Chromatin accessibility at the ALS-associated *C9orf72* repeat expansion

Monika HM Schmidt

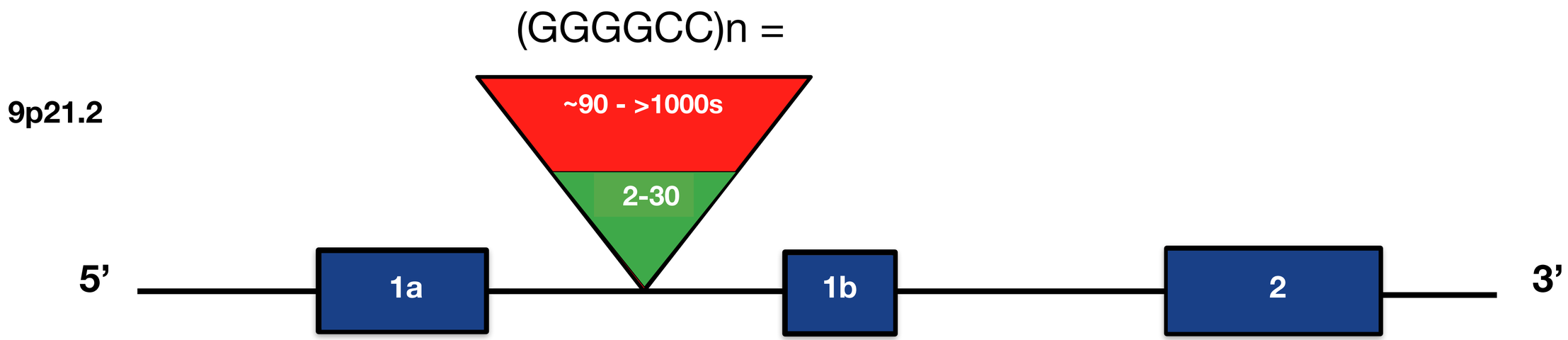
Lab of Dr. Christopher E. Pearson

ALS Canada Research Forum
April 28th, 2019.

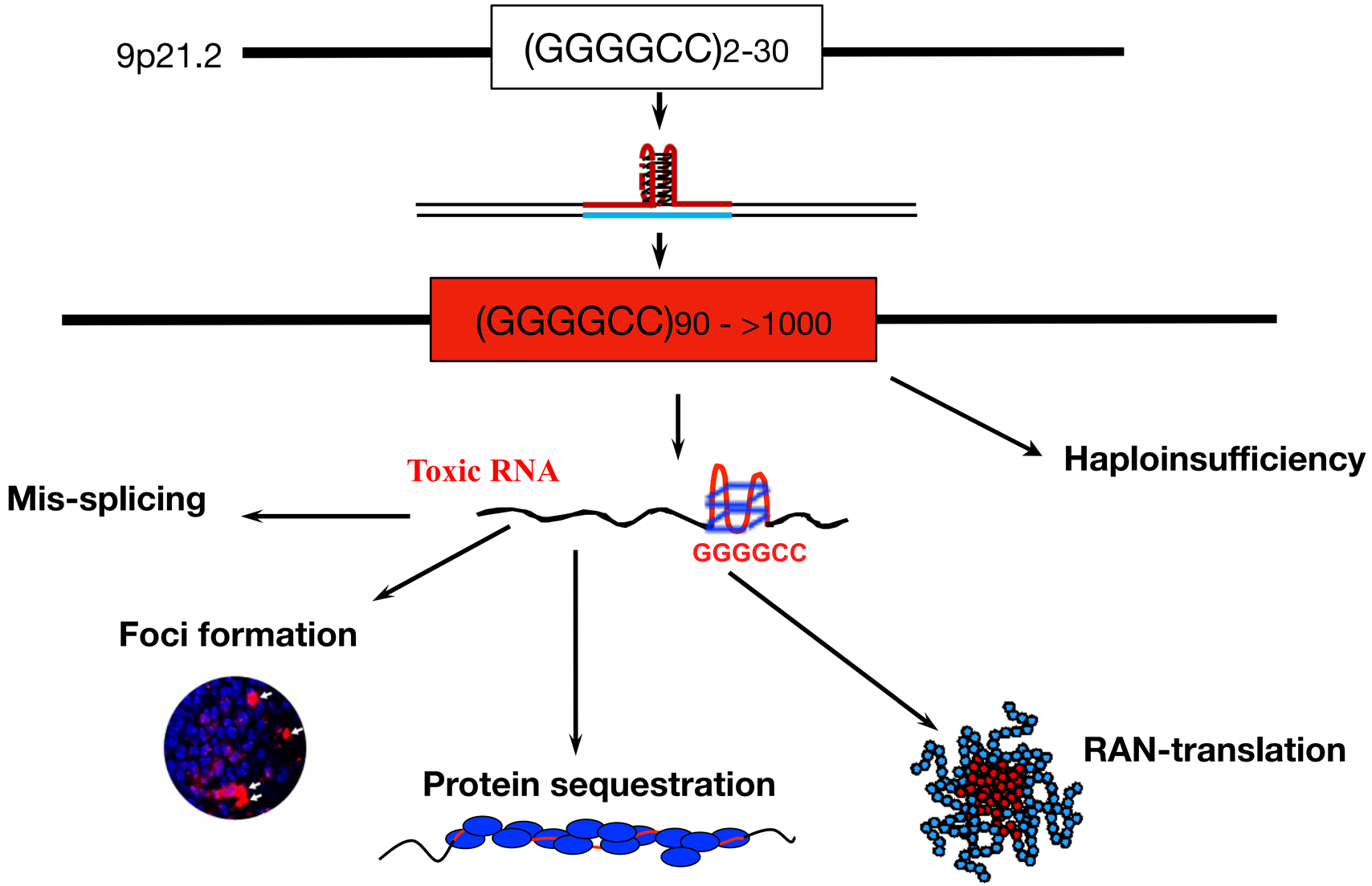
Disease-Associated DNA Repeat Instability

>48 human neurological, neurodegenerative, and neuromuscular inherited diseases are caused by unstable repeat sequences in specific genes…

