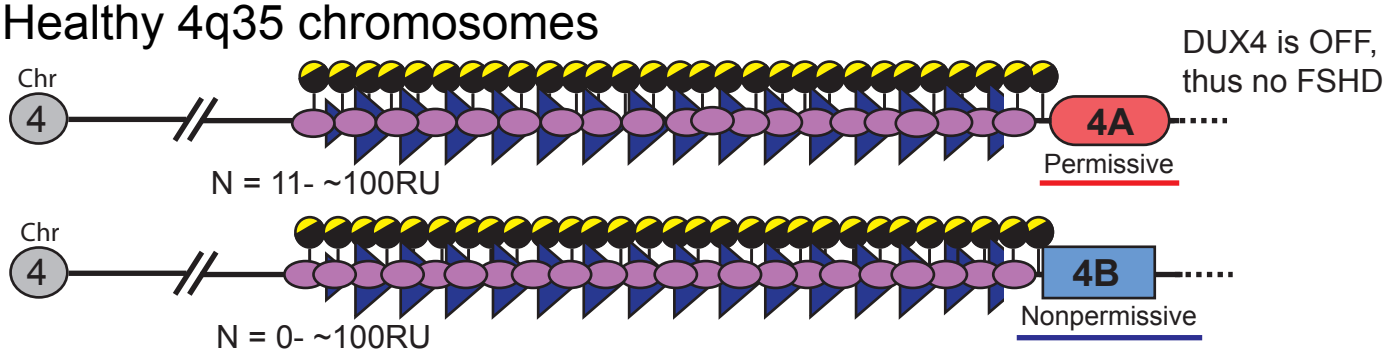
FSHD Epigenetic Diagnostics (Pt 1)

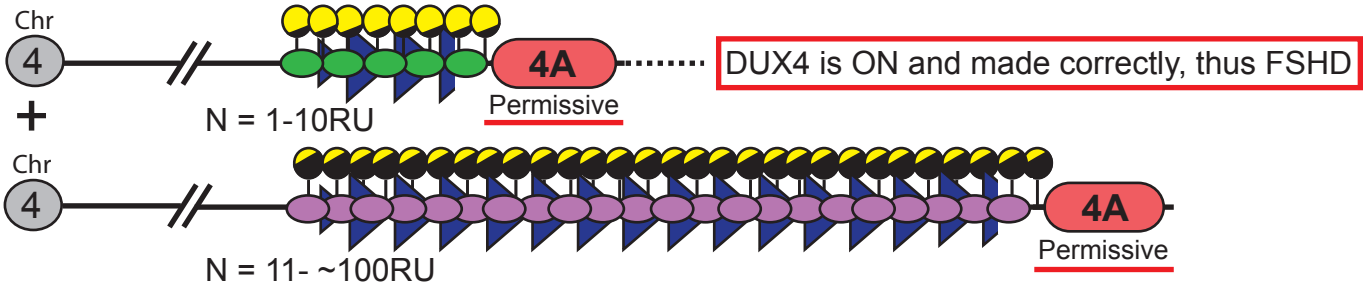
Epigenetic diagnostics are based on the FSHD-specific methylation levels of the chromosome 4q and 10q D4Z4 arrays.

