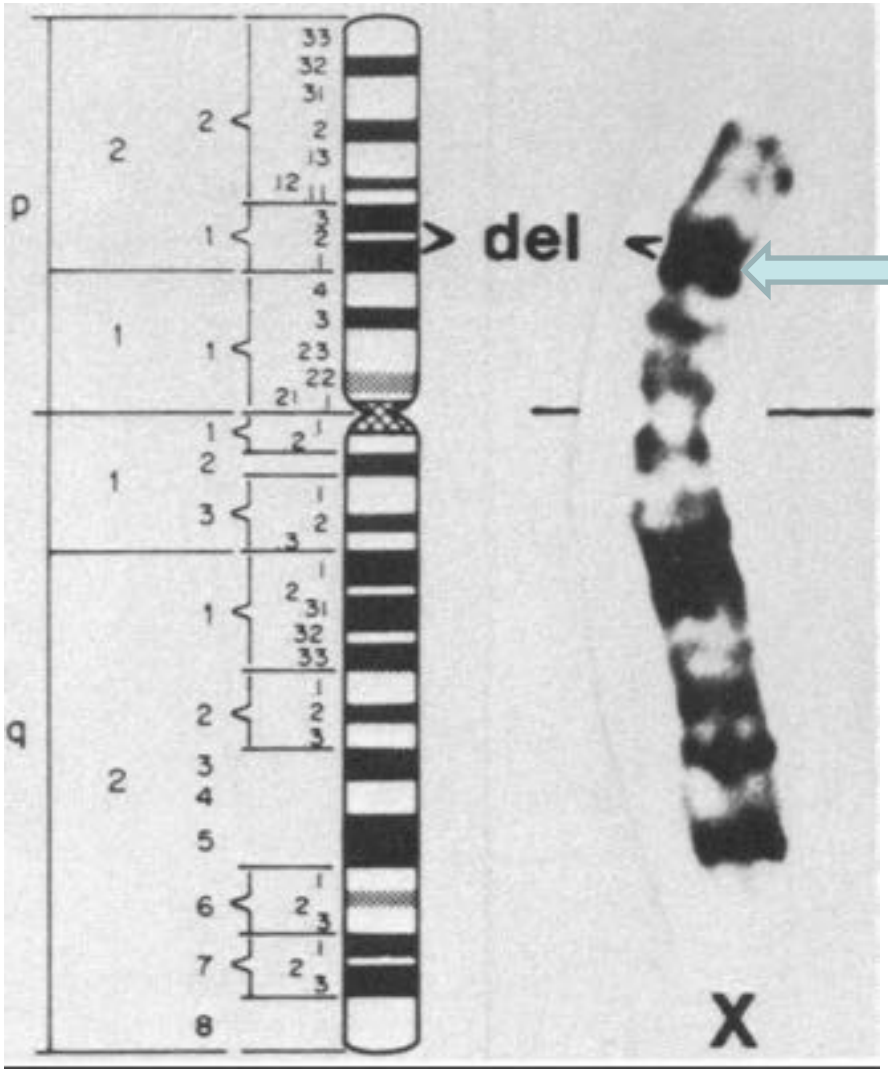
Science. 1984 Jun 29;224(4656):1447-9.
Duchenne muscular dystrophy involving translocation of the dmd gene next to ribosomal RNA genes.
Worton RG, Duff C, Sylvester JE, Schmickel RD, Willard HF.

Verellen-Dumoulin C, Freund M, De Meyer R, Laterre C, Frédéric J, Thompson MW, Markovic VD, Worton RG. **Expression of an X-linked muscular dystrophy in a female due to translocation involving Xp21 and non-random inactivation of the normal X chromosome.** Hum Genet. 1984;67(1): 115-9. translocation t(X;21) (p21;p12)



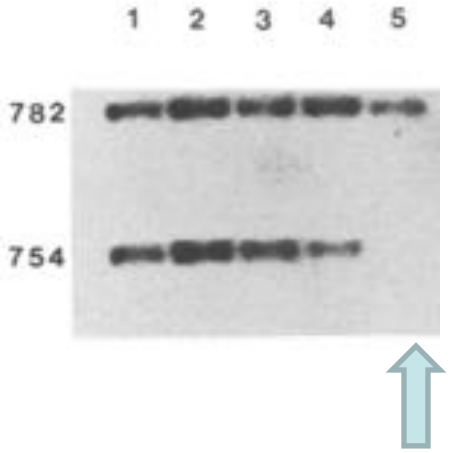
MINOR XP21 CHROMOSOME DELETION IN A MALE ASSOCIATED WITH EXPRESSION OF DUCHENNE MUSCULAR-DYSTROPHY, CHRONIC GRANULOMATOUS-DISEASE, RETINITIS PIGMENTOSA, AND MCLEOD SYNDROME

Francke, U., Ochs, H. D., Demartinville, B., Giacalone, J., Lindgren, V., Disteche, C., Pagon, R. A., Hofker, M. H., Van-Ommen, G. J., Pearson, P. L., Wedgewood, R. J. AMERICAN JOURNAL OF HUMAN GENETICS 1985; 37 (2): 250-267



White space of
Xp21 missing in BB

Deletion confirmed by southern using probe 754



BB

Cloning of the breakpoint of an X;21 translocation associated with Duchenne muscular dystrophy.

Ray PN, Belfall B, Duff C, Logan C, Kean V, Thompson MW, Sylvester JE, Gorski JL, Schmickel RD, Worton RG.

Nature. 1985 Dec 19-1986 Jan 1;318(6047):672-5.

Genetics Department and Research Institute, The Hospital for Sick Children and The Departments of Medical Genetics and Biophysics, University of Toronto, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada

Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment.

Monaco AP, Bertelson CJ, Middlesworth W, Colletti CA, Aldridge J, Fischbeck KH, Bartlett R, Pericak-Vance MA, Roses AD, Kunkel LM.

Nature. 1985 Aug 29-Sep 4;316(6031):842-5

Division of Genetics, Mental Retardation Program, Department of Pediatrics, Harvard Medical School, The Children's Hospital, Boston, Massachusetts 02115, USA

