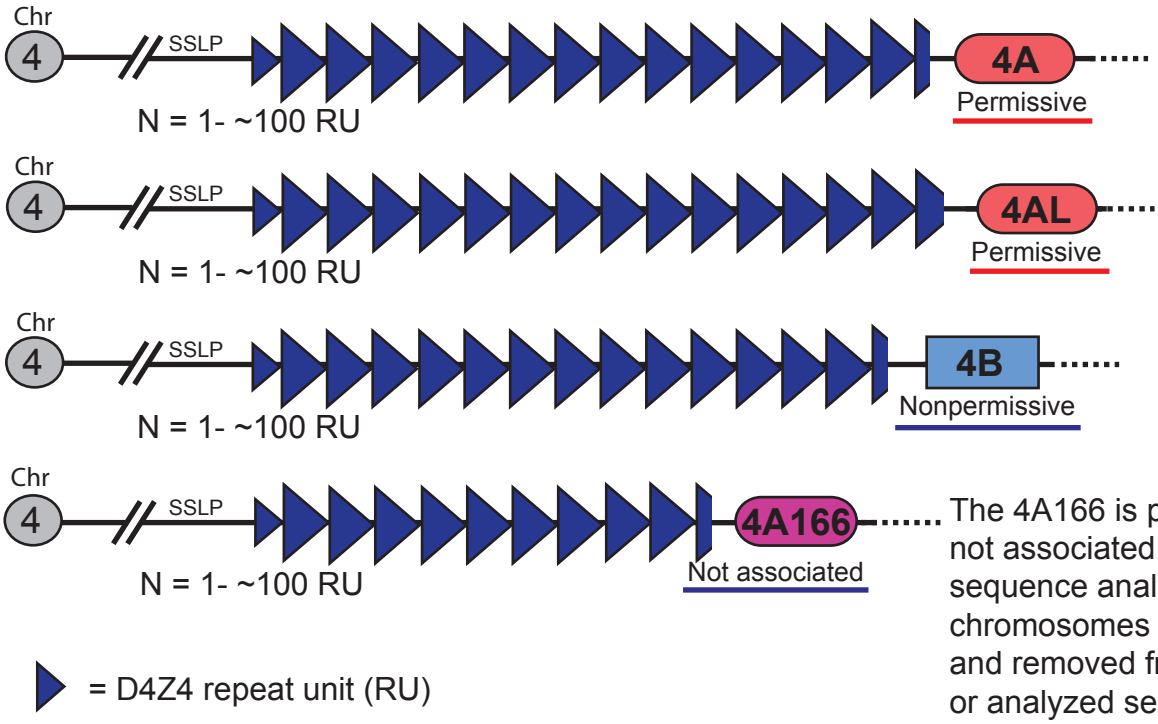
Testing for FSHD using epigenetics

(Step 1, Genetic analysis)

Step 1: This epigenetic testing is based on a special type of DNA sequencing, called bisulfite sequencing (BSS). The BSS analysis is targeted to specific DNA sequences. Thus, one must know what sequences you have in order to do the correct analysis. The FSHD region has three general possibilities, 4A, 4AL, and 4B, and several subtle variations within. The first step is to determine the distal regions as being 4A, 4AL, or 4B.