Healthy 4q35 chromosomes

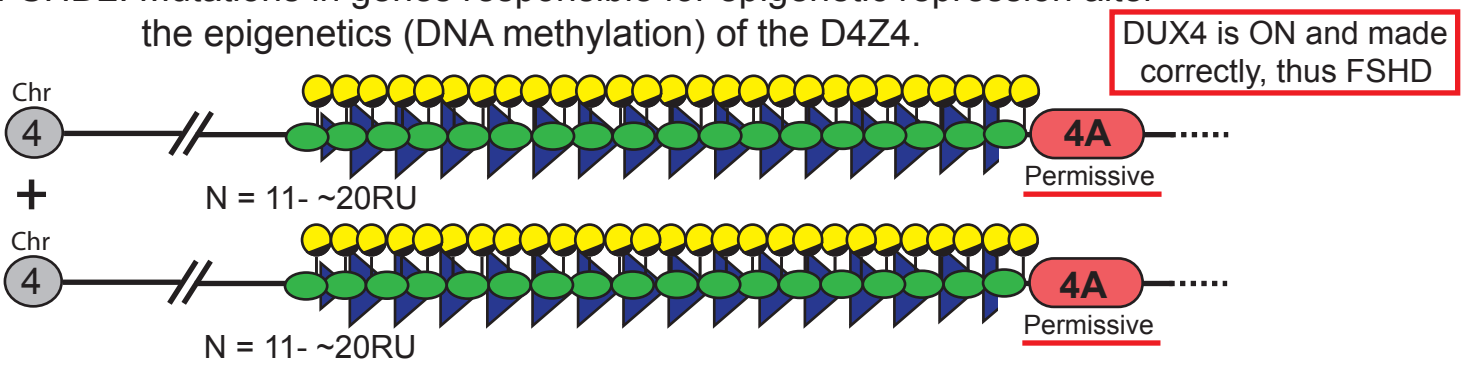


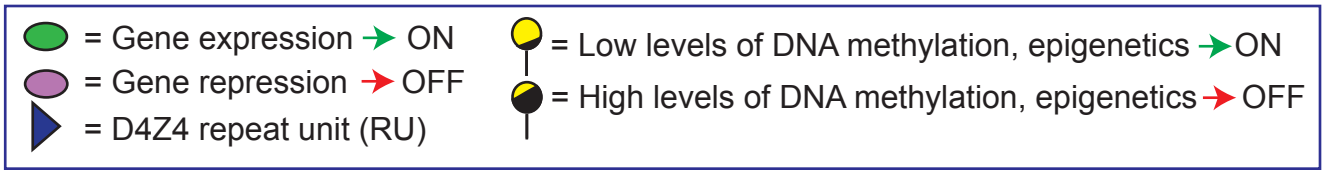
FSHD 4q35 chromosomes

FSHD1: DNA deletions alter the epigenetics (DNA methylation) of the D4Z4.



FSHD2: Mutations in genes responsible for epigenetic repression alter the epigenetics (DNA methylation) of the D4Z4.





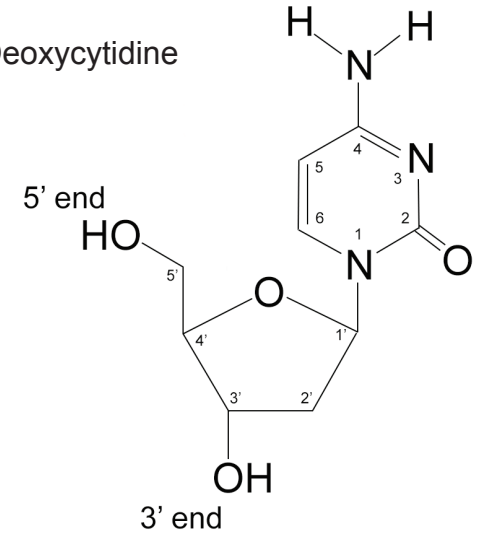
FSHD Epigenetic Diagnostics (Pt 2)

Epigenetic diagnostics are based on the methylation levels of the chromosome 4q and 10q D4Z4 arrays.

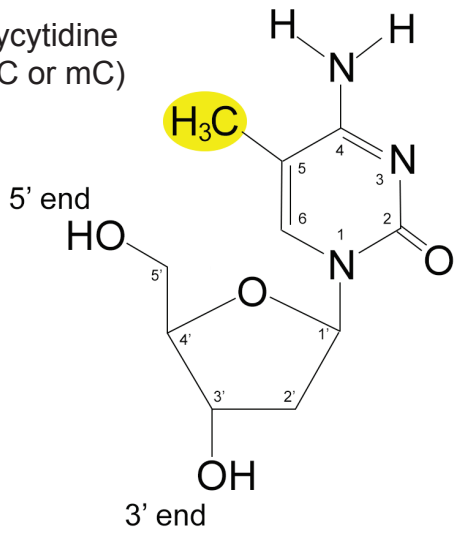
Bisulfite genomic sequencing (BSS) is a technique that identifies the methylation status of each deoxycytidine (C) in the region of interest. But first, we need to understand some basics about DNA methylation.

In mammals (such as people), deoxycytidine (C) is the only DNA base that is methylated (mC) and only when paired with a deoxyguanosine (G). Thus, CG is the only one of the 16 possible DNA base pairs that can be methylated (CG or mCG).

Deoxycytidine



5-methyl-deoxycytidine
(aka 5-methyl-C or mC)

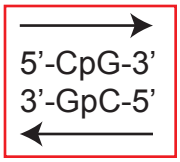


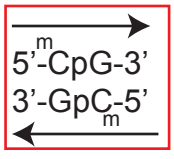
DNA ribonucleotides are connected by a phosphodiester backbone, signified by a "p". DNA also has direction, 5' to 3', based on carbon positions in the nucleotide sugar-ring. Thus DNA convention is CpG dinucleotide oriented 5'-CpG-3'.