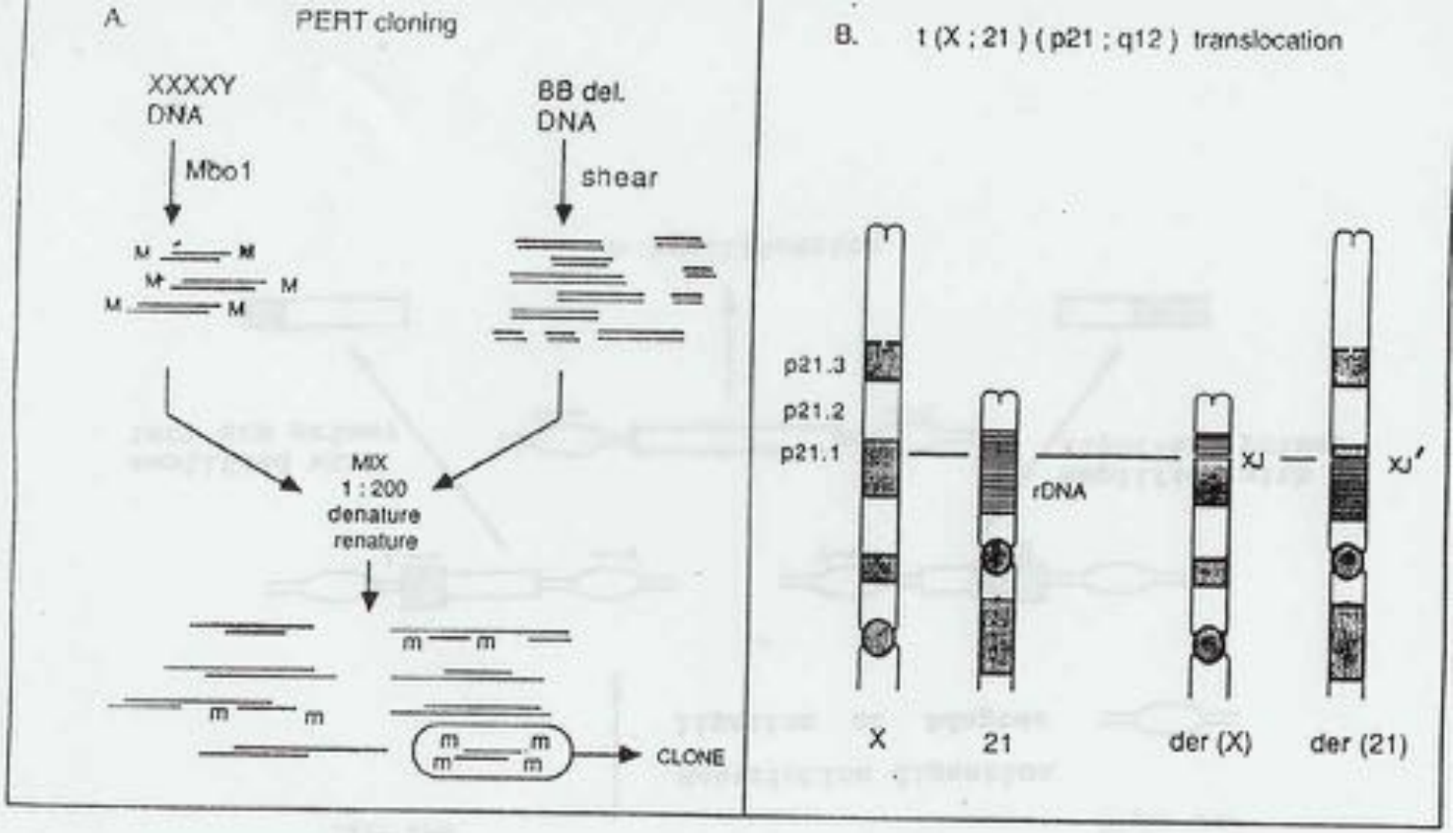
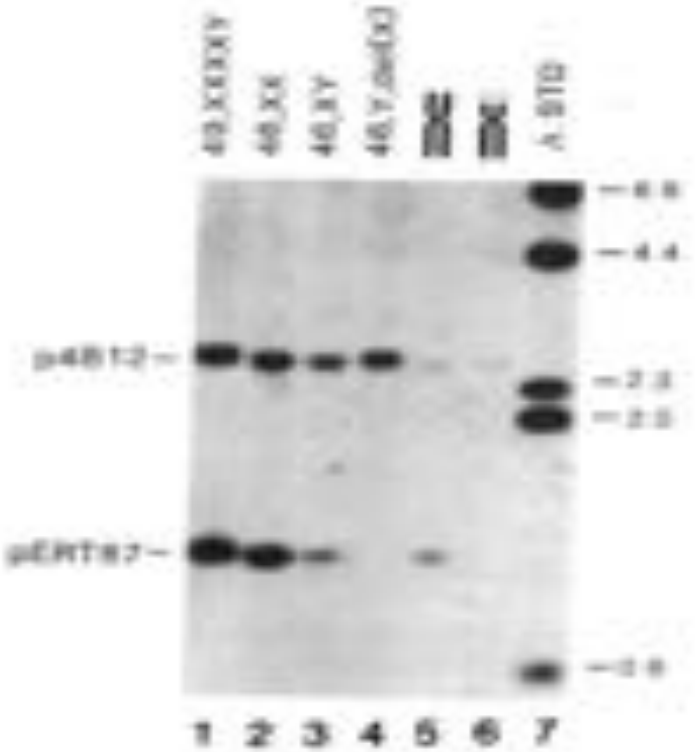


Figure 2 Schematic to illustrate the two successful cloning strategies.

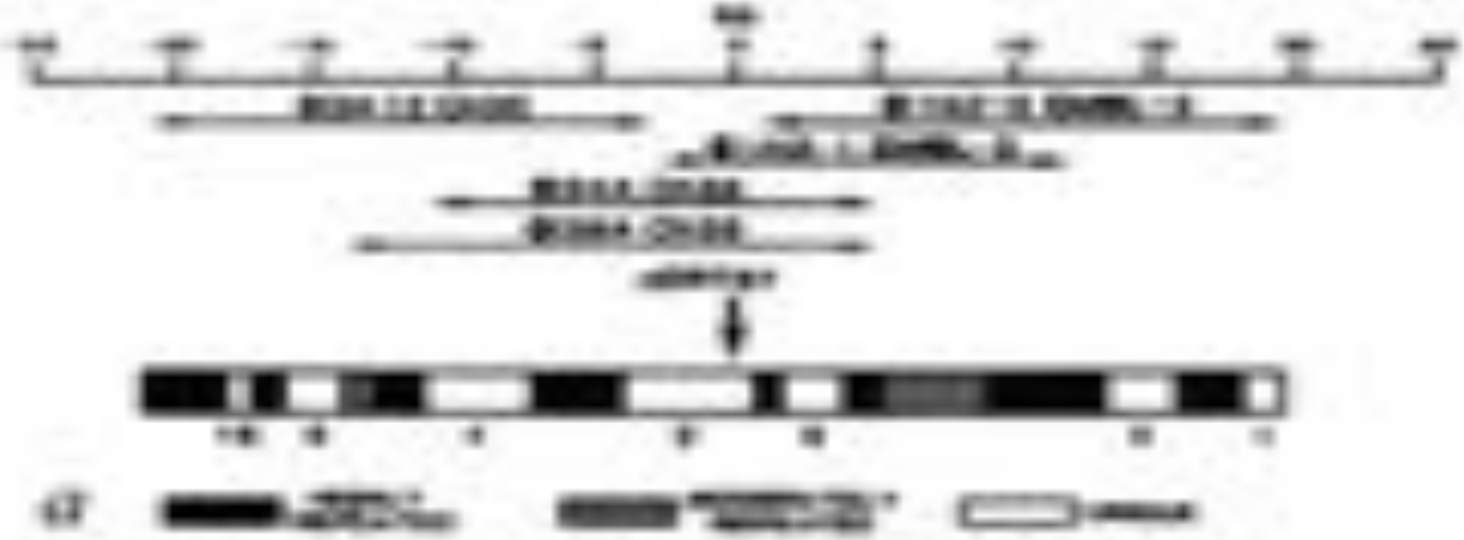
A. The pERT cloning scheme is described in the text. The ends of the *Mbo*I-digested molecules are indicated as M (double stranded) or m (single stranded). The ends of the sheared molecules are unlabeled. Among the reassociated molecules, only the perfectly aligned *Mbo*I-digested molecules are clonable, and these are enriched for sequences missing from the DNA of patient BB.

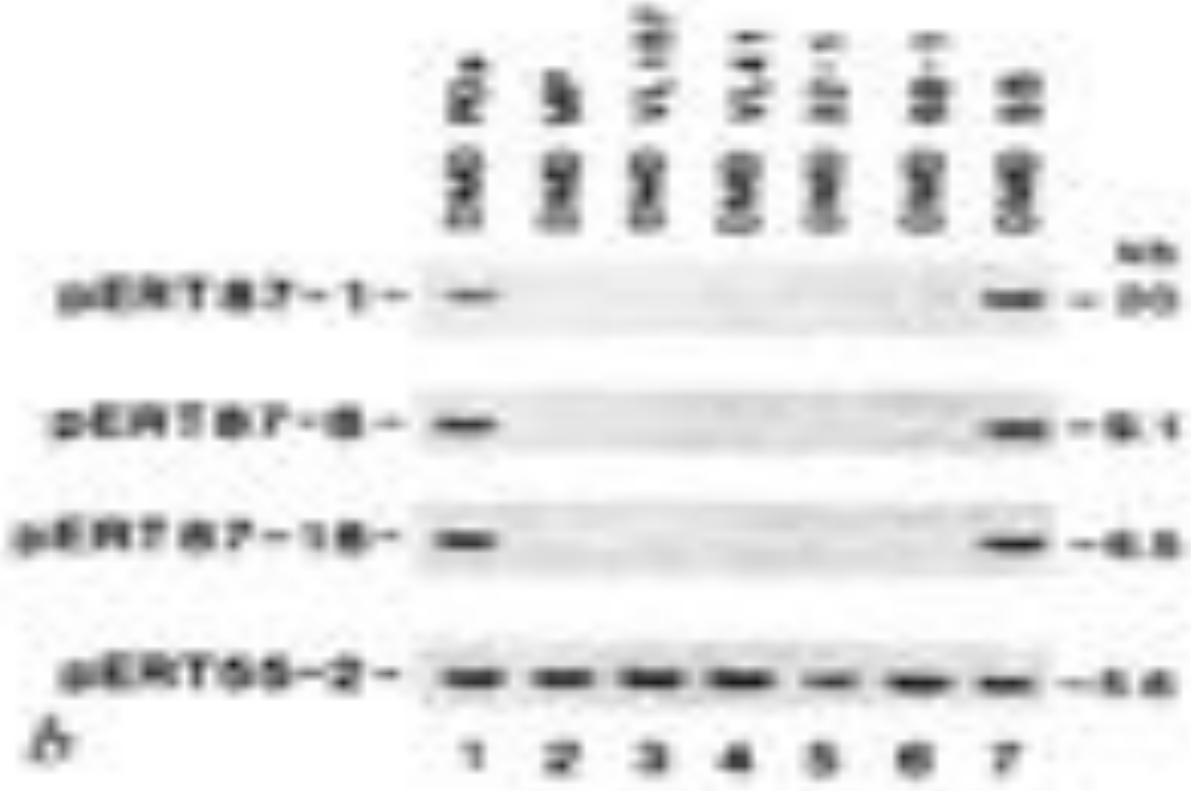
Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion