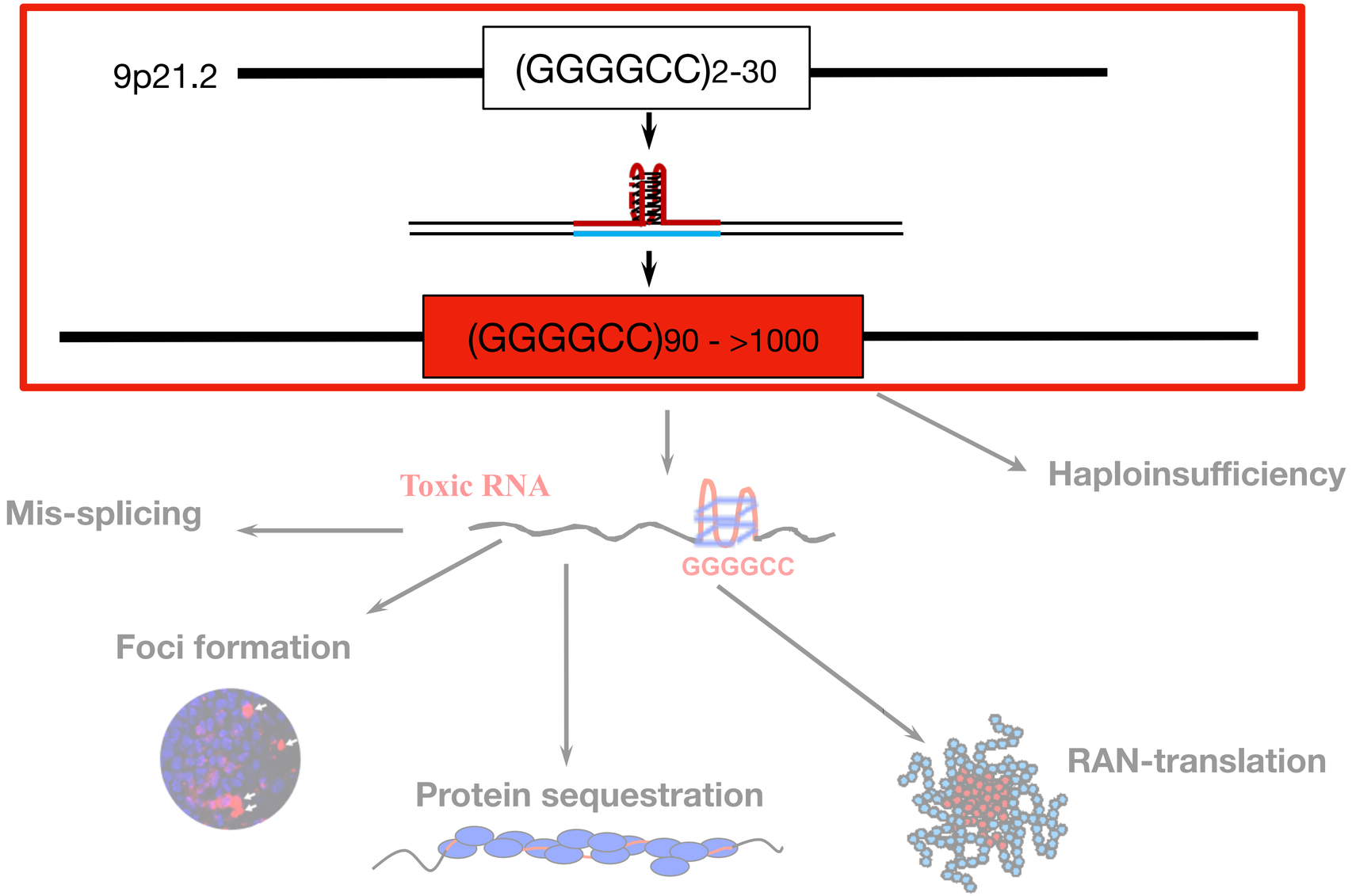
C9orf72 ALS & FTD



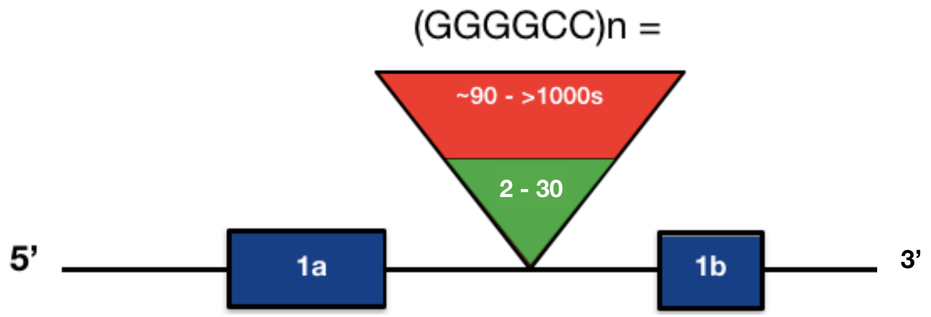
C9orf72 repeat expansion disease pathways



C9orf72 repeat expansion disease pathways



Somatic instability at expanded *C9orf72* repeat tract

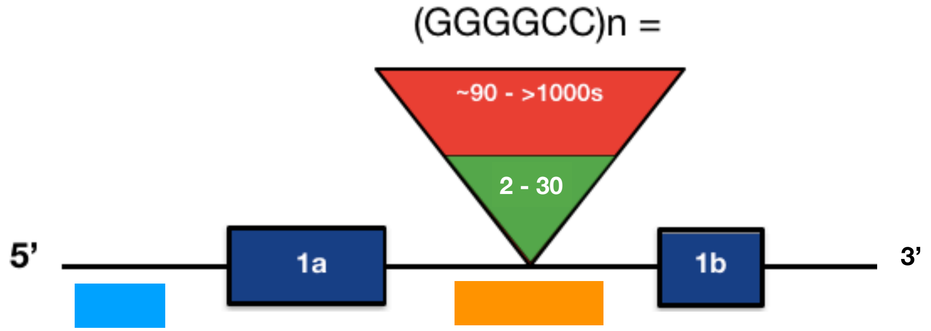


The Lancet Neurology, 2013.

Association between repeat sizes and clinical and pathological characteristics in carriers of *C9ORF72* repeat expansions (Xpansize-72): a cross-sectional cohort study

Marka van Blitterswijk, Mariely DeJesus-Hernandez, Ellis Niemantsverdriet, Melissa E Murray, Michael G Heckman, Nancy N Diehl, Patricia H Brown, Matthew C Baker, NiCole A Finch, Peter O Bauer, Geidy Serrano, Thomas G Beach, Keith A Josephs, David S Knopman, Ronald C Petersen, Bradley F Boeve, Neill R Graff-Radford, Kevin B Boylan, Leonard Petrucelli, Dennis W Dickson, Rosa Rademakers

Methylation at expanded *C9orf72* repeat tract



CpG methylation
~36% of patients

Methylation of
expanded repeat
100% of patients

Acta Neuropathologica, 2015.