The complementary DNA strand runs the other direction.

CpG is the only dinucleotide that is the same in both strands!

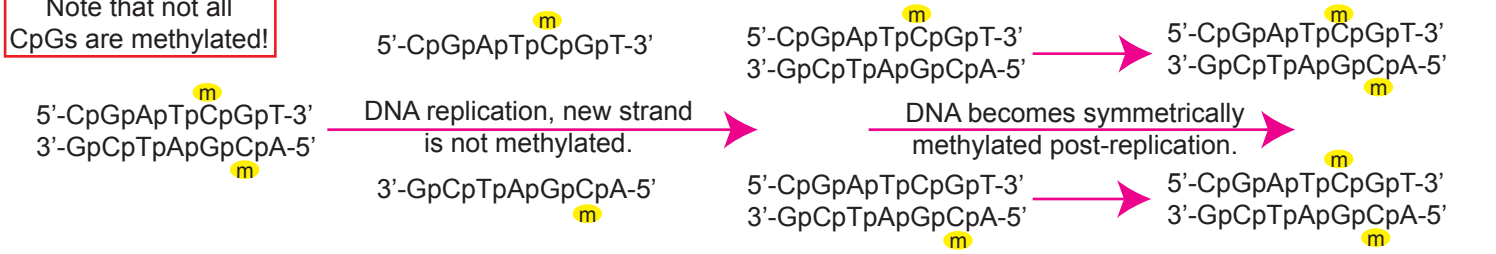
Methylation of C in a CpG dinucleotide is always symmetrical. Thus, the methylation mark is maintained after replication. This is what makes CpG DNA methylation "epigenetic"; its pattern is heritable!





The original methylation pattern is maintained after DNA replication.

Note that not all CpGs are methylated!



FSHD Epigenetic Diagnostics (Pt 3)

Epigenetic diagnostics are based on the methylation levels of the chromosome 4q and 10q D4Z4 arrays.

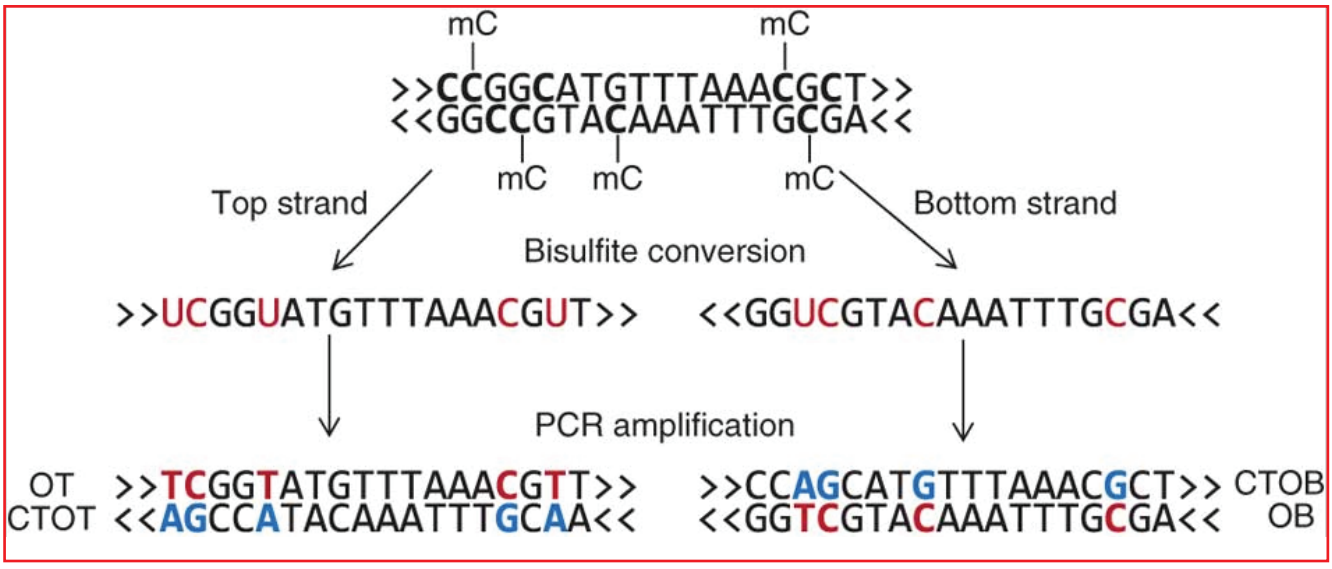
Bisulfite genomic sequencing (BSS) is a technique that identifies the methylation status of each deoxycytidine (C) in the region of interest.