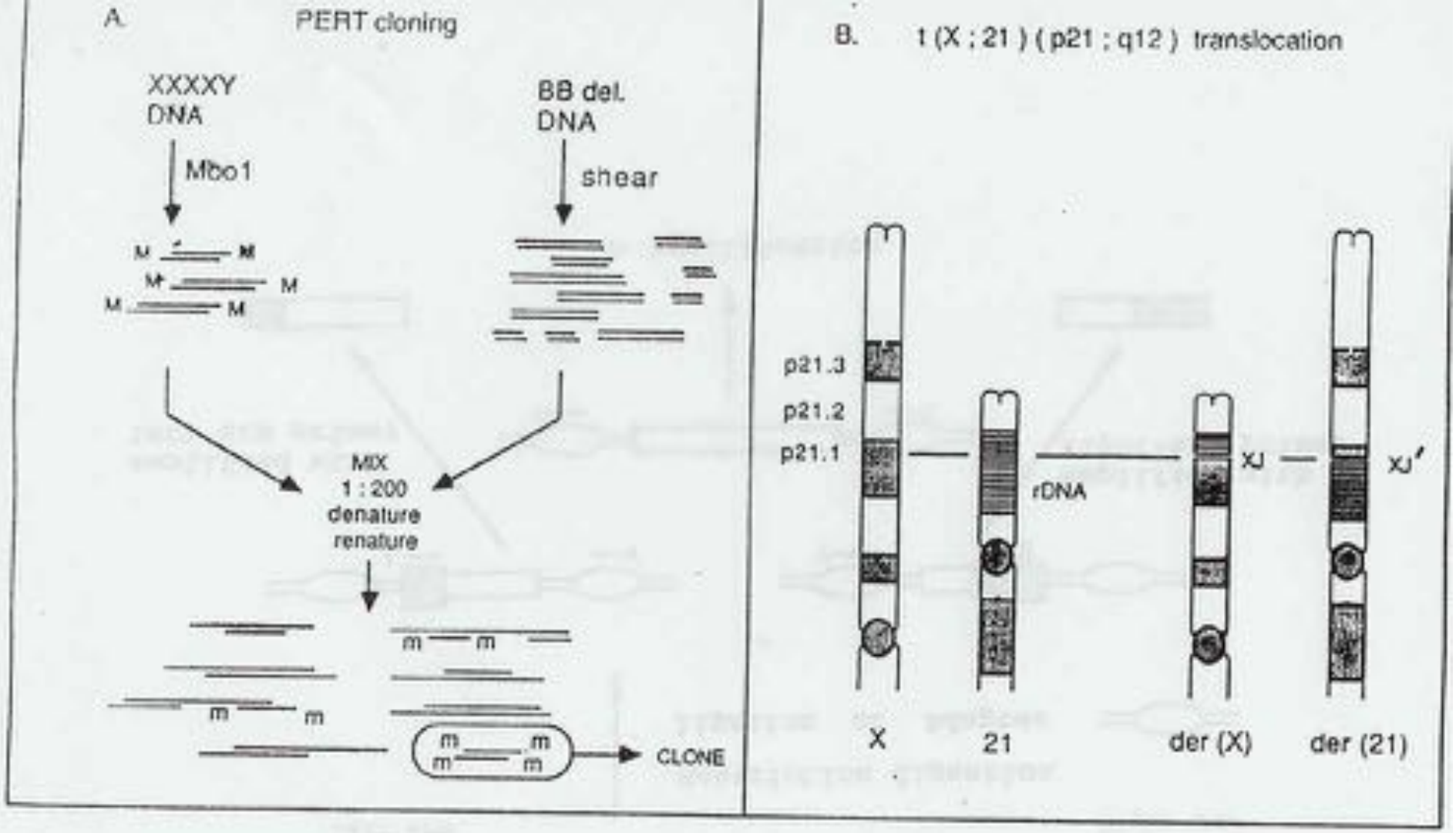
Louis M. KUNKEL, ANTHONY P. MONACO, WILLIAM MIDDLESWORTH, HANS D. OCHS, AND SAMUEL A. LATT Proc. Natl. Acad. Sci. USA Vol. 82, pp. 4778-4782, July 1985



Royer-Pokora, B., L.M. Kunkel, A.P. Monaco, S.C. Goff, P.E. Newburger, R.L. Baehner, ES. Cole, J.T. Curnutte, and S.H. Orkin. 1986. Cloning the gene for an inherited human disorder-chronic granulomatous disease-on the basis of its chromosomal location. Nature 322: 32.




pERT837-1
pERT837-6
pERT837-16
pERT855-2
1
2
3
4
5
6
7



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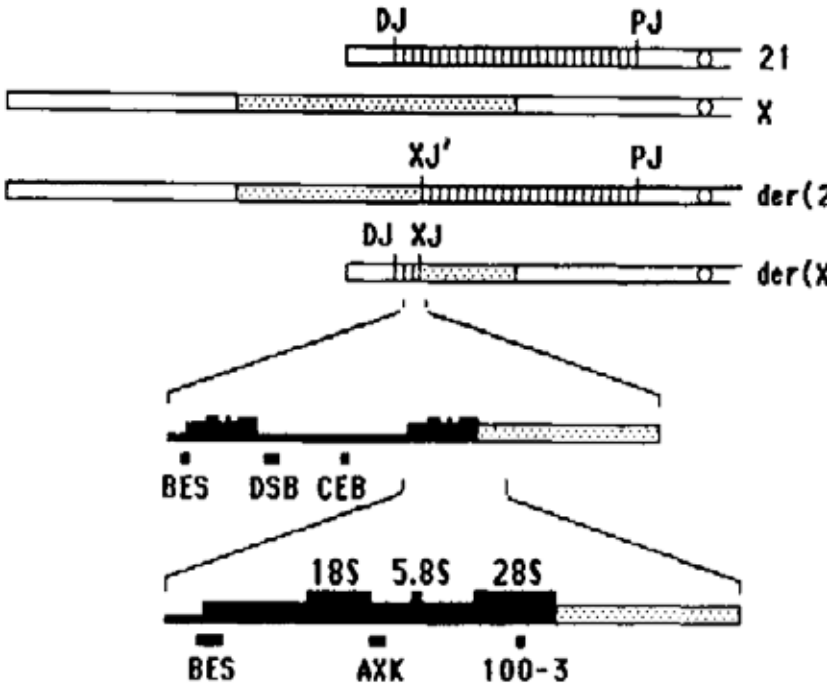
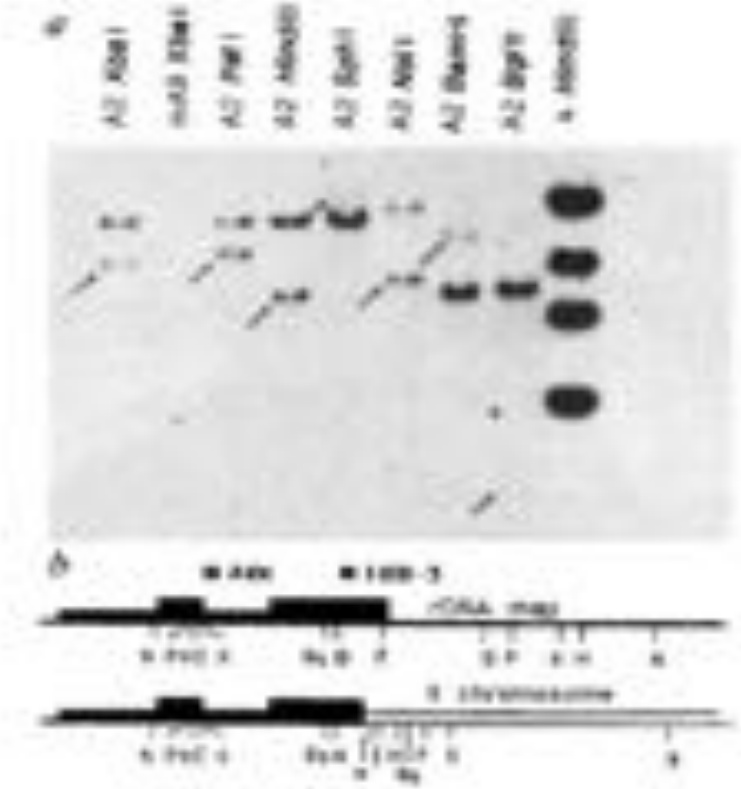
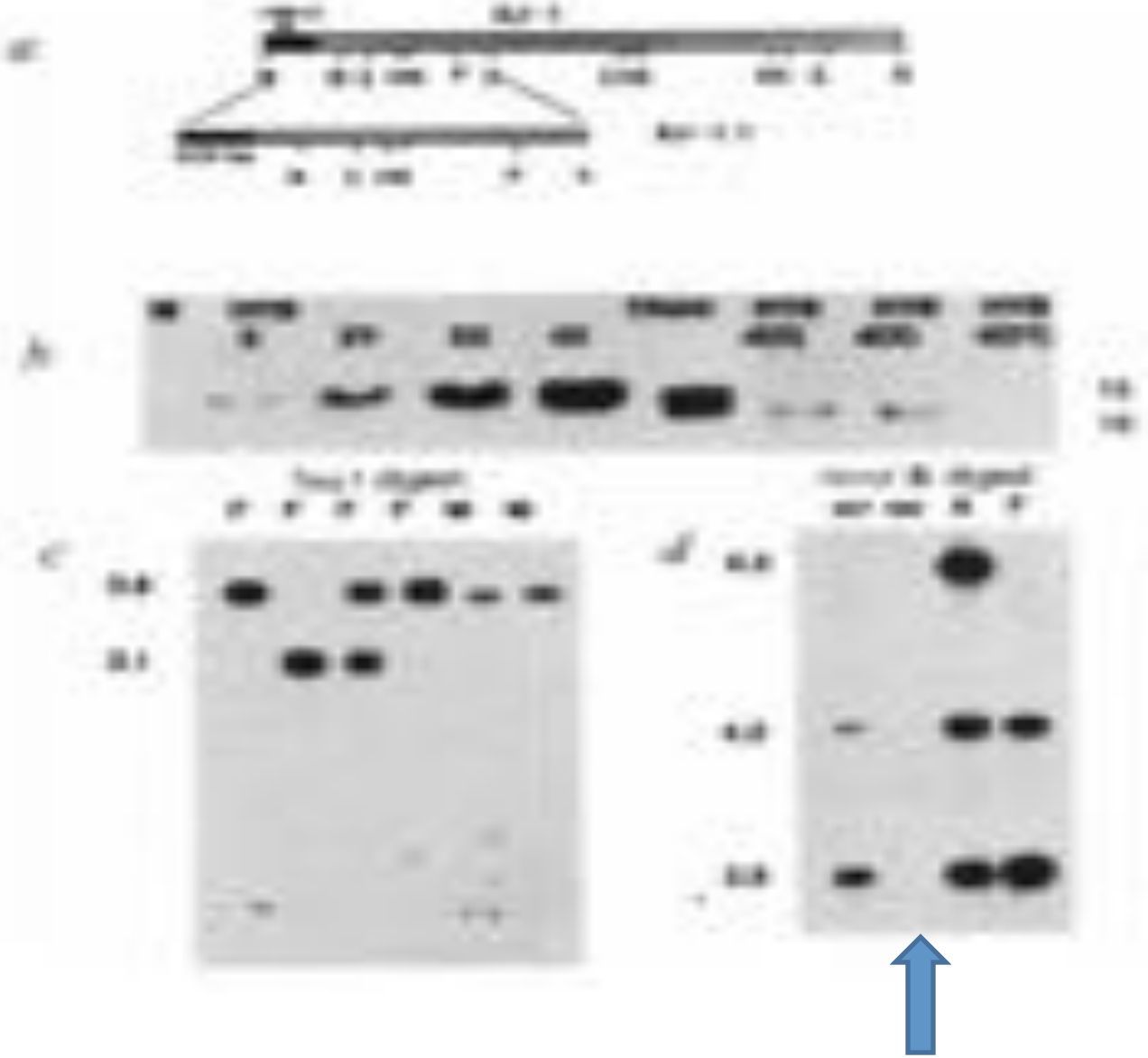
Translocation Junction Cloned
Ray et al.