ORIGINAL PAPER

The *C9orf72* repeat expansion itself is methylated in ALS and FTLD patients

Zhengrui Xi · Ming Zhang · Amalia C. Bruni · Raffaele G. Maletta · Rosanna Colao · Pietro Fratta · James M. Polke · Mary G. Sweeney · Ese Mudanohwo · Benedetta Nacmias · Sandro Sorbi · Maria Carmela Tartaglia · Innocenzo Rainero · Elisa Rubino · Lorenzo Pinessi · Daniela Galimberti · Ezequiel I. Surace · Philip McGoldrick · Paul McKeever · Danielle Moreno · Christine Sato · Yan Liang · Julia Keith · Lorne Zinman · Janice Robertson · Ekaterina Rogaeva

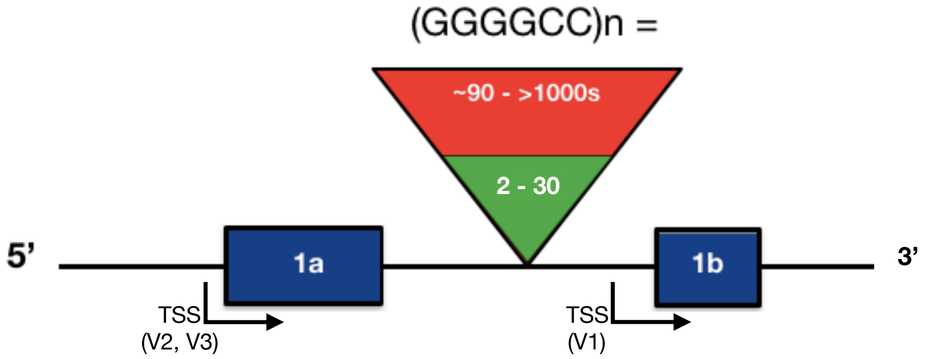
American Journal of Human Genetics, 2013.

REPORT

Hypermethylation of the CpG Island Near the G_4C_2 Repeat in ALS with a *C9orf72* Expansion

Zhengrui Xi,[1] Lorne Zinman,[2] Danielle Moreno,[1] Jennifer Schymick,[1] Yan Liang,[1] Christine Sato,[1] Yonglan Zheng,[3] Mahdi Ghani,[1] Samar Dib,[1] Julia Keith,[2] Janice Robertson,[1] and Ekaterina Rogaeva[1,4,*]

Transcription occurs across the expanded *C9orf72* repeat tract



***c9orf72* Disease-Related Foci are Each Composed of One Mutant Expanded Repeat RNA**

Jing Liu[1], Jiaxin Hu[1], Andrew Ludlow[2], Jackie Pham[3], Jerry W. Shay[2], Jeffrey D. Rothstein[3], and David R. Corey[1]

Cell Chem Biol., 2017.

Acta Neuropathologica, 2013.

ORIGINAL PAPER

Reduced *C9orf72* gene expression in c9FTD/ALS is caused by histone trimethylation, an epigenetic event detectable in blood

Veronique V. Belzil · Peter O. Bauer · Mercedes Prudencio · Tania F. Gendron · Caroline T. Stetler · Irene K. Yan · Luc Pregent · Lillian Daughrity · Matthew C. Baker · Rosa Rademakers · Kevin Boylan · Tushar C. Patel · Dennis W. Dickson · Leonard Petrucelli

Methylation and aberrant chromatin modification in DM1

Research Article

Journal of Nucleic Acids, 2013.

Transcriptionally Repressive Chromatin Remodelling and CpG Methylation in the Presence of Expanded CTG-Repeats at the DM1 Locus

Judith Rixt Brouwer,[1,2] Aline Huguet,[1,2] Annie Nicole,[1,2] Arnold Munnich,[1,2] and Geneviève Gourdon[1,2]

Histone Modifications Depict an Aberrantly Heterochromatinized *FMR1* Gene in Fragile X Syndrome

Bradford Coffee,[1] Fuping Zhang,[2,3] Stephanie Ceman,[2] Stephen T. Warren,[2,3,*] and Daniel Reines[1]

American Journal of Human Genetics, 2002.

Acetylated histones are associated with *FMR1* in normal but not fragile X-syndrome cells

Bradford Coffee[1], Fuping Zhang[2], Stephen T. Warren[2] & Daniel Reines[1]

Nat. Gen., 1999.

Aberrant chromatin compaction observed as assessed by nuclease accessibility

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 5465–5469, June 1995
Medical Sciences

Triplet repeat expansion in myotonic dystrophy alters the adjacent chromatin structure

A. D. OTTEN AND S. J. TAPSCOTT

Clinical Research Division, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104

Effect of Triplet Repeat Expansion on Chromatin Structure and Expression of *DMPK* and Neighboring Genes, *SIX5* and *DMWD*, in Myotonic Dystrophy

Richard Frisch,* Kenneth R. Singleton,* Priscilla A. Moses,* Iris L. Gonzalez,* Paul Carango,* Harold G. Marks,*† and Vicky L. Funanage*†‡

Molecular Genetics and Metabolism, 2001.

Nuclease Sensitivity of Permeabilized Cells Confirms Altered Chromatin Formation at the Fragile X Locus

Derek E. Eberhart and Stephen T. Warren

Somatic Cell and Molecular Genetics, 1996.

Aberrant chromatin compaction observed as assessed by nuclease accessibility

Proc. Natl. Acad. Sci. USA
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Medical Sciences

Triplet repeat expansion in myotonic dystrophy alters the adjacent chromatin structure

A. D. OTTEN AND S. J. TAPSCOTT

Clinical Research Division, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104