Sodium bisulfite treatment leads to deamination of deoxycytidine (C) to deoxyuracil (U); 5-methyl-deoxycytidine is protected from deamination and remains mC.

Thus, bisulfite treatment causes methylation-specific changes to the DNA sequence.

unmethylated C becomes a T
C ➤ U ➤ T

methylated C remains C
mC ➤ C ➤ C



When one does BSS, you compare the obtained DNA sequence with the predicted DNA sequence. Anywhere you still have a C where there is a C predicted, you know that C was protected from deamination and thus methylated. Anywhere you have a T where there is a C predicted, you know that C was deaminated by the bisulfite and thus not methylated.

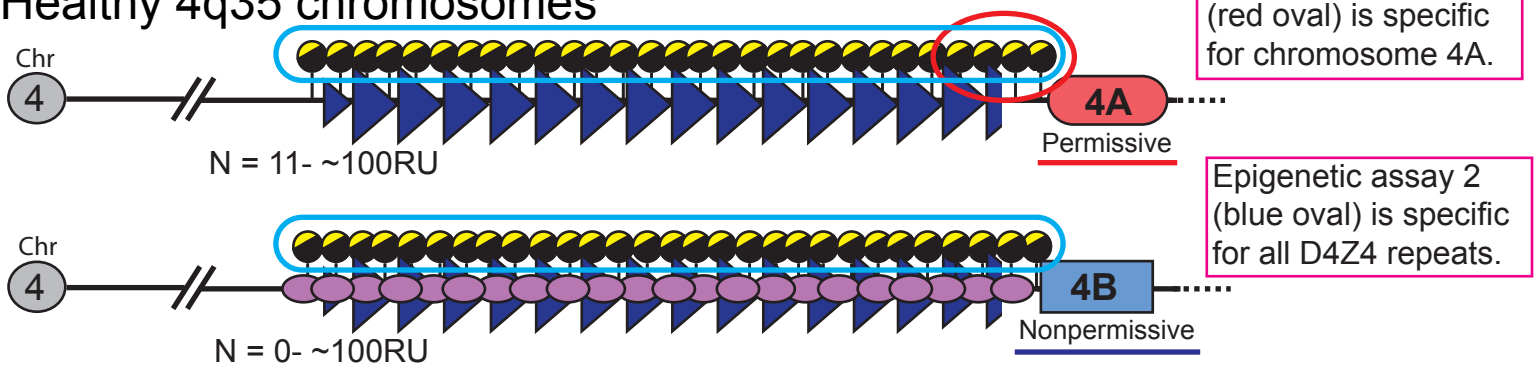
The resulting BSS is a linear representation of the base-by-base methylation status of the region of interest.

BSS is used to determine the methylation status of the FSHD-associated D4Z4 array and the *DUX4* gene. They are methylated in healthy individuals and unmethylated in FSHD.

FSHD Epigenetic Diagnostics (Pt 4)

Epigenetic diagnostics are based on the FSHD-specific methylation levels of the chromosome 4q and 10q D4Z4 arrays.

Healthy 4q35 chromosomes

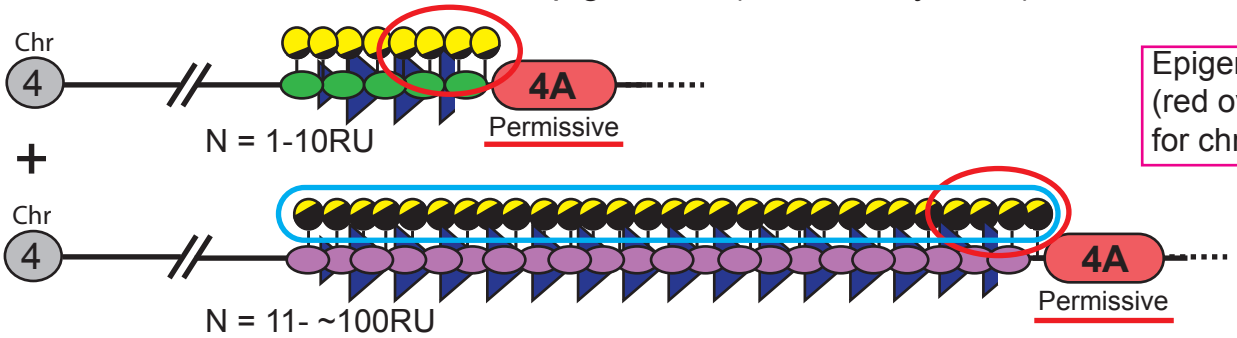


Epigenetic assay 1 (red oval) is specific for chromosome 4A.