Probe XJ 1.1 Detects deletions in patients with just DMD



DMD with deletion

Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene

ANTHONY P. MONACO*†, RACHAEL L. NEVE*†, CHRIS COLLETTI-FEENER*, CORLEE J. BERTELSON*, DAVID M. KURNIT* & LOUIS M. KUNKEL*†‡

*Division of Genetics, Mental Retardation Program, Department of Pediatrics, Harvard Medical School, The Children's Hospital, Boston, Massachusetts 02115, USA

†The Program in Neuroscience, Harvard University, Cambridge, Massachusetts 02138, USA

‡To whom correspondence should be addressed.

Nature. 1986 Oct 16-22;323(6089):646-50

A cDNA clone from the Duchenne/Becker muscular dystrophy gene

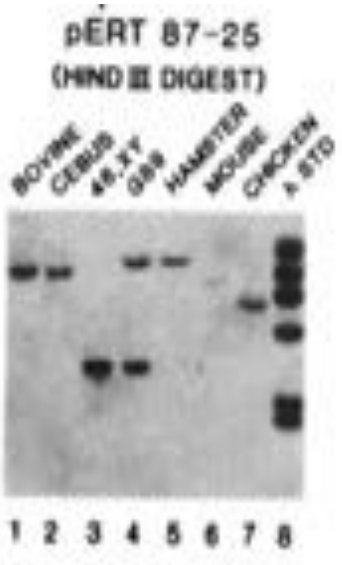
ARTHUR H. M. BURGHES, CAIRINE LOGAN, XIUYUAN HU, BONNIE BELFALL, RONALD G. WORTON & PETER N. RAY

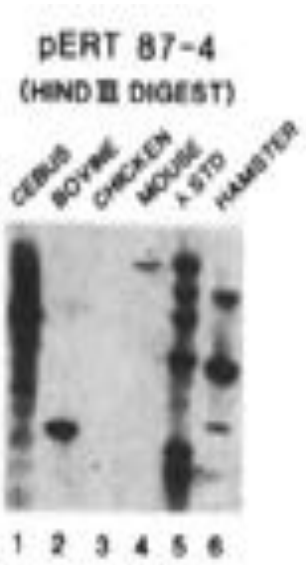
Genetics Department and Research Institute, The Hospital for Sick Children and The Departments of Medical Genetics and Biophysics, University of Toronto, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada

Nature. 1987 Jul 30-Aug 5;328(6129):434-7

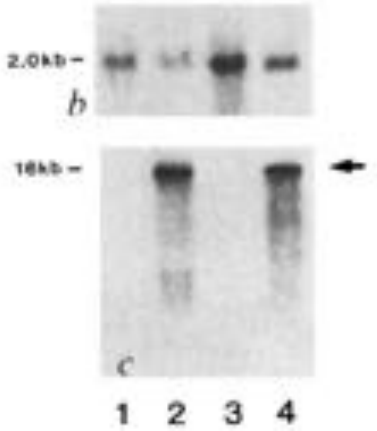
Identification of DMD mRNA Monaco et al

Identification of conserved segments in PERT 87 region
pERT87-25 detected mRNA and cDNA