Effect of Triplet Repeat Expansion on Chromatin Structure and

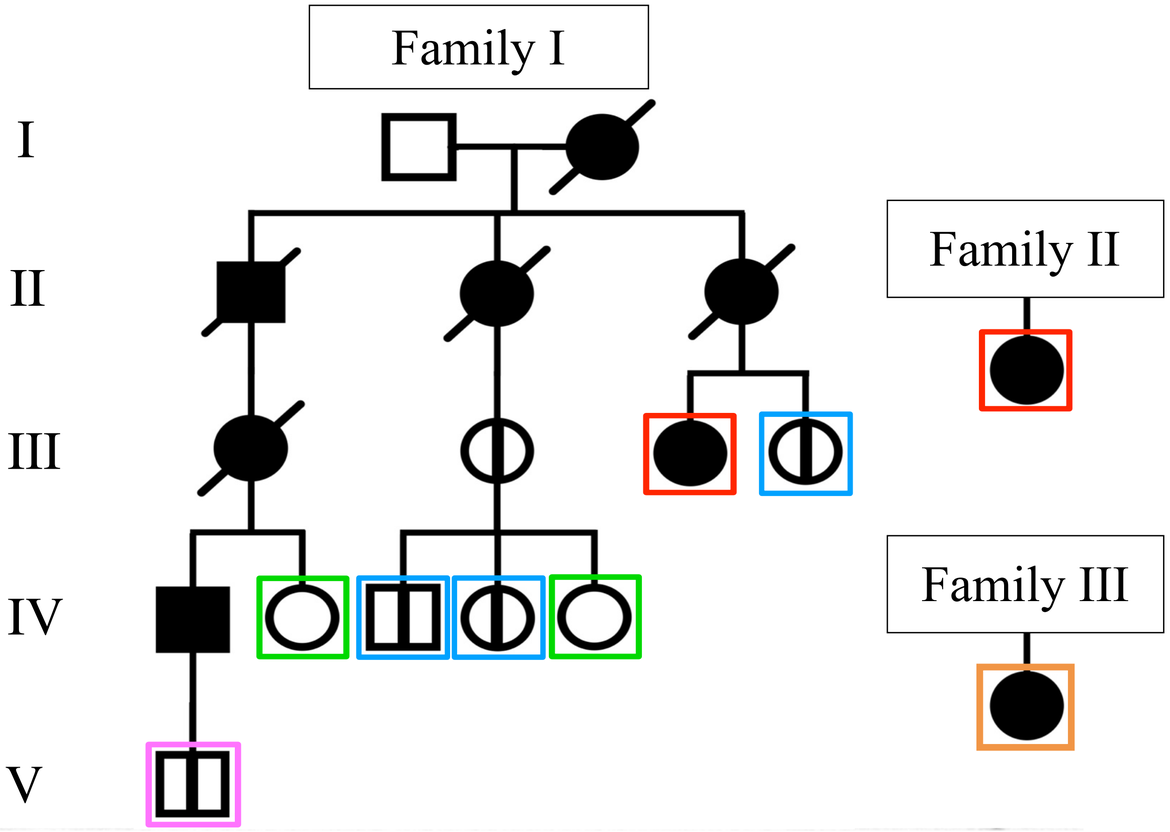
Does chromatin accessibility differ between alleles of *C9orf72*-ALS patients?

Molecular Genetics and Metabolism, 2001.

Nuclease Sensitivity of Permeabilized Cells Confirms Altered Chromatin Formation at the Fragile X Locus

Derek E. Eberhart and Stephen T. Warren

Somatic Cell and Molecular Genetics, 1996.



Expanded	Methyl	Affected
✔	✔	✔
✔	✔	
✔		✔
✔		

E:

- Expanded

EM:

- Expanded
- Methylated

EA:

- Expanded
- Affected

EMA:

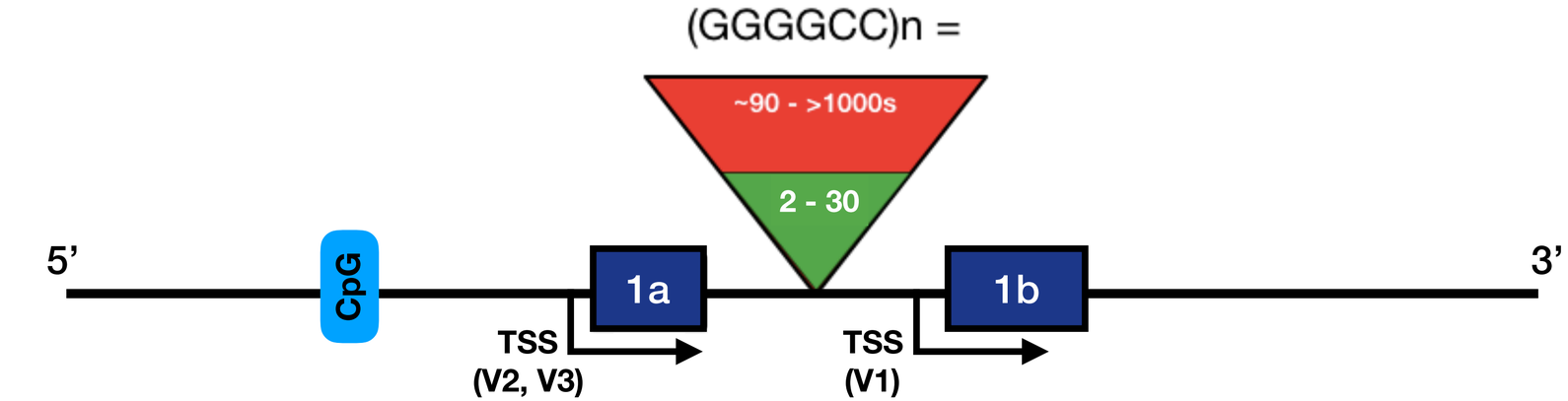
- Expanded
- Methylated
- Affected

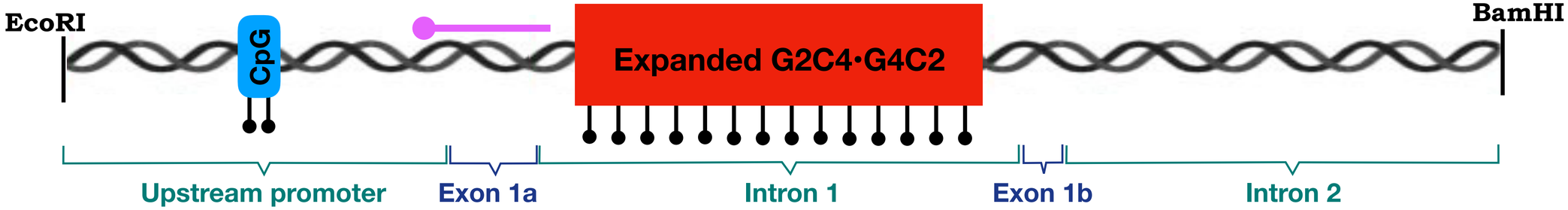
WT:

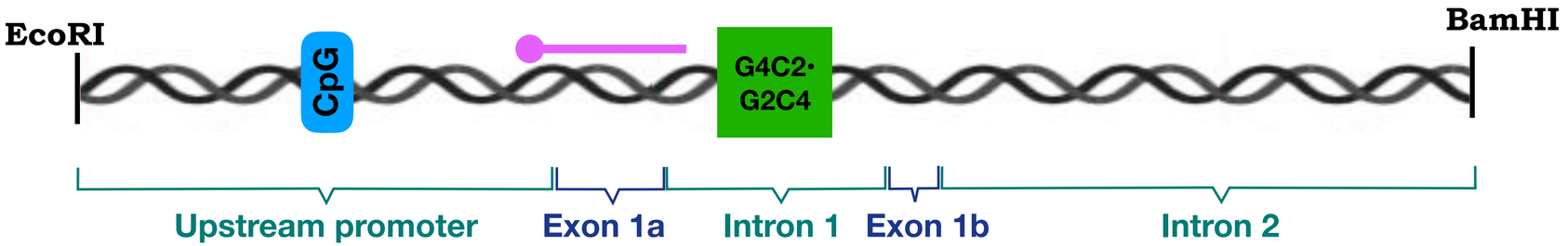
- No expansion

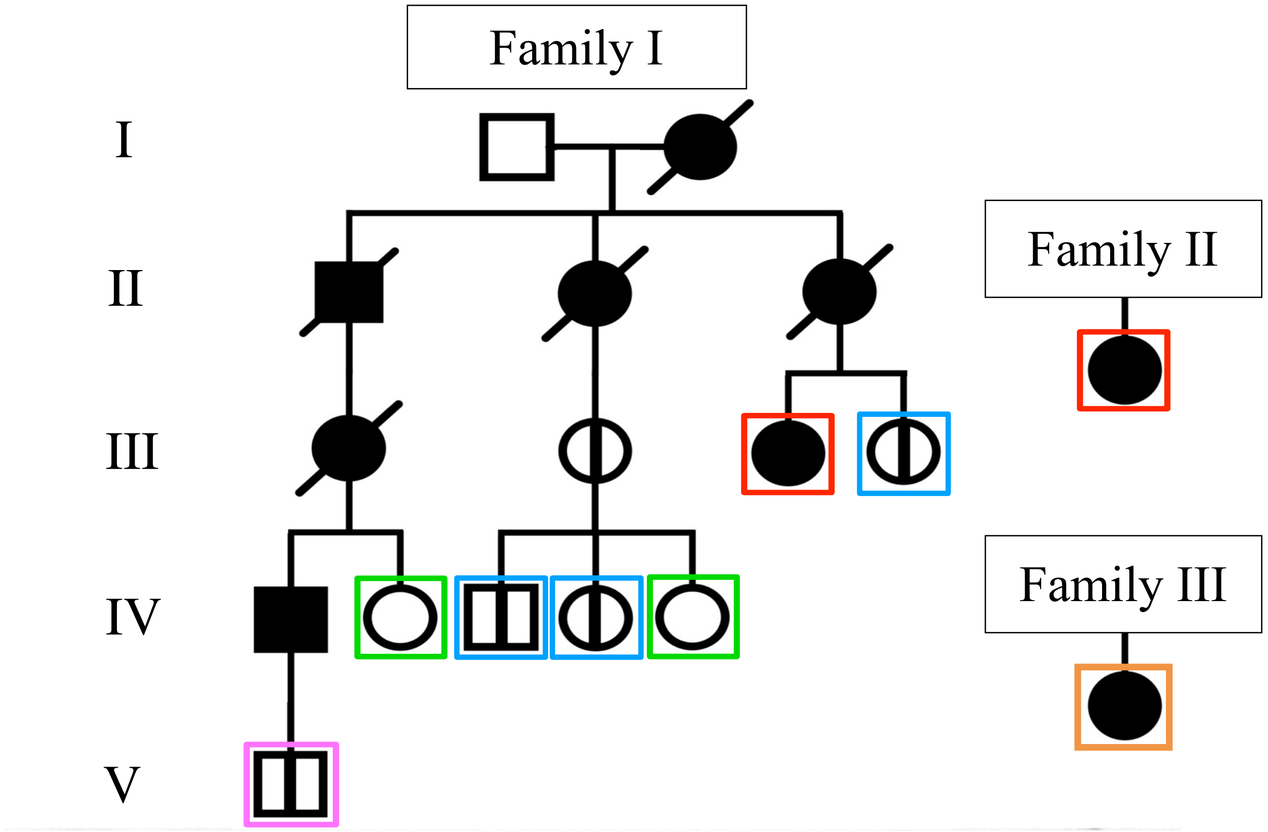
Cell lines - Dr. Guy Rouleau
Methylation data - Dr. Ekaterina Rogaeva
Southern blots in collaboration with Dr. Tanya Prasolava