Epigenetic assay 2 (blue oval) is specific for all D4Z4 repeats.

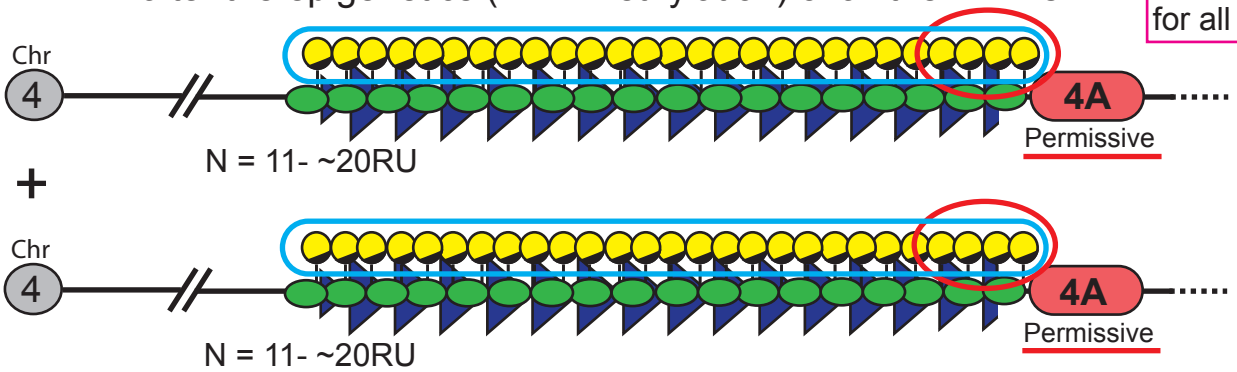
FSHD 4q35 chromosomes

FSHD1: DNA deletions alter the epigenetics (DNA methylation) of the D4Z4.



Epigenetic assay 1 (red oval) is specific for chromosome 4A.

FSHD2: Mutations in genes responsible for epigenetic repression alter the epigenetics (DNA methylation) of all the D4Z4s.



Epigenetic assay 2 (blue oval) is specific for all D4Z4 repeats.

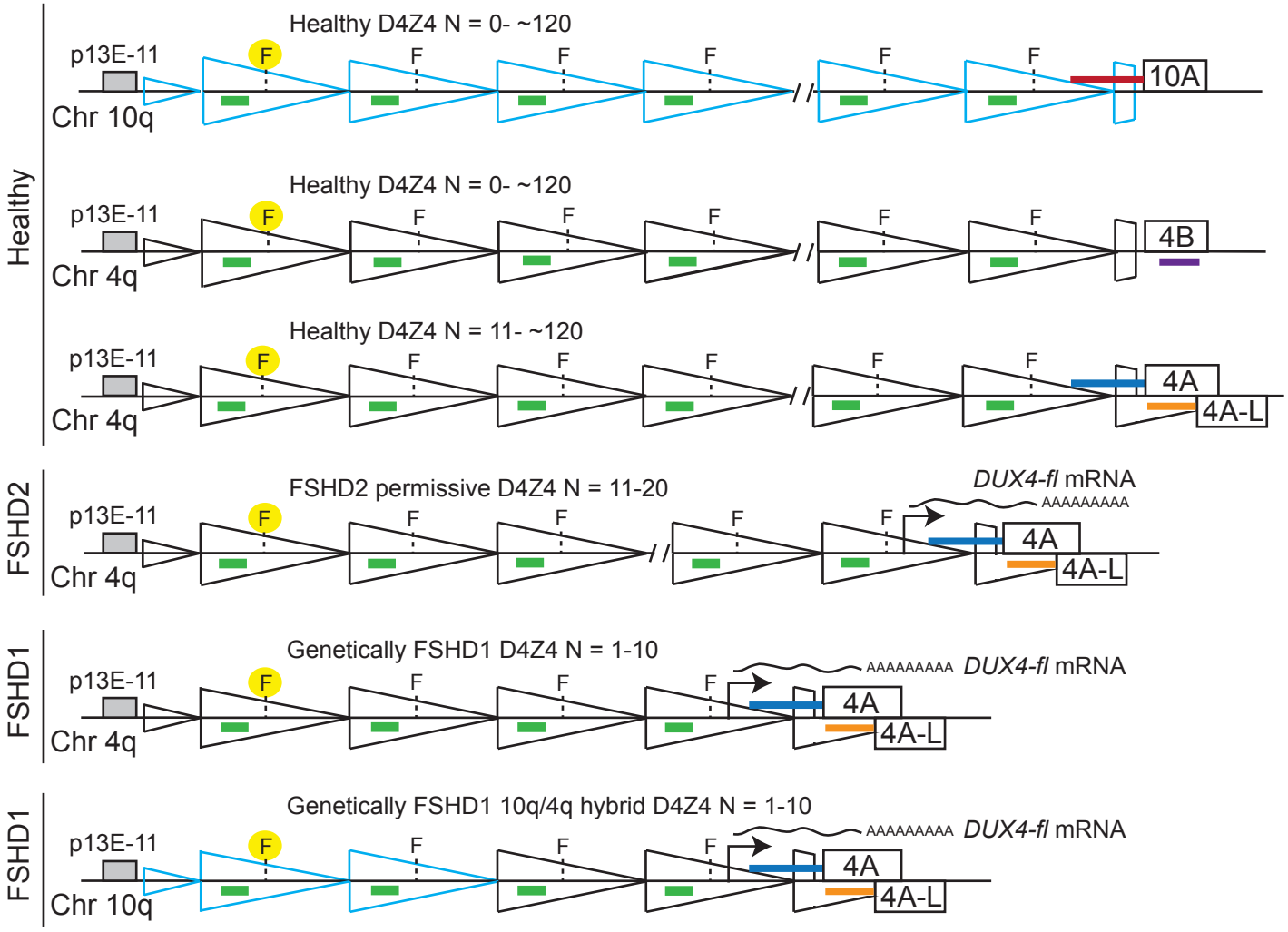
= D4Z4 repeat unit (RU)