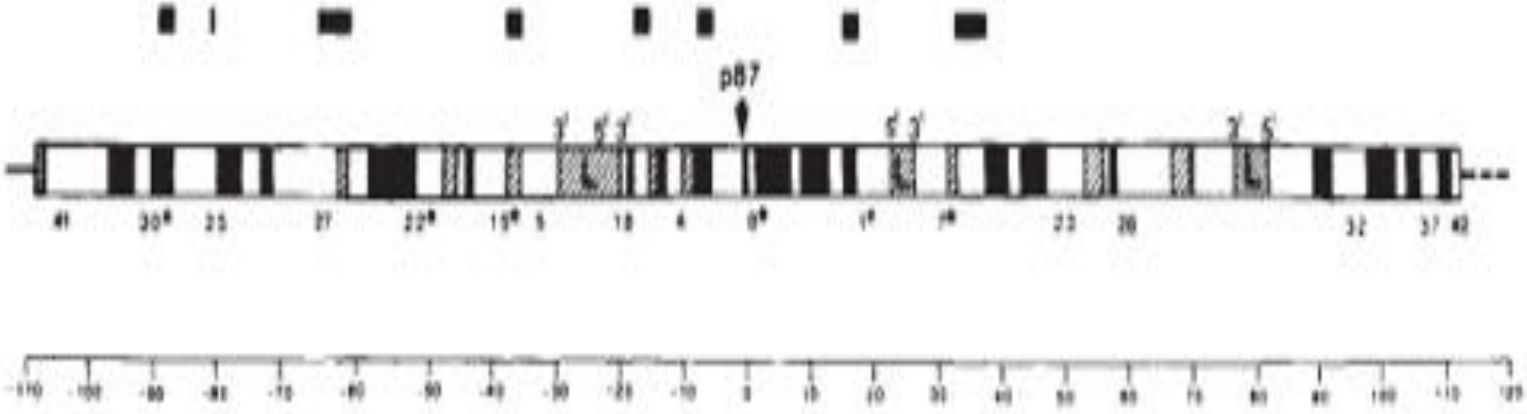




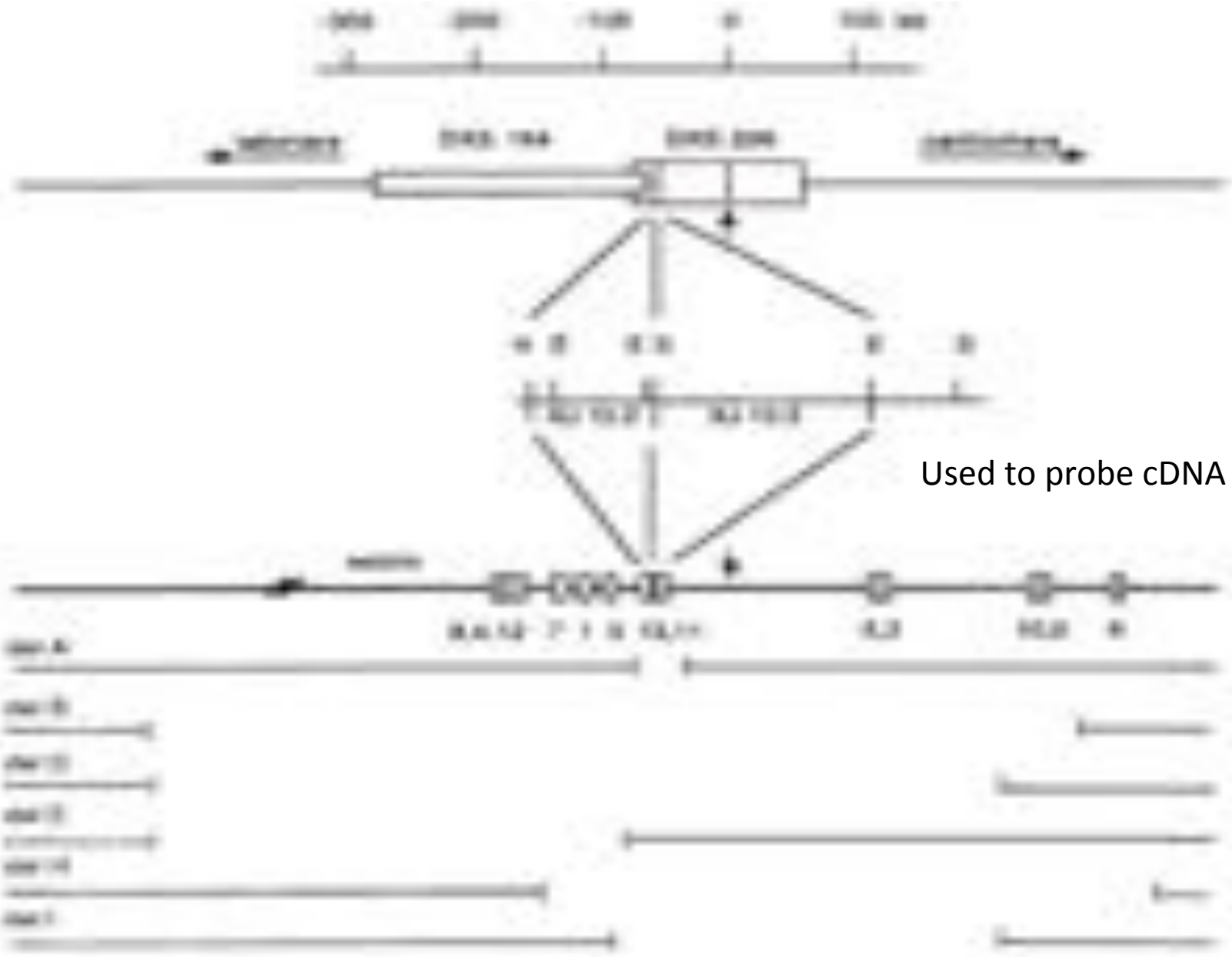
14kb dystrophin mRNA



Distribution of dystrophin exons in pERT region

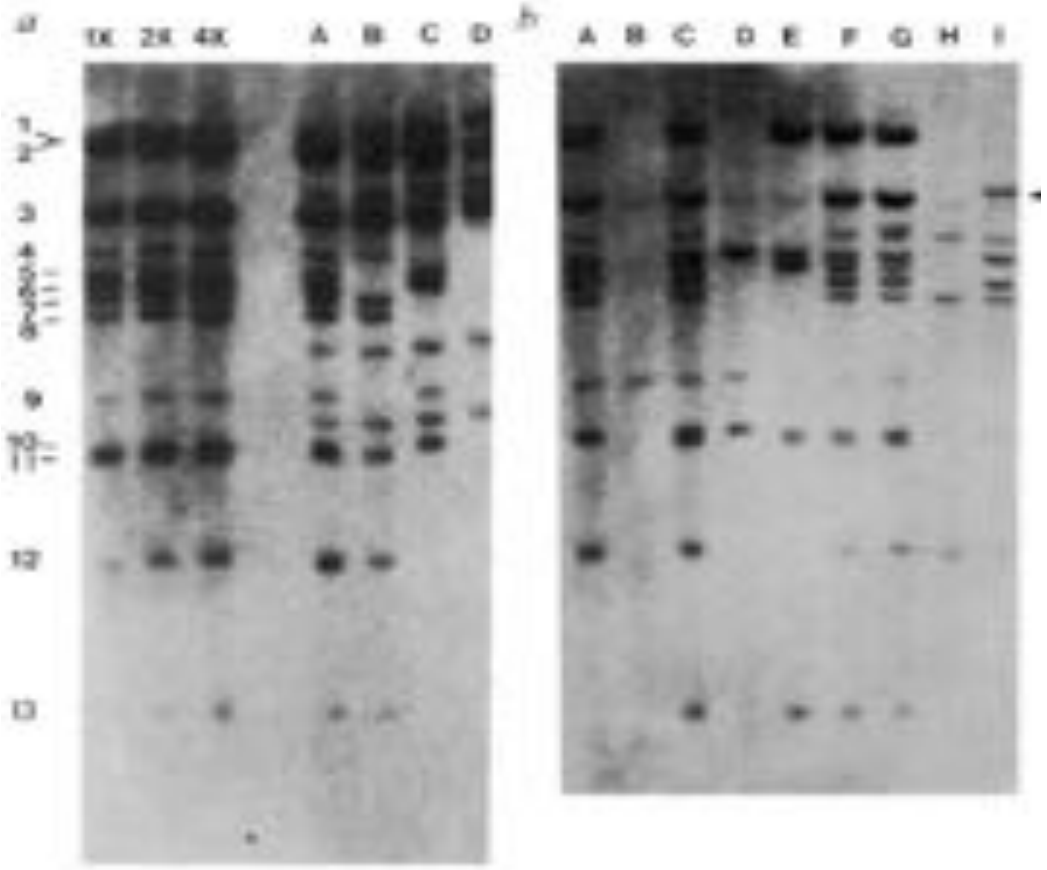


Identification of cDNA/mRNA from DX206 and 110kb intron of dystrophin



Used to probe cDNA

Identification of multiple exons and deletions at the five prime end of dystrophin

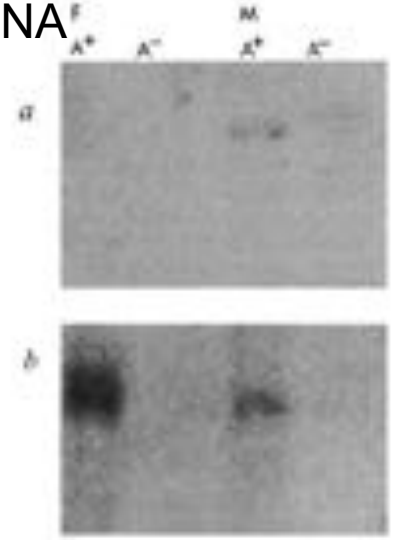


Southern blot of patient DNA probed with 2.0kb Dystrophin cDNA

Table 1 Exon mapping using translocation hybrids and deletion patients

Exon*	Fragment size (kb)	Translocation chromosome Der(X)	Translocation chromosome Der(21)	Deletion patients A	B	C	D	E	F	G	H	I
E8	5.3		+	+		+			+	+	+	+
E4	3.7		+	+		+			+	+	+	+
E12	0.8		+	+		+			+	+	+	+
E7	4.0		+	+		+			+	+		+
E1	15		+	+		+			+	+		J
E3*(+)	6.6		+	+		+			+	+		
E13†	0.7		+			+		+	+	+		
E11†	1.9		+			+		+	+	+		
t(X;21)												
E6	4.3	+		+		+		+	+	+		
E2	13.5	+		+		+		+	+	+		
E10	2.1	+		+		+	+	+	+	+		+
E5	4.6	+		+		+	+	+	+	+		+
E9	2.6	+		+	+	+	+	+	+	+		+

