FSHD1 Diagnostics: PFGE and Southern blotting (Pt 1)

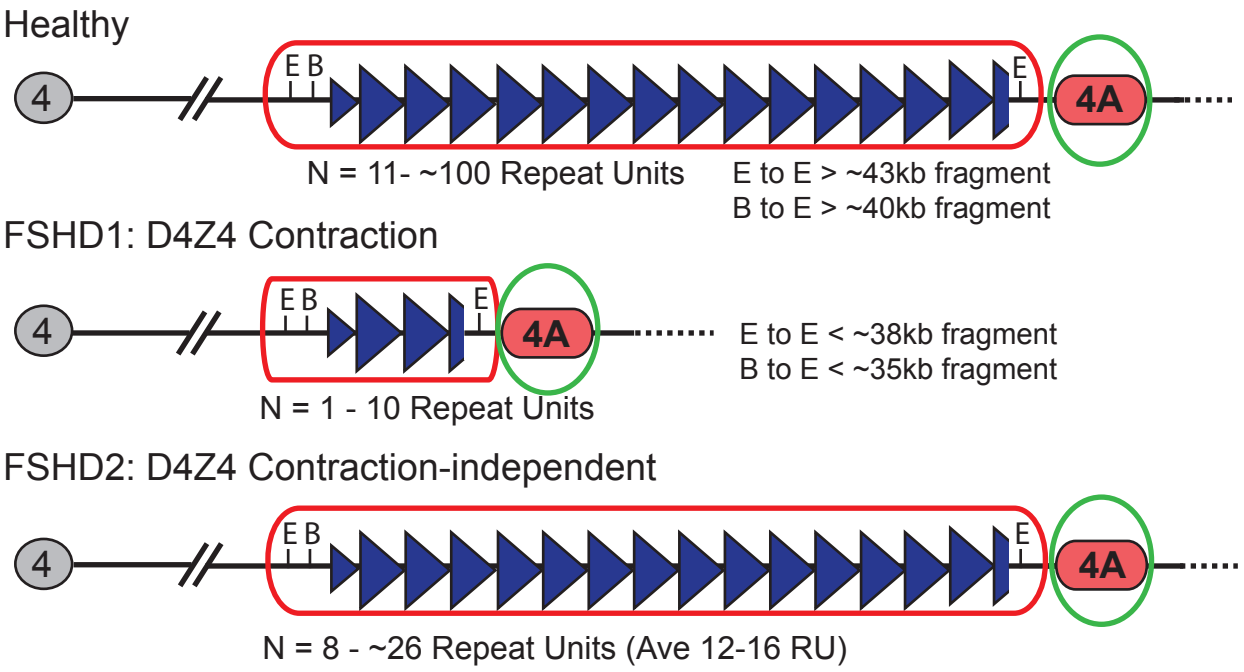
PFGE and Southern blotting D4Z4 deletion testing.

The original genetic testing for FSHD was based on detecting a deletion within the chromosome 4q35 D4Z4 repeat array using a combination of restriction enzymes (enzymes that cut DNA at specific DNA sequences), pulsed-field gel electrophoresis (PFGE -- a technique for separating very large DNA fragments), and Southern blotting (a technique for identifying DNA sequences using labeled DNA probes).

This measures large pieces of DNA, thus genomic DNA must be carefully isolated to prevent fragmentation and requires DNA from blood or cells. This cannot be performed on saliva DNA, which is too fragmented.

Lemmers *et al*. (2004) Facioscapulohumeral muscular dystrophy. Clinical medicine and molecular cell biology. M Upadhyaya and DN Cooper editors. Oxn, UK: Garland Science/BIOS Scientific Publishers p. 211-34.

Lemmers *et al*. (2012) Best practice guidelines on genetic diagnostics of Facioscapulohumeral muscular dystrophy: workshop 9th Jun 2010, LUMC, Leiden, The Netherlands. *Neuromuscular Disorders* 22:463-70.

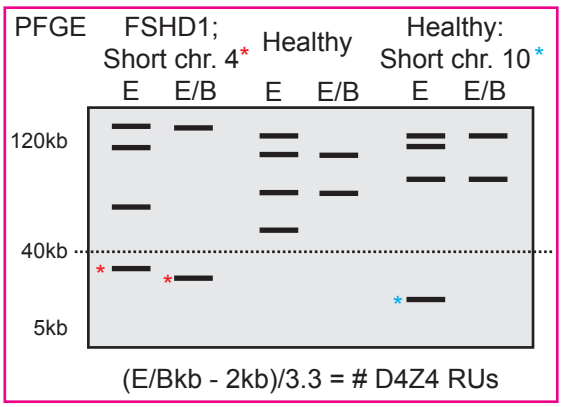


Determining the size of your chromosome 4 D4Z4 arrays:
Collect ~15ml blood sample, isolate cells and HMW DNA.