= Low levels of DNA methylation, epigenetics → ON

= High levels of DNA methylation, epigenetics → OFF

FSHD Epigenetic Diagnostics (Pt 5)

Targeted bisulfite genomic sequencing (BSS) readily distinguishes healthy, FSHD1, and FSHD2.



- BSSA assay analyzes the distal most D4Z4 RU when it is 4qA.
- BSSL assay analyzes the distal most D4Z4 RU when it is 4qA-L.
- BSSB assay analyzes the distal most D4Z4 RU when it is 4qB.
- BSSX assay analyzes all 4q and 10q D4Z4 RUs regardless of haplotype.
- BSS10A assay analyzes the distal most D4Z4 RU when it is 10qA.
- F — MSRE assay analyzes the 4 proximal most D4Z4 RUs regardless of haplotype.

The first assay (BSSA or BSSL) determines the methylation level of the last 4qA D4Z4, which is methylated (>35%) in healthy and unmethylated (<25%) in FSHD1 and FSHD2.

The second assay (BSSX) determines if all four D4Z4 arrays are unmethylated (FSHD2) or if just one is unmethylated (FSHD1).

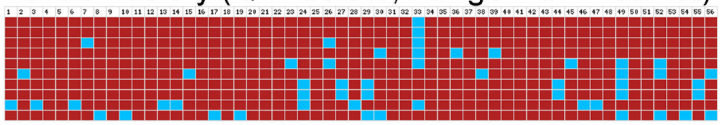
FSHD Epigenetic Diagnostics (Pt 6)

Targeted bisulfite genomic sequencing (BSS) readily distinguishes healthy, FSHD1, and FSHD2.

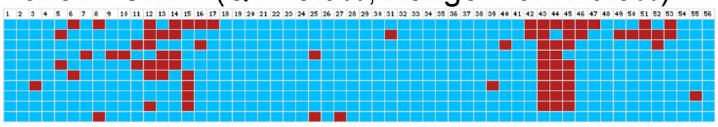
The first assay (BSS Assay 1; red circle below) determines the methylation level of the last 4qA D4Z4, which is methylated (>35%) in healthy and unmethylated (<25%) in FSHD.