Northern detecting 14kb Dystrophin mRNA



Subcellular fractionation of dystrophin to the triads of skeletal muscle

ERIC P. HOFFMAN*, C. MICHAEL KNUDSON‡, KEVIN P. CAMPBELL‡ & LOUIS M. KUNKEL*†

* Department of Pediatrics, Harvard Medical School and Division of Genetics, Children's Hospital, and

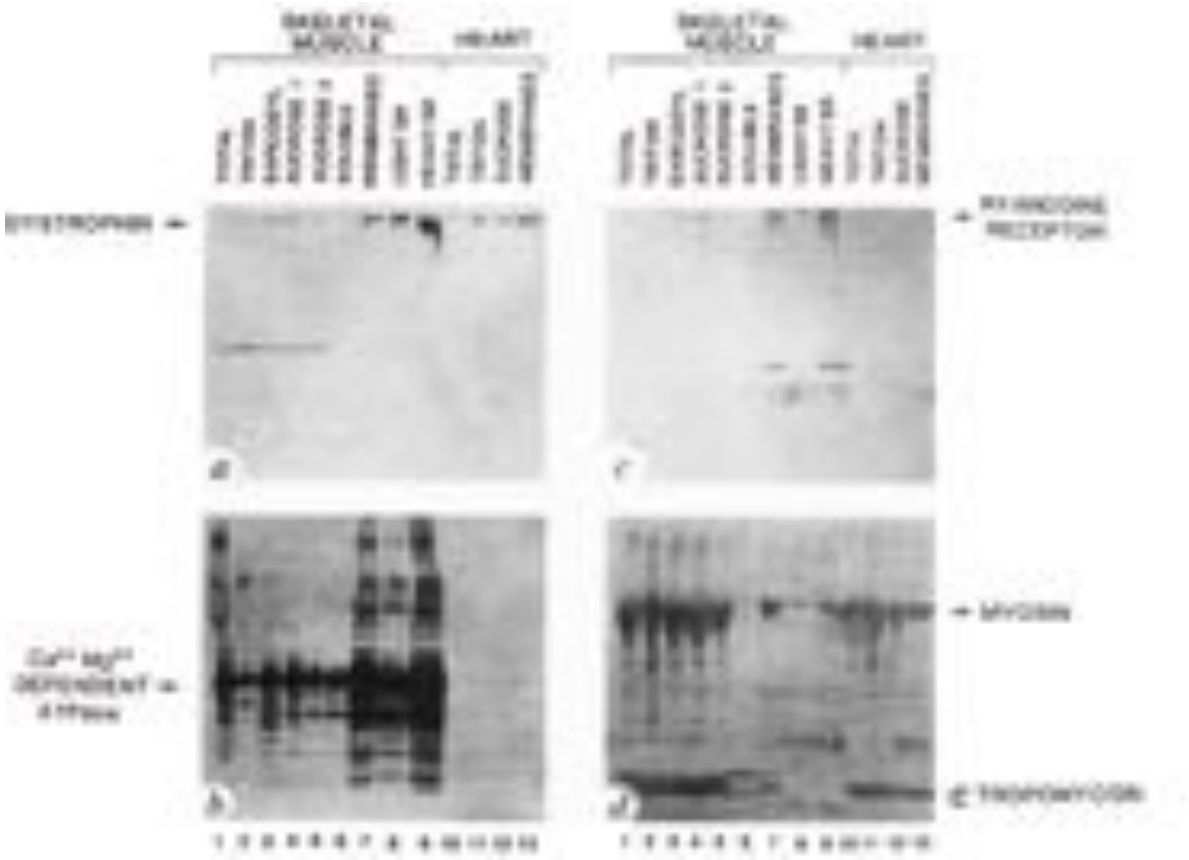
† Howard Hughes Medical Institute, Boston, Massachusetts 02115, USA

‡ Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242, USA

Here we show that dystrophin is associated with the triadic junctions in skeletal muscle, and is therefore probably involved with Ca^{2+} homoeostasis.

Nature. 1987 Dec 24-31;330(6150):754-8

Nature 330 1987

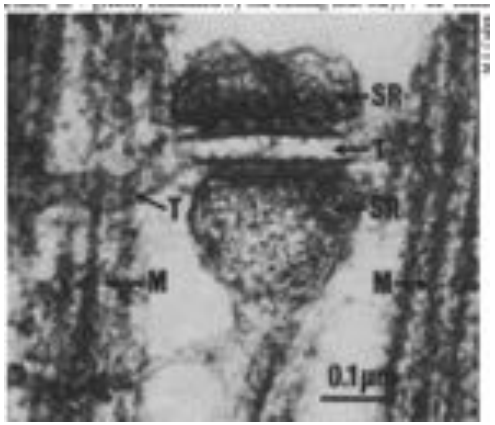


News and views Nature

Muscular dystrophy

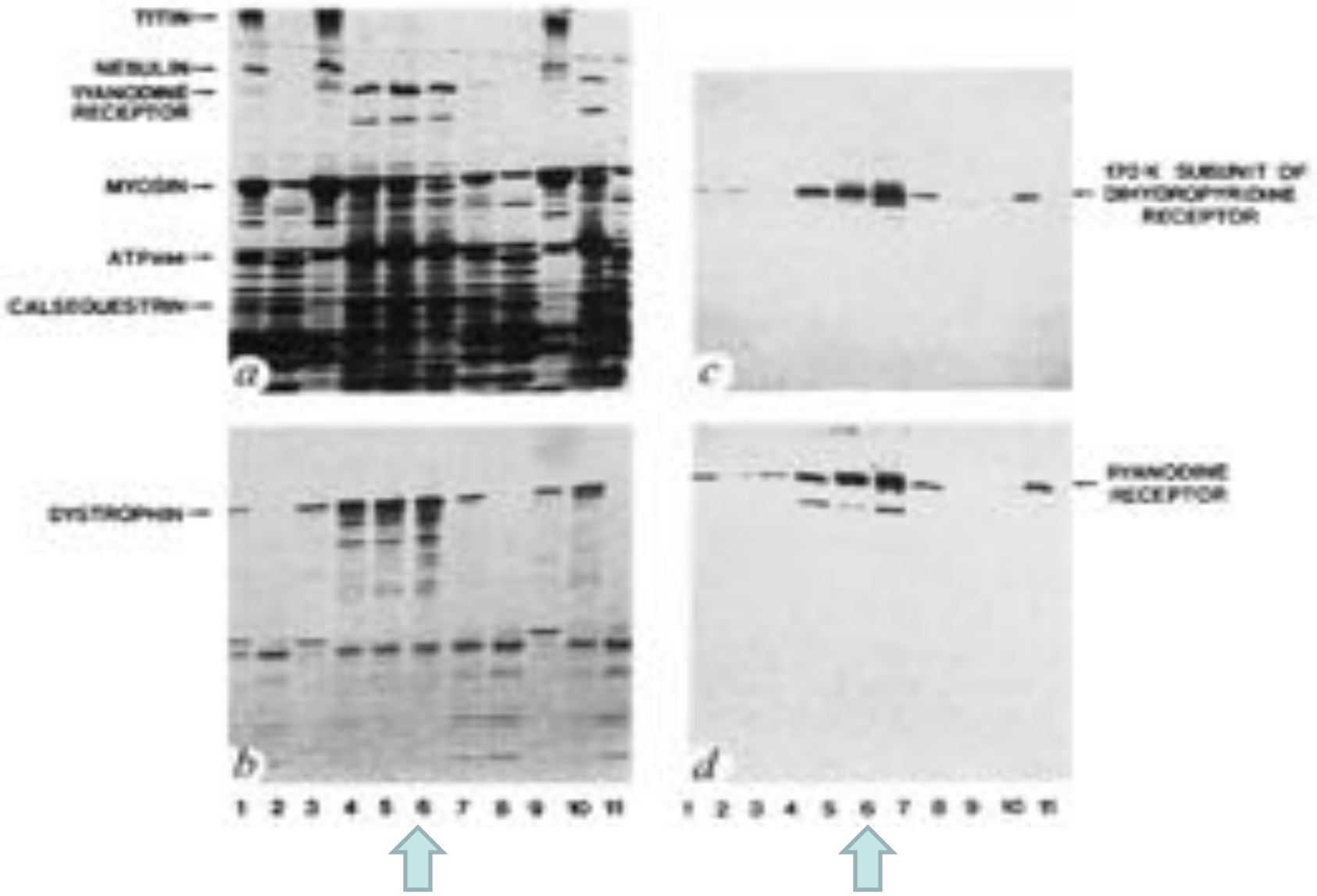
The missing link in DMD?