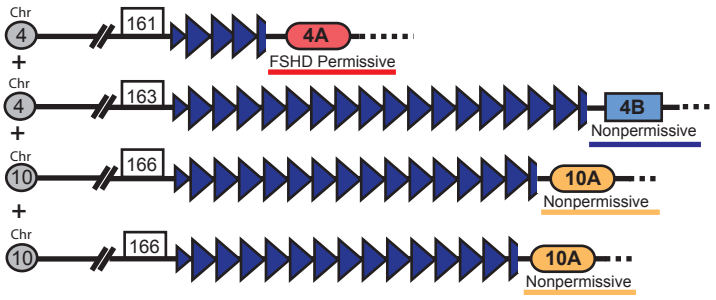
Types of 4q35 chromosomes



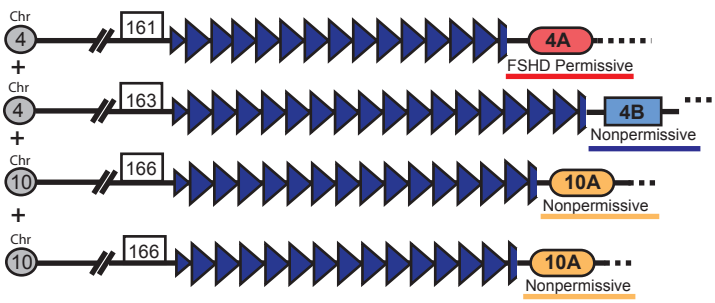
Testing for FSHD: Step 1, genetic analysis to determine the FSHD region haplotype.
→ Do you have an FSHD permissive chr 4A or 4AL? If yes, go to Step 2.