= Methylated CpG
= Unmethylated CpG

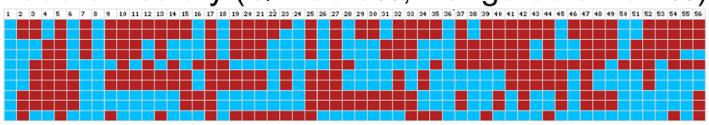
75195 Healthy (Q1= 89.3%; Range = 82.1-98.2%)



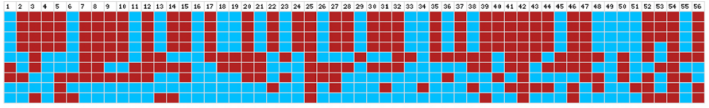
75204 FSHD (Q1= 8.9%; Range = 5.4-26.8%)



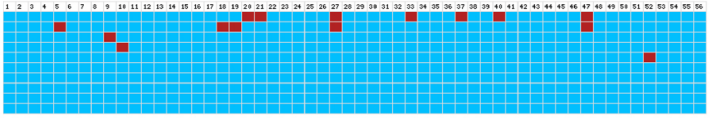
1090-6 Healthy (Q1=53.6%; Range 33.9-73.2%)



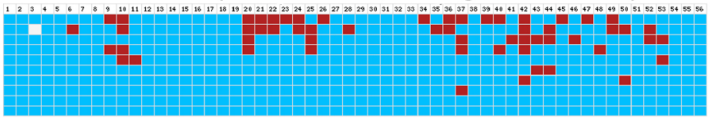
1090-8 Healthy (Q1= 51.6%; Range 23.2-62.5%)



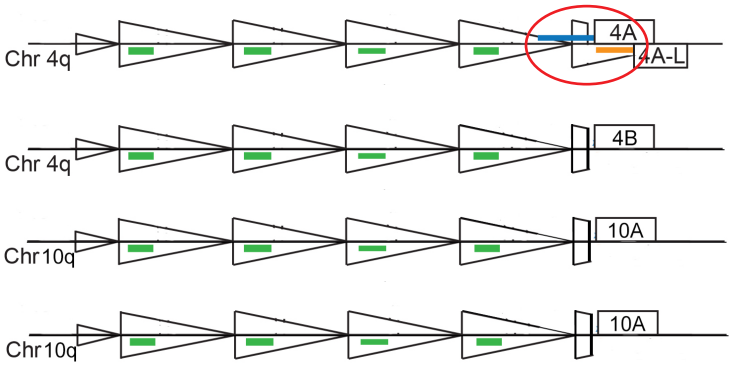
1090-1 FSHD (Q1=2.7%; Range 0-12.5%)



1090-3 FSHD (Q1=10.4%; Range 0-30.4%)



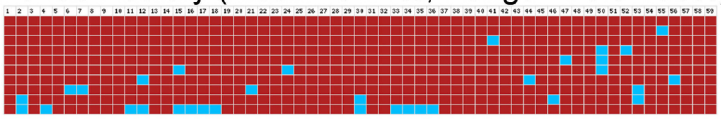
BSS Assay 1



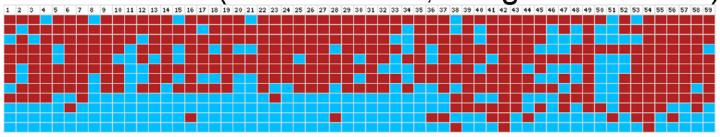
The second assay (BSS Assay 2; blue circles below) determines if all four D4Z4 arrays are unmethylated (FSHD2) or if just one is unmethylated (FSHD1).

= Methylated CpG
= Unmethylated CpG

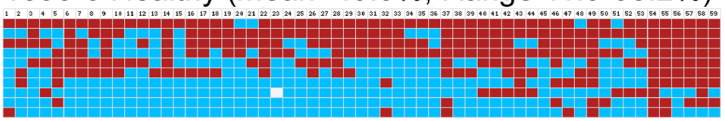
75195 Healthy (Mean= 94.4%; Range= 78.0-100%)



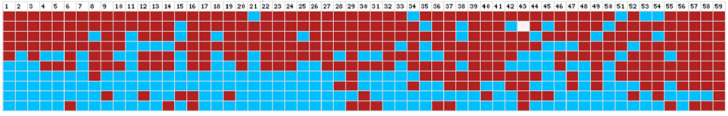
75204 FSHD1 (Mean= 59.3%; Range= 5.1-88.1%)



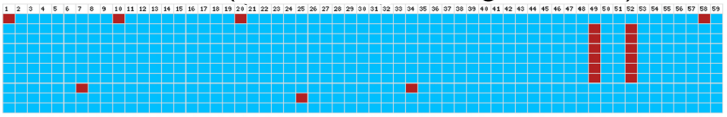
1090-6 Healthy (Mean=49.9%; Range 11.9-93.2%)