Clarke R. Slater



This protein species is called dystrophin because of its identification by molecular-genetic analysis of affected individuals. Here we show that dystrophin is associated with the triadic junctions in skeletal muscle, and is therefore probably involved with Ca^{2+} homoeostasis.

400kd protein not Nebulin but dystrophin



But no plasma membrane marker such as muscle Sodium Channel

The Duchenne muscular dystrophy gene product is localized in sarcolemma of human skeletal muscle

Elizabeth E. Zubrzycka-Gaarn*, Dennis E. Bulman*, George Karpati†, Arthur H. M. Burghes*, Bonnie Belfall*, Henry J. Klamut*, Jim Talbot‡, Robert S. Hodges‡, Peter N. Ray* & Ronald G. Worton*

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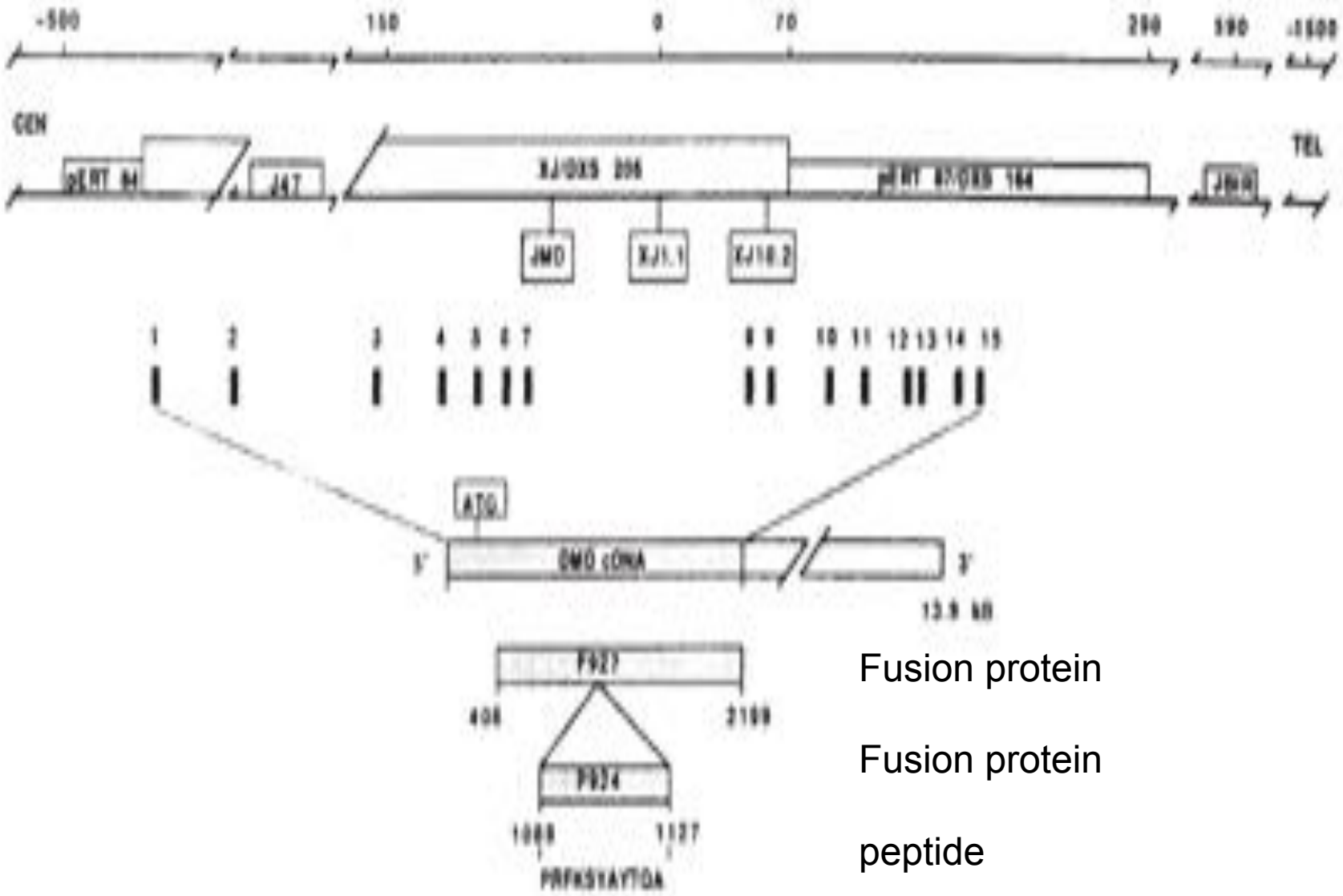
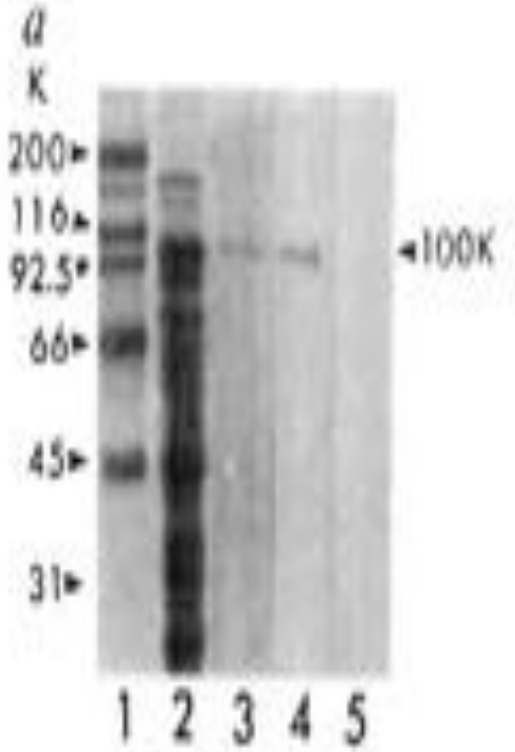


Fusion protein

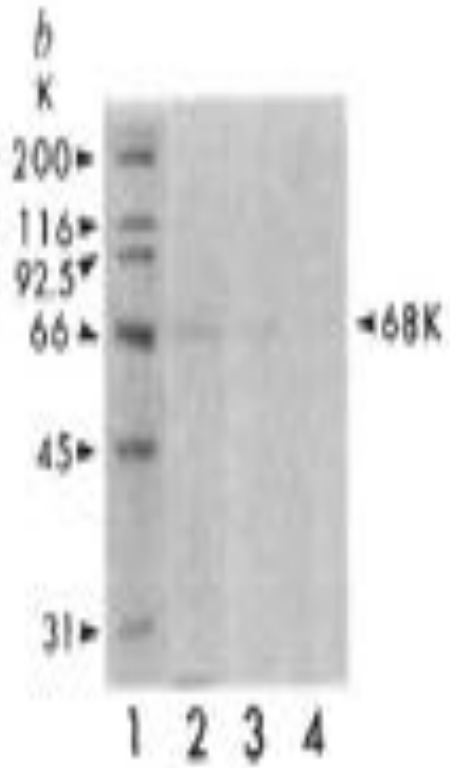
Fusion protein

peptide

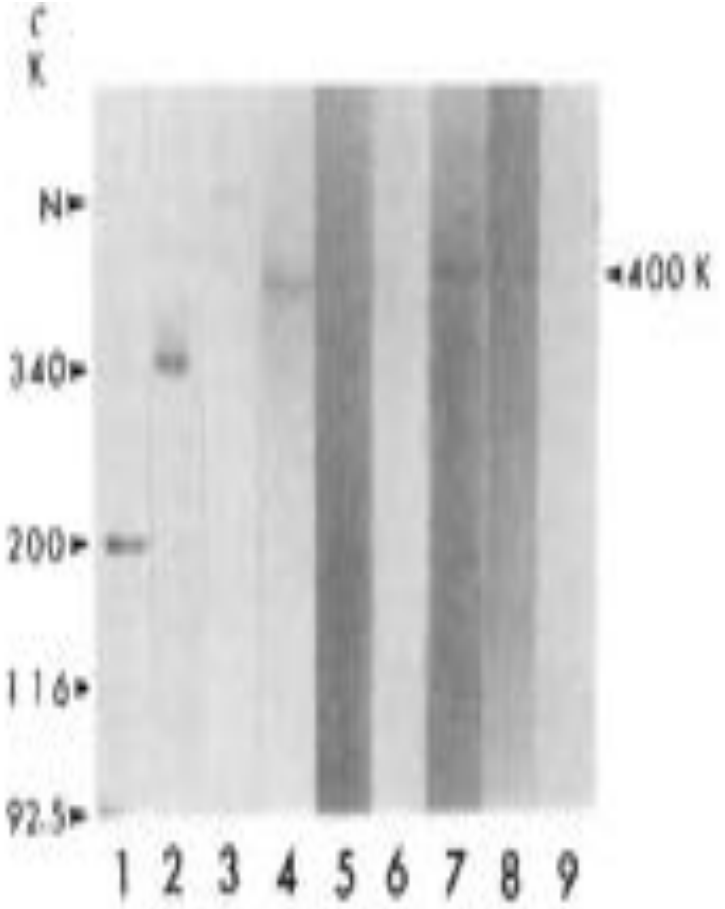
Antibodies do recognize dystrophin



E.Coli Fusion protein
of DMD cDNA
In pATH and PRIT vector
3)Peptide P924
4)Fusion F927
5) pre-immune