Embed DNA in special agarose and soak with specific restriction enzymes (EcoRI, and EcoRI + BlnI).

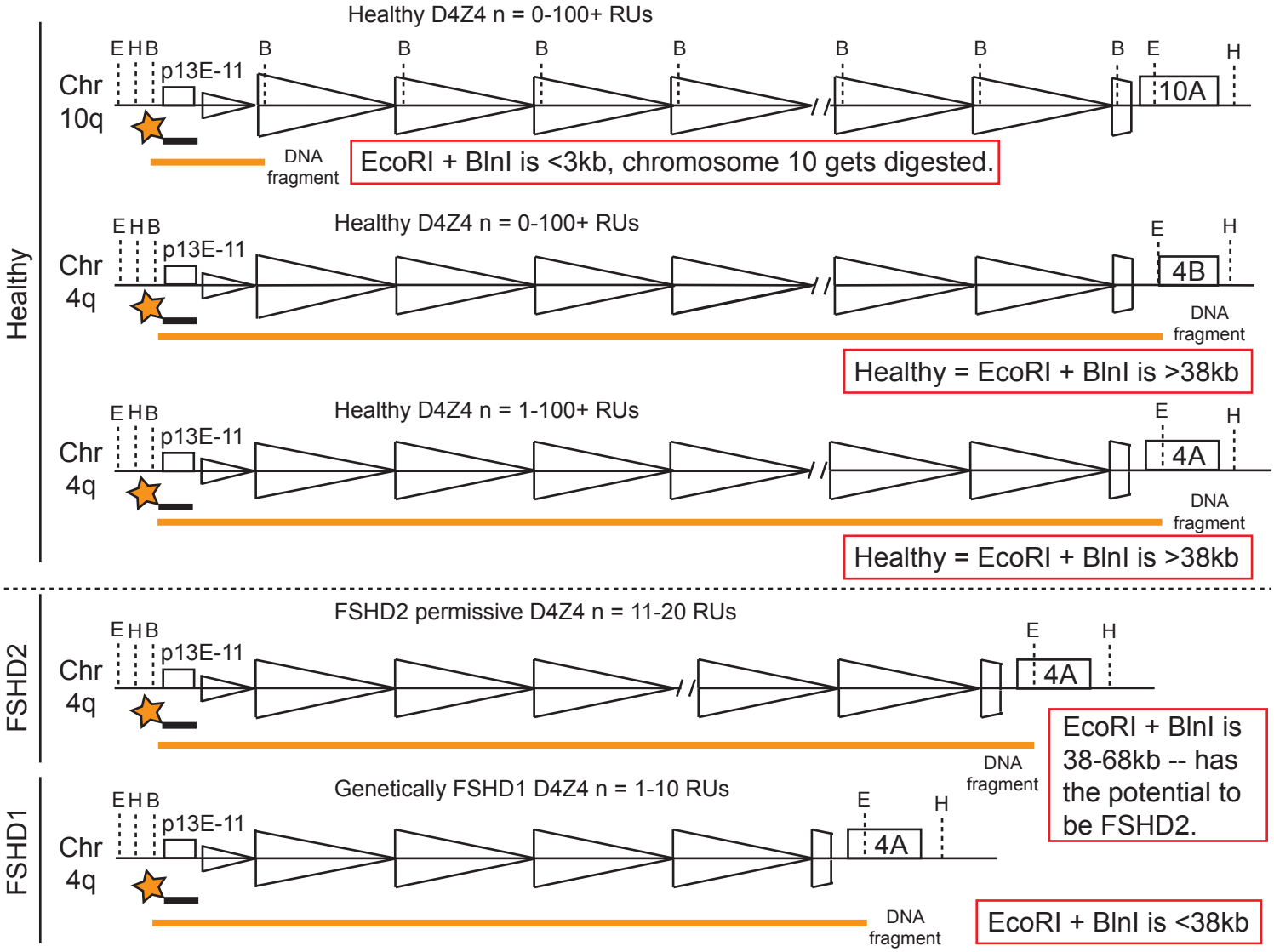
Run PFGE and Southern blot with probe p13E-11.

Identify the chromosome 4 band sizes and determine if one is short (<40kbp or <40,000 bp).



FSHD1 Diagnostics: PFGE and Southern blotting (Pt 2)

PFGE and Southern blotting FSHD diagnostics, Step 1: Determining the sizes of the chromosome 4 D4Z4 arrays.



FSHD1 deletion analysis by PFGE and Southern blotting

p13E-11 is the DNA sequence used to probe Southern blots specifically for the 4q and 10q fragments.

The radioactive p13E-11 DNA sequence probe for Southern blots to identify the 4q and 10q fragments.

The EcoRI/BlnI cut DNA fragment specific for the chromosome 4q35 D4Z4 array (probes with p13E-11).

E = EcoRI restriction enzyme cut sites. These fragments are for sizing the chr 4 and chr 10 D4Z4 arrays.