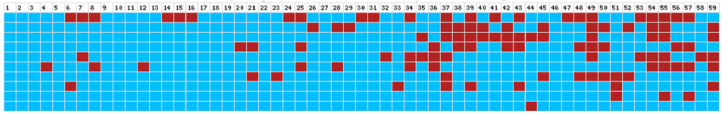
1090-8 Healthy (Mean=59.3%; Range 22.0-91.5%)



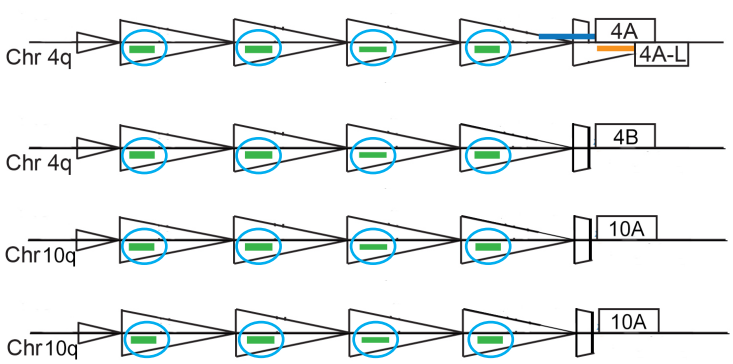
1090-1 FSHD2 (Mean=3.2%; Range 0-6.8%)



1090-3 FSHD2 (Mean=18.5%; Range 1.7-39%)

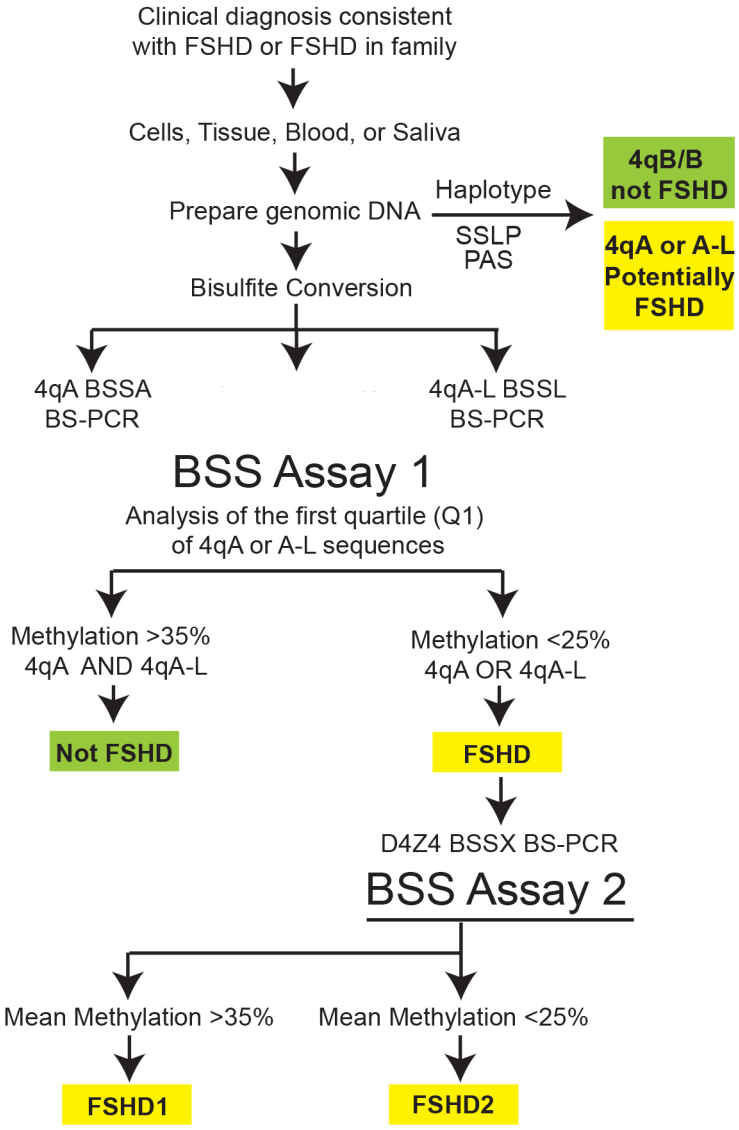


BSS Assay 2



FSHD Epigenetic Diagnostics (Pt 7)

Targeted bisulfite genomic sequencing (BSS) readily distinguishes healthy, FSHD1, and FSHD2.



Ref: T. Jones *et al*. (2014) *Clinical Epigenetics* 6:23