Haplotype = SSLP + subtelomere

Example case with FSHD1: 4A161/4B163



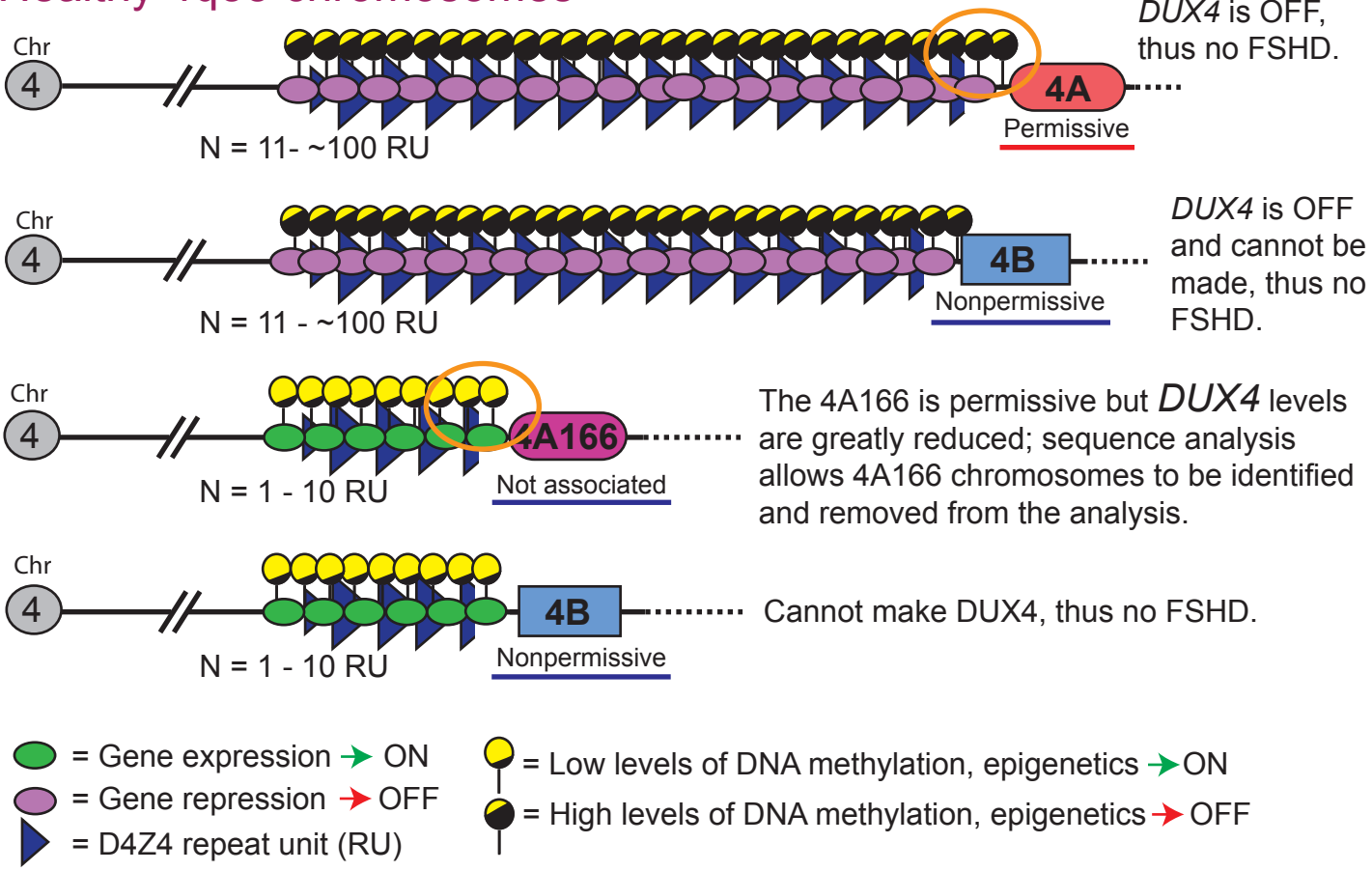
Example case being healthy: 4A161/4B163



Testing for FSHD using epigenetics
(Step 2.1)

Step 2: The epigenetic component of this testing has two parts. The first assay is specific for FSHD permissive chromosomes (4A and 4AL). Nonpermissive chromosome 4Bs are not assayed. Analysis determines if the epigenetic status (DNA methylation) is consistent with FSHD or not consistent with FSHD (healthy or at least not FSHD).

Healthy 4q35 chromosomes



= Gene expression → ON
= Gene repression → OFF
= D4Z4 repeat unit (RU)

= Low levels of DNA methylation, epigenetics → ON
= High levels of DNA methylation, epigenetics → OFF

Testing for FSHD: Step 1, genetics: Do you have a permissive chr 4A? If yes, go to Step 2.

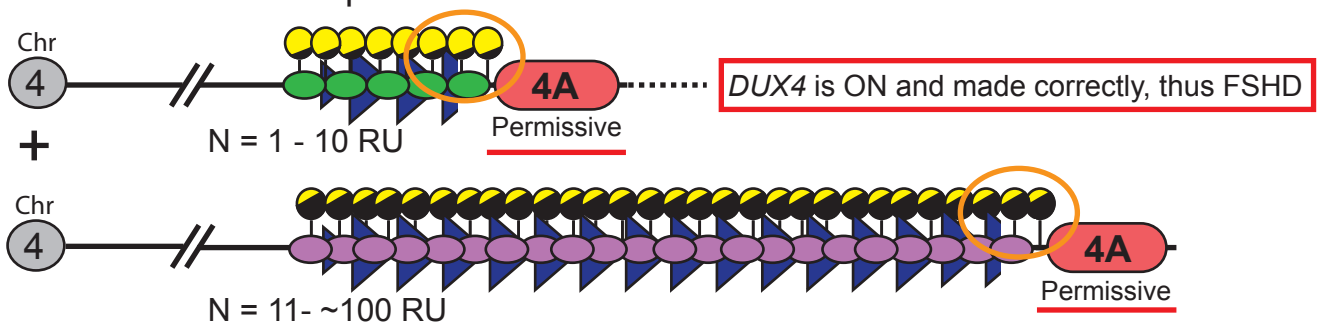
Step 2, do you have FSHD epigenetics? (FSHD BSS Assay 1) → Are you similar to healthy or FSHD?

Testing for FSHD using epigenetics
(Step 2.2)