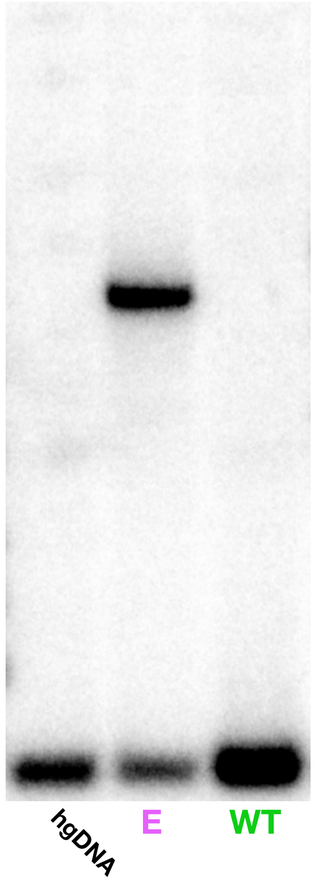
Southern blot probe design





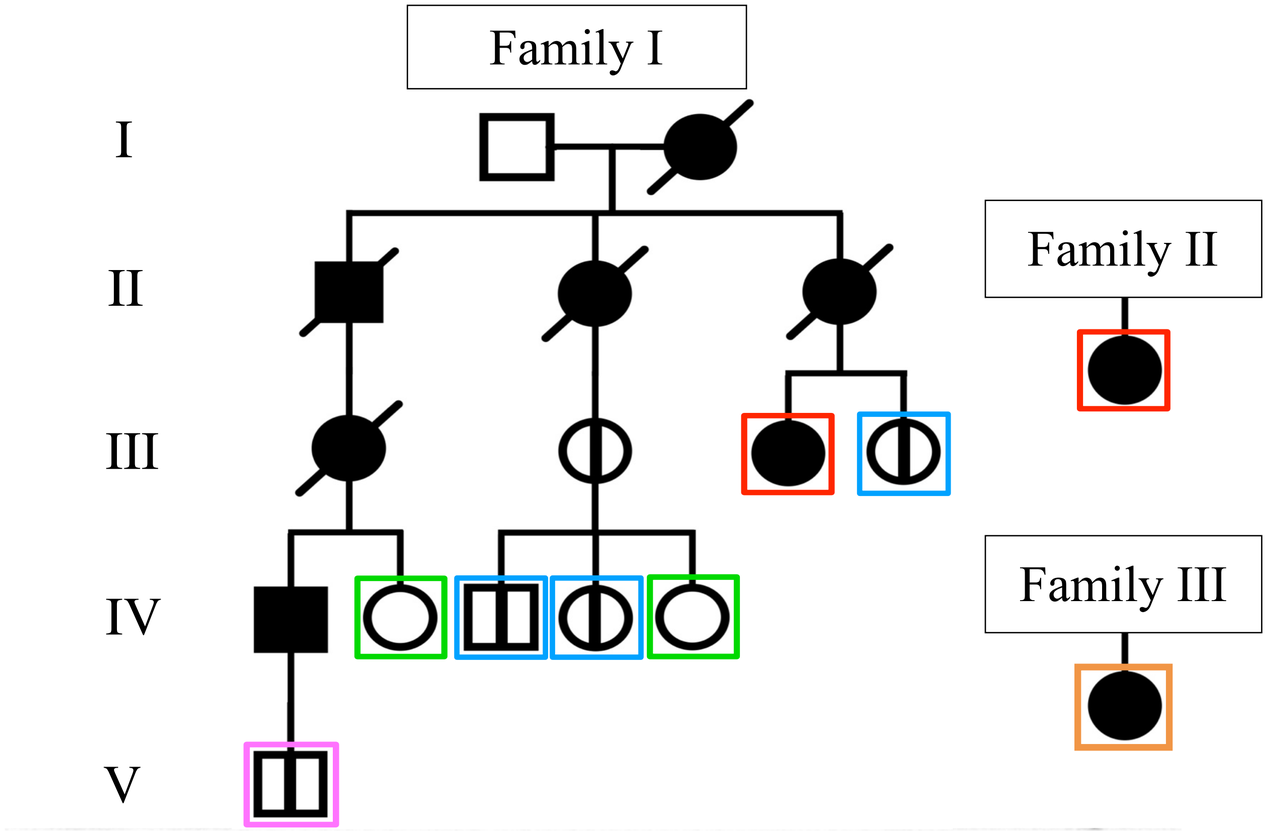




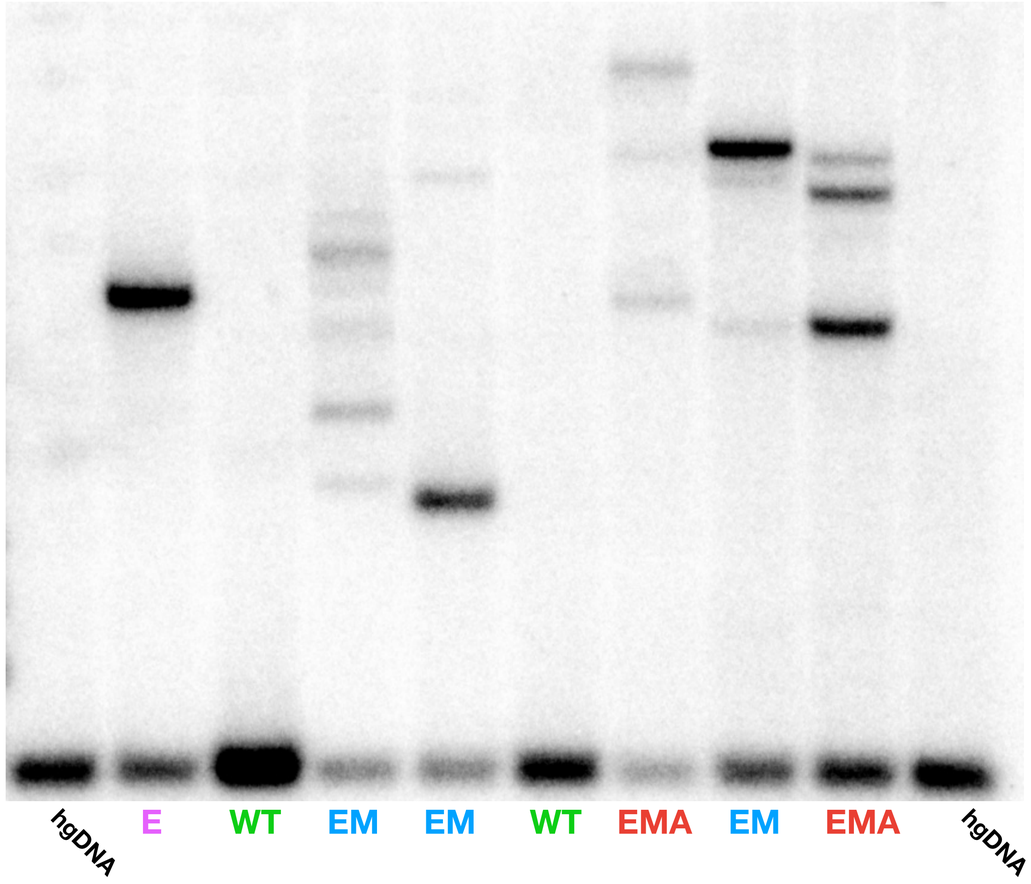


Expanded	Methyl	Affected
✔	✔	✔
✔	✔	
✔		✔
✔		

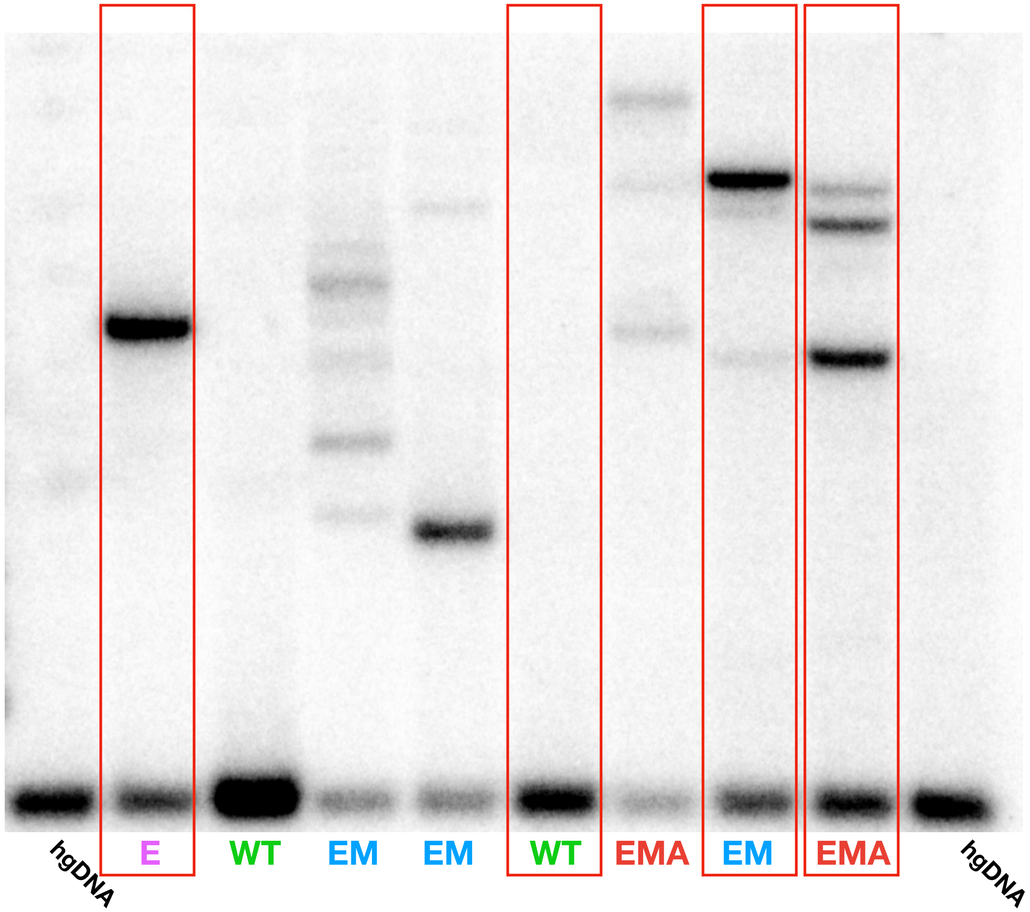
Cell lines - Dr. Guy Rouleau
Methylation data - Dr. Ekaterina Rogaeva
Southern blots in collaboration with Dr. Tanya Prasolava