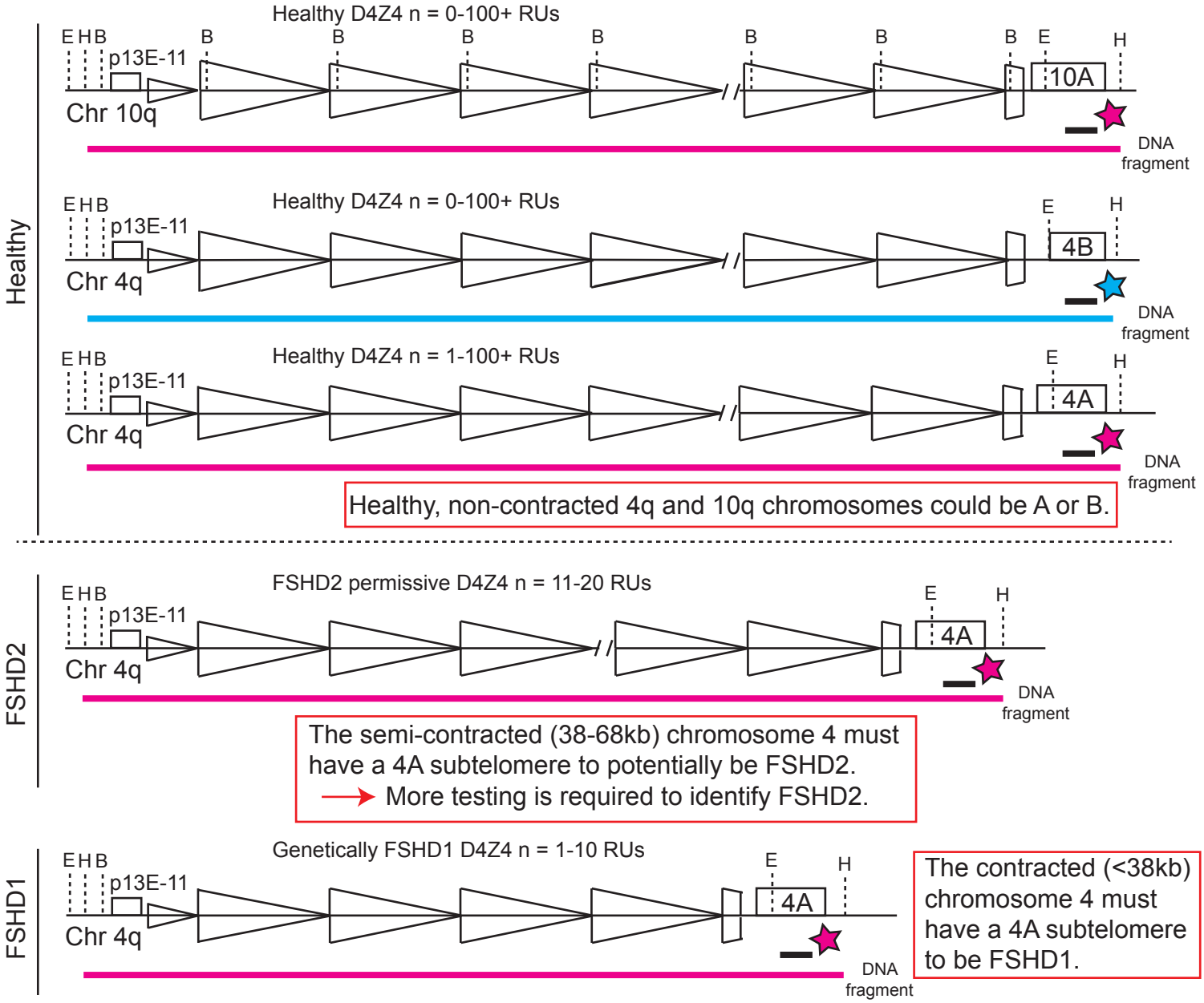
B = BlnI restriction enzyme cut sites. Cutting with BlnI eliminates chr 10; chr 4 is resistant to cutting.

In the USA, available at several facilities including:
University of Iowa Diagnostics Laboratory
Athena Diagnostics
Quest Diagnostics

In Europe, available at:
University of Leiden, The Netherlands

FSHD1 Diagnostics: PFGE and Southern blotting (Pt 3)

PFGE and Southern blotting FSHD diagnostics, Step 2: Determining the subtelomere (A or B) for each fragment.



FSHD subtelomere analysis by PFGE and Southern blotting

- The HindIII cut DNA fragment specific for the A subtelomere (probed with A fragment).
- The radioactive DNA probe specific for the 4A and 10A subtelomere.
- The HindIII cut DNA fragment specific for the B subtelomere (probed with B fragment).
- The radioactive DNA probe specific for the 4B subtelomere.

H = HindIII restriction enzyme cut sites. These fragments are for A or B testing.