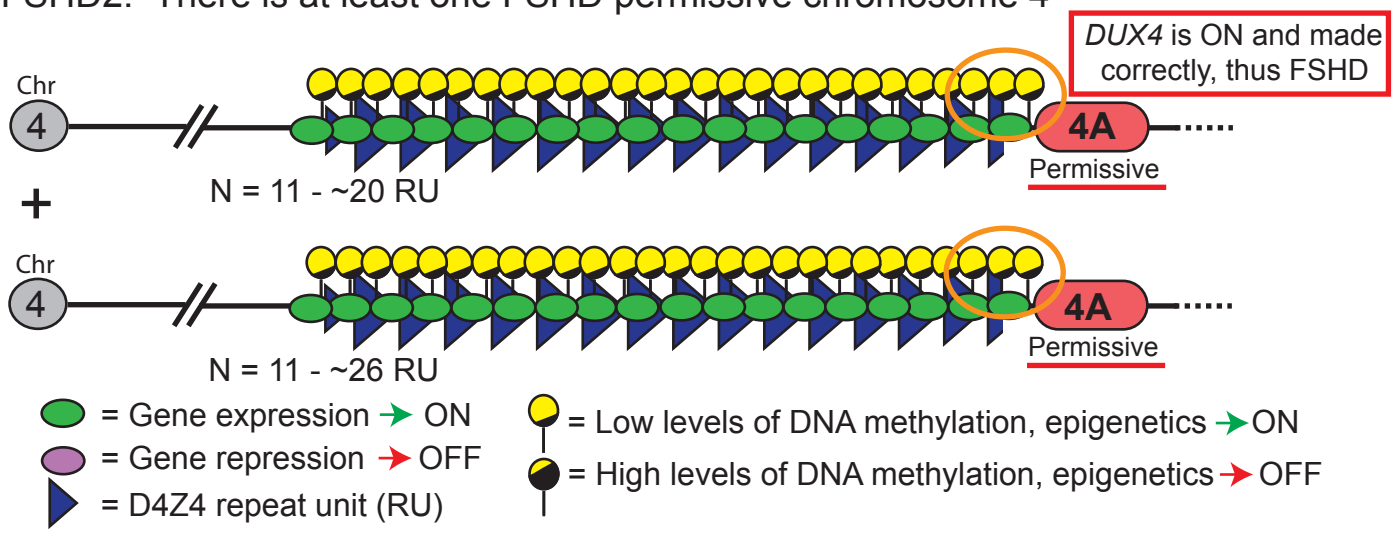
Step 2: The epigenetic component of this testing has two parts. The first assay is specific for FSHD permissive chromosomes (4A and 4AL). Nonpermissive chromosome 4Bs are not assayed. Analysis determines if the epigenetic status (DNA methylation) is consistent with FSHD or not consistent with FSHD.

Examples for your pair of chromosome 4s for FSHD types 1 and 2.

FSHD1: One FSHD permissive chromosome 4 is contracted to 1-10 D4Z4 RUs



FSHD2: There is at least one FSHD permissive chromosome 4



Testing for FSHD: Step 1, genetics: Do you have a permissive chr 4A? If yes, go to Step 2.

Step 2, do you have FSHD epigenetics? (FSHD BSS Assay 1) → Are you similar to healthy or FSHD?

