Introduction
The hexanucleotide repeat expansion (RE) in C9orf72 is the most common genetic biomarker of familial ALS-FTLD. In unaffected populations, the repeat region is short (2-24 repeats of the G4C2 hexanucleotide repeat unit) and can be identified with routine DNA sequencing. In contrast, ALS-FTLD patients have REs with hundreds to thousands of repeats. In ALS, longer REs can correlate with the age of onset, the severity of clinical symptoms, or the mechanism of disease. The heterogeneity of clinical phenotypes in ALS also suggests the possibility of disparate responses to therapeutics, so accurate methods for RE characterization could have great benefit for ALS research, diagnostics, and therapy management.

We present a simple RE typing procedure that uses the SageELF electrophoretic platform to size-fractionate and collect DNA. Briefly, genomic DNA is digested with restriction enzymes that cleave in single copy regions flanking the repeat. The digest is separated into 12 consecutive size fractions on an automated preparative electrophoresis system. The size fractions containing the RE region are identified by qPCR, using a single-copy amplification target located adjacent to the repeat. The length of the repeat expansion can be determined directly from the size fraction in which it is located. All assay steps (digestion, fractionation, and qPCR) can be carried out in a day.
**SageELF RE Screen Workflow**

The schematic below shows an overview of the workflow used. The entire process can be completed in approx. 8 hours. DNA size fraction collection with the SageELF requires 2-3 hours for these size ranges, but may require longer for larger RE screens.

- **Blood or tissue sample**
  - Isolate HMW DNA
  - Restriction digest – cut single-copy sequences on both sides of repeat region.
    - 1.5 hrs
  - Electrophoretic size selection (separate unexpanded and expanded repeat fragments), isolate of DNA from 12 sequential sections of gel lane.
    - 2.3 hrs
  - qPCR using single copy target on fragment containing repeat region.
    - qPCR target lies on one side of repeat – not across repeat
    - 2.5 hrs

Determine presence/size of repeat expansion from position of qPCR signal in elution fractions.

(Confirm positive tests by repeat-primed PCR or sequencing.)

**SageELF Electrophoresis Conditions**

The wt C9 XbaI fragment is 2.4kb in size, when 2-3 G4C2 repeats are present. Southern blot analyses suggest that most ALS patient have expansions ranging in hundreds to thousands of repeats. XbaI fragments carrying such expansions would be found in elution modules 2-4.

**Results**

Results indicate the presence of target in collected size fractions of the XbaI digest. These are indicative of the C9orf72 ALS RE in the expected elution modules. The positive indications were confirmed by RP-PCR.

**Genomic Map of C9orf72 Repeat Region**

XbaI was used to excise the first exon region of C9orf72. In the hg38 reference, this XbaI fragment is 2377 bp, and there are two G2C4 repeats. The 105 bp qPCR amplicon used for detection of the C9 XbaI fragment is located ~1kb to the right of the G2C4 repeats.

**Discussion**

Our assay combines the benefits of Southern blotting for RE sizing, with the sensitivity of PCR, without the need to amplify through the repetitive 100% GC-rich repeat region. Since the electrophoretic resolution can be tailored to different size ranges by changing gel concentration, voltage, and run time, our assay may also be useful for characterizing repeat lengths in other RE diseases. This could include diseases with smaller repeat expansions such as Huntington’s Disease, Fragile X syndrome, Friedrich’s ataxia, etc.