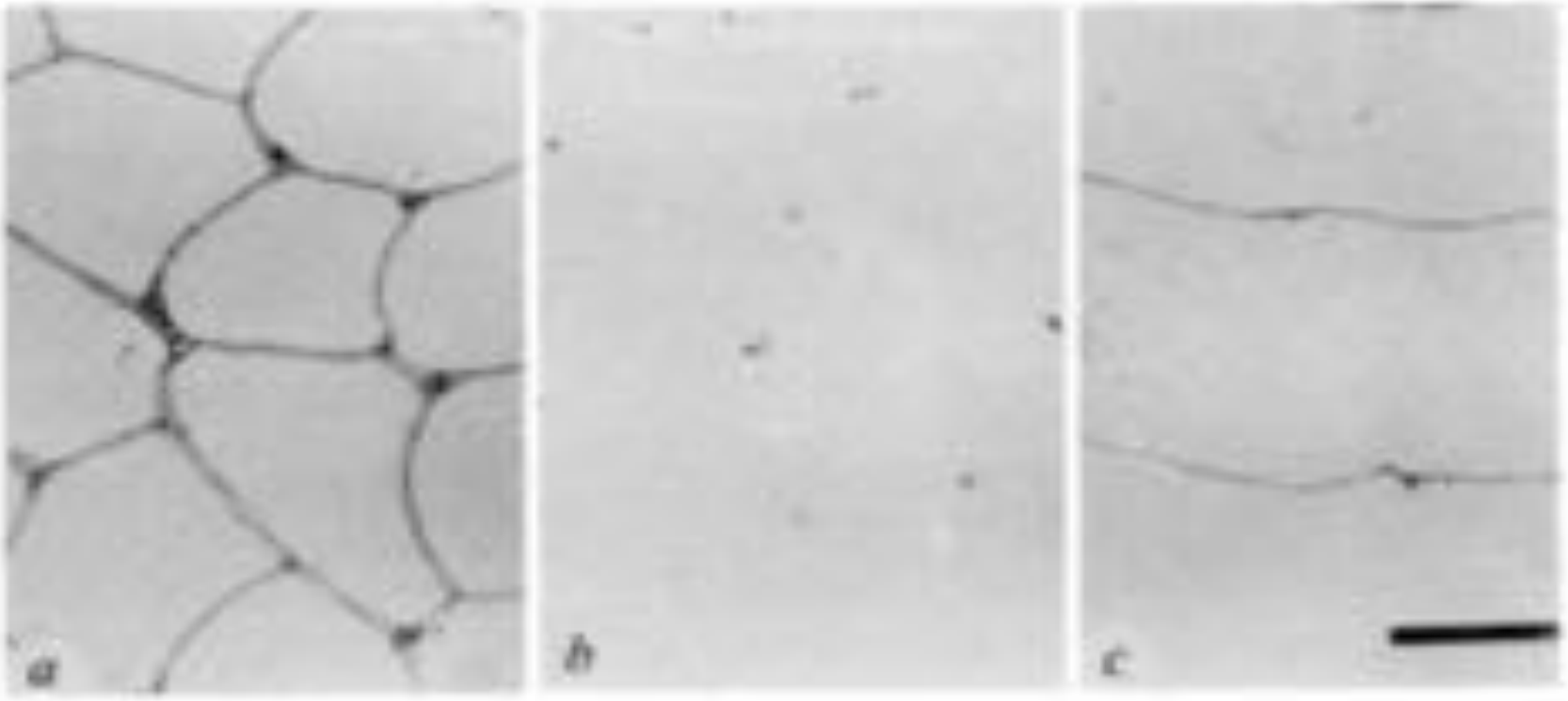


In-vitro translation
of DMD cDNA



Muscle fractionated
(Not a whole leg)
4)P924
7)F927

Dystrophin is at the membrane

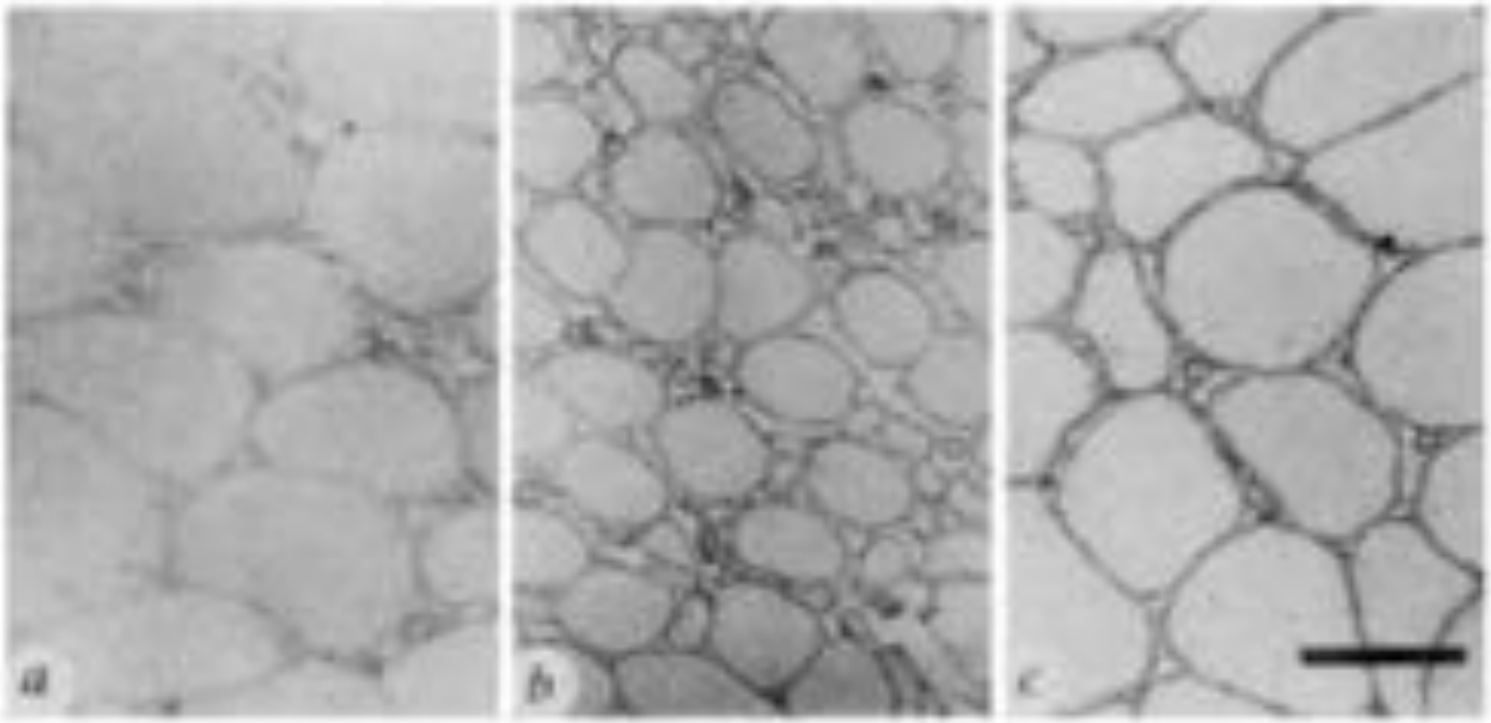


Normal muscle stained with F927

Normal muscle stained With pre-immune serum

Normal muscle longitudinal stained with F927

F927 immunostaining shows absence of dystrophin in DMD



5 year old DMD

Nemaline rod myopathy

Dermatomyostitis

Immunostaining of skeletal and cardiac muscle surface membrane with antibody against Duchenne muscular dystrophy peptide

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Immunoelectron microscopic localization of dystrophin in myofibres

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Two historical references with some of the information

Duchenne Muscular Dystrophy
Alan Emery and Francesco Muntoni

Genome: The Story of the Most Astonishing Scientific Adventure of Our Time--The Attempt to Map All the Genes in the Human Body
By Jerry Bishop and Michael Waldholz