Expanded	Methyl	Affected
✔	✔	✔
✔	✔	
✔		✔
✔		



Cell lines - Dr. Guy Rouleau
Methylation data - Dr. Ekaterina Rogaeva
Southern blots in collaboration with Dr. Tanya Prasolava

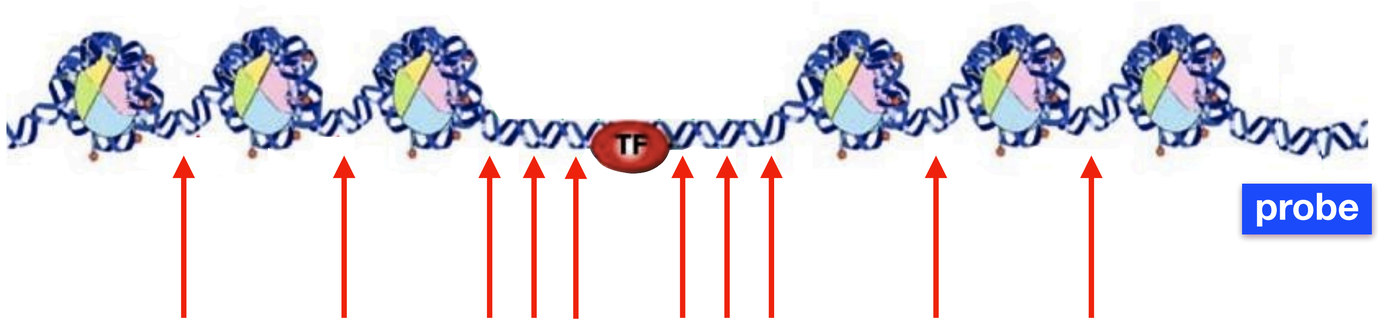


Is chromatin accessibility altered between expanded and non-expanded alleles?

Cell lines - Dr. Guy Rouleau
Methylation data - Dr. Ekaterina Rogaeva
Southern blots in collaboration with Dr. Tanya Prasolava

Micrococcal Nuclease (MNase) Chromatin Accessibility Assay

MNase is widely used to assess chromatin compaction.



Adapted from Tsompana and Buck, 2014.