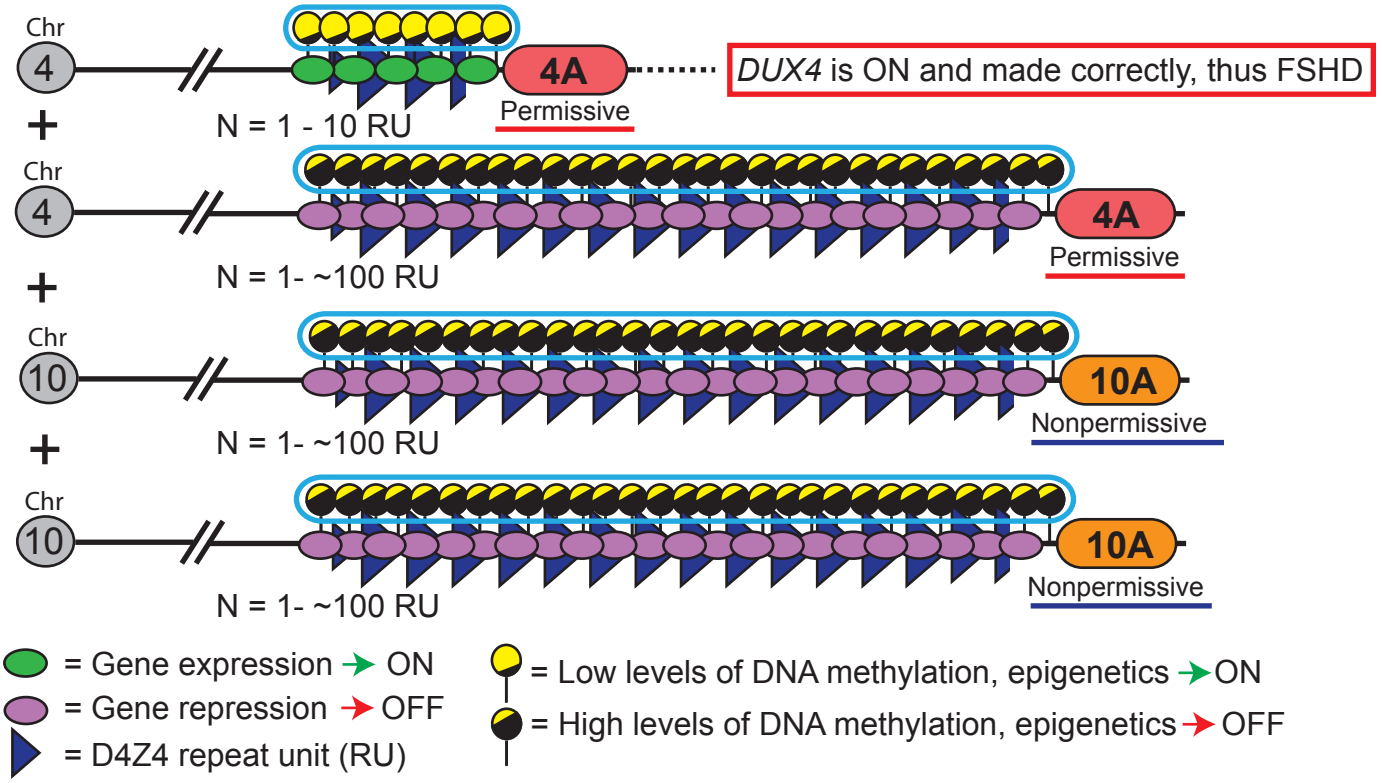
Testing for FSHD using epigenetics
(Step 3.1)

Step 3: The second epigenetic assay determines if the epigenetic status (DNA methylation) is consistent with FSHD1 or FSHD2. This also confirms the results of the first assay, if healthy. In this assay, the DNA methylation status of all D4Z4 repeats on both of your chromosome 4s and 10s are assayed. In FSHD1, only a small fraction of your total D4Z4 repeats are unmethylated; in FSHD2, most of your D4Z4 repeats are unmethylated.

Chromosomes 4q35 and 10q26 have D4Z4 repeat arrays.

FSHD1: DNA deletions within the chromosome 4 D4Z4 alter the epigenetics (DNA methylation) of the contracted D4Z4 while the noncontracted chromosome 4 D4Z4 array and both chromosome 10 D4Z4 arrays remain normally methylated.

Example FSHD1 case:



Testing for FSHD: Step 1, genetics: Do you have a permissive chr 4A? If yes, go to Step 2.
Step 2, do you have FSHD epigenetics? If yes, go to Step 3.

Step 3, do you have FSHD1 or FSHD2 epigenetics?

FSHD BSS Assay 2: If FSHD, is it FSHD1 or 2?