Micrococcal Nuclease (MNase) Chromatin Accessibility Assay

Collect cells

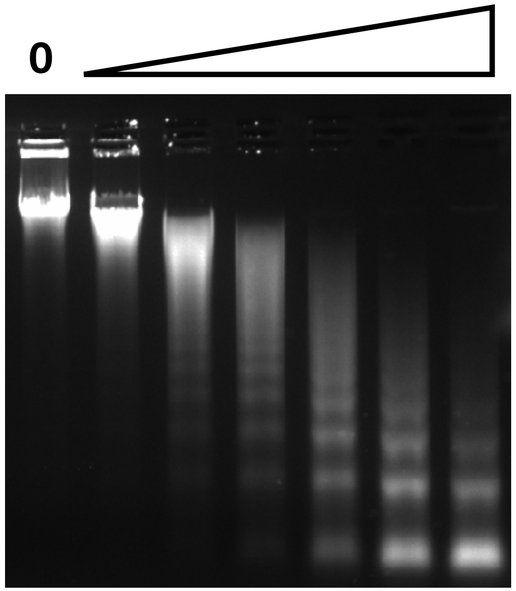
Permeabilize membrane

Treat nuclear chromatin with MNase

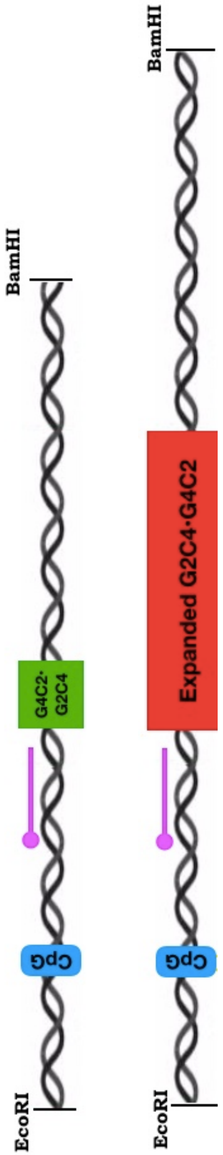
Purify gDNA
Restrict to completion

MNase treat to over-digestion

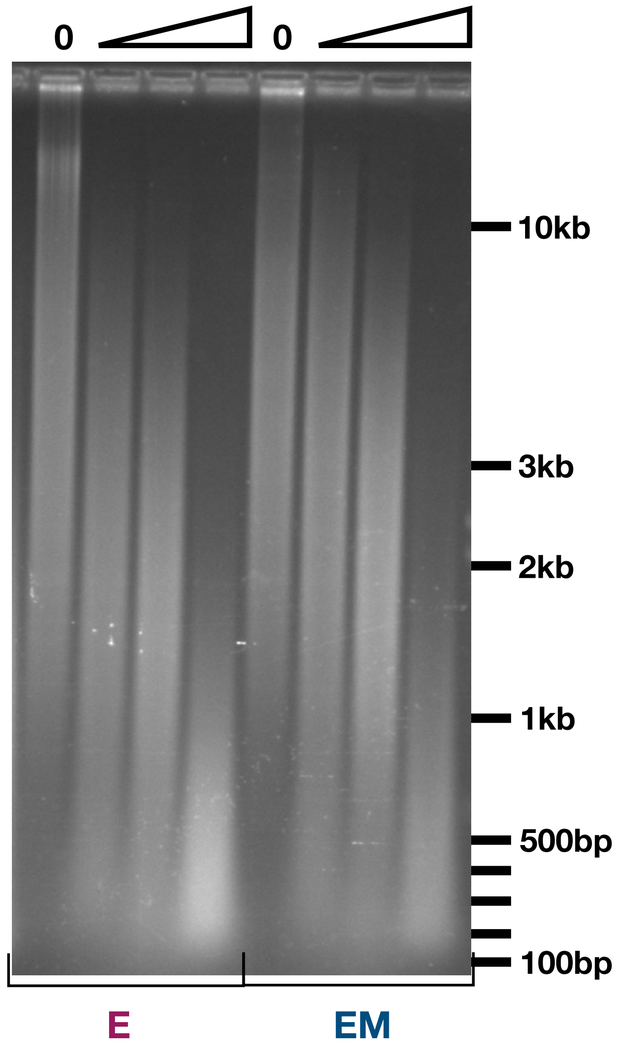
0



Southern Blot



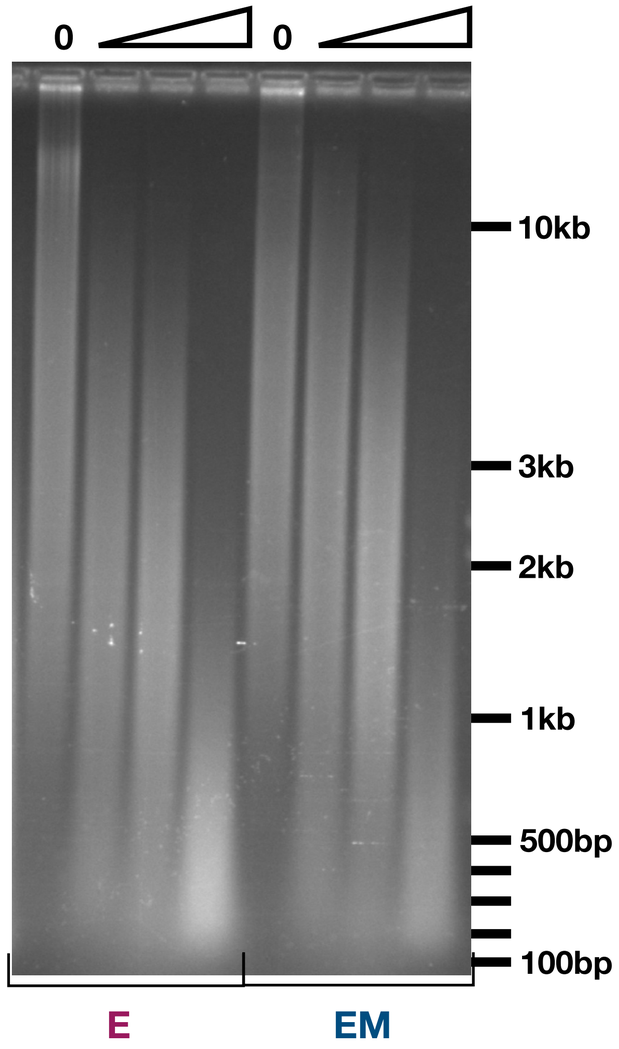
Are purified *C9* gDNA alleles equally digestible by MNase?

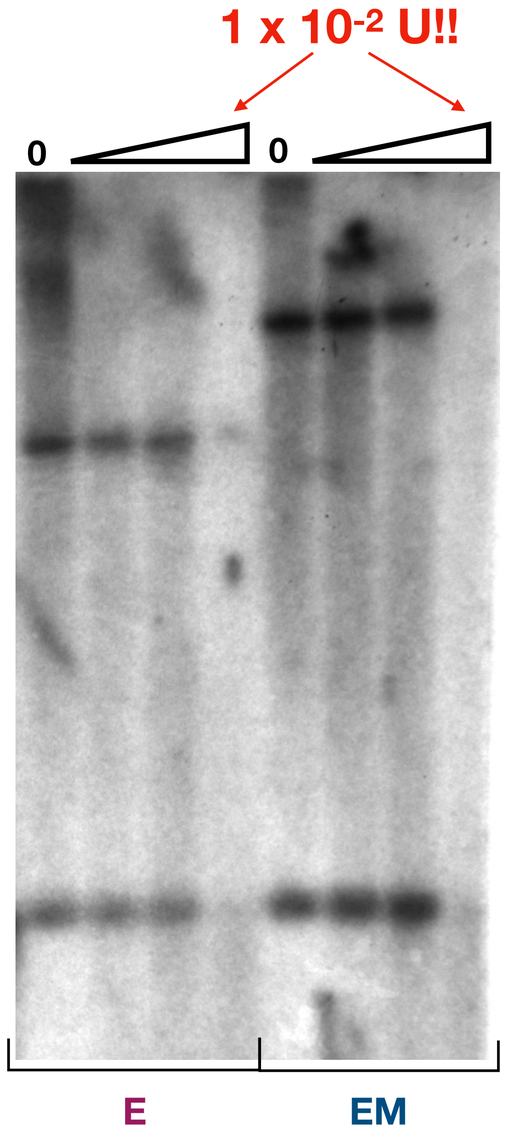


MNase to over-digestion of non-chromatinized

DNA does not yield nucleosomal ladders.

Both *C9* alleles are equally digestible by MNase





MNase-digestible irrespective of expansion of methylation status.

Summary: *C9orf72* chromatin accessibility

- Non-expanded *C9orf72* allele is largely accessible throughout to MNase

- *C9orf72* repeat expansion restricts MNase accessibility
  - increasing repeat size —> increasing MNase resistance

- CpG methylation of the expanded allele enhances MNase resistance

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2806–2810, May 1984
Genetics

Methylation of the hypoxanthine phosphoribosyltransferase locus on the human X chromosome: Implications for X-chromosome inactivation

(dosage compensation/"housekeeping" genes/5-azacytidine/X-chromosome reactivation/mouse–human hybrids)

STANLEY F. WOLF*, DOUGLAS J. JOLLY†, KEITH D. LUNNEN*, THEODORE FRIEDMANN†, AND BARBARA R. MIGEON*

Clusters of CpG dinucleotides implicated by nuclease hypersensitivity as control elements of housekeeping genes

Stanley F. Wolf* & Barbara R. Migeon†

Nature, 1985.

Acknowledgements

Pearson Lab Members

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Dr. Paul M. McKeever
Dr. Lorne Zinman

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