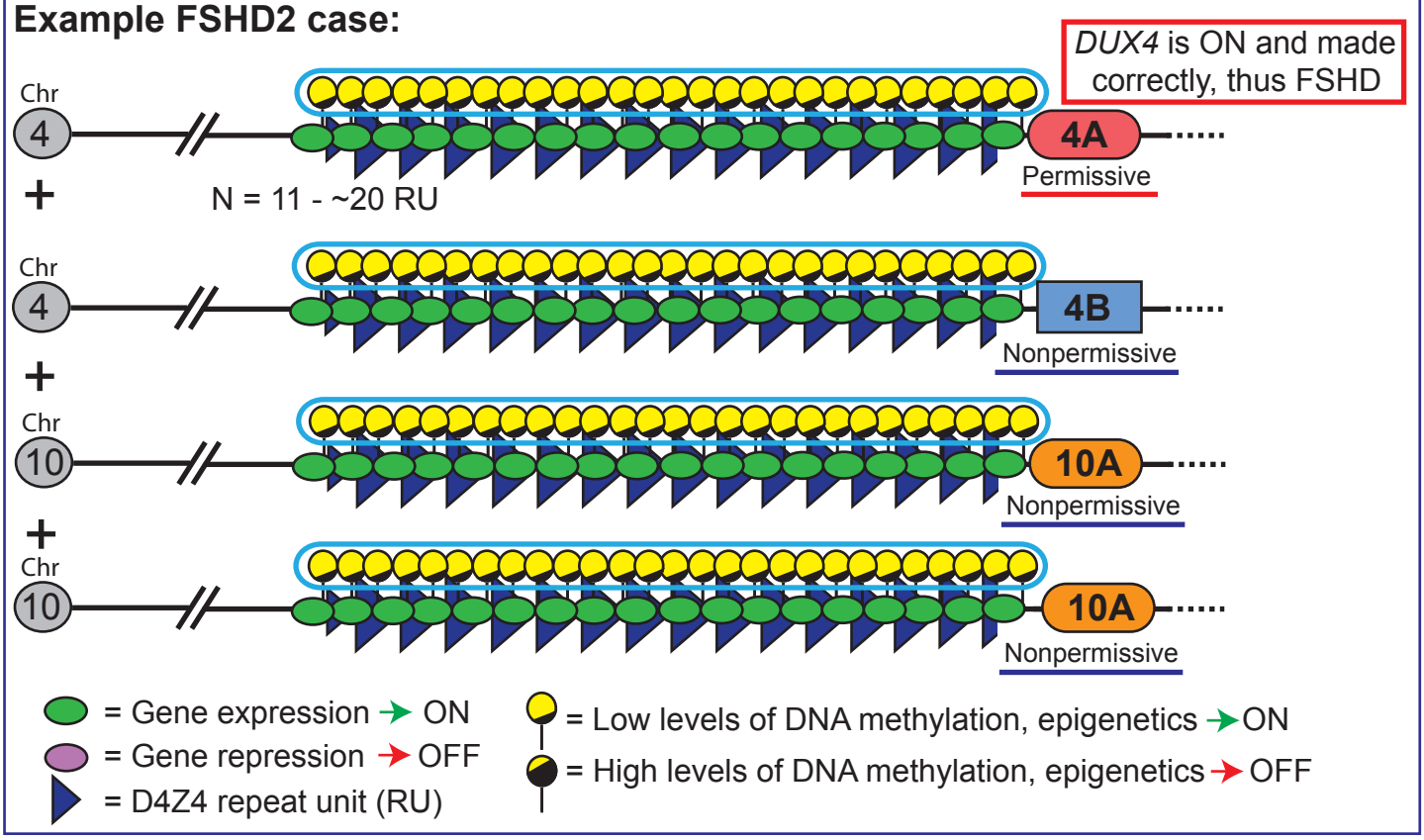
Testing for FSHD using epigenetics
(Step 3.2)

Step 3: The second epigenetic assay determines if the epigenetic status (DNA methylation) is consistent with FSHD1 or FSHD2. This also confirms the results of the first assay, if healthy. In this assay, the DNA methylation status of all D4Z4 repeats on both of your chromosome 4s and 10s are assayed. In FSHD1, only a small fraction of your D4Z4 repeats are unmethylated; in FSHD2, most of your D4Z4 repeats are unmethylated.

Chromosomes 4q35 and 10q26 have D4Z4 repeat arrays

FSHD2: Mutations in genes responsible for epigenetic repression at the D4Z4 alter the epigenetics (DNA methylation) of the D4Z4s on chromosomes 4 and 10.

Example FSHD2 case:



Testing for FSHD: Step 1, genetics: Do you have a permissive chr 4A? If yes, go to Step 2.
Step 2, do you have FSHD epigenetics? If yes, go to Step 3.

Step 3, do you have FSHD1 or FSHD2 epigenetics?

FSHD BSS Assay 2: If FSHD, is it FSHD1 or 2?