A Simple Screening Assay for C9orf72 ALS Repeat Expansions

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Abstract

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 sequencing. In contrast, ALS-FTLD patients have RE’s with 100’s to 1000’s 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 a novel electrophoretic method. Briefly, genomic DNA is digested with restriction enzymes or customized Cas9 nucleases 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.

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’s 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.

Conventional methods for C9orf72 repeat expansion

These existing methods are labor-intensive and lengthy (in the case of Southern blotting), or require expensive specialized equipment (in the case of RP-PCR).

A new screening assay based on SageELF preparative platform

The SageELF system performs preparative DNA size selection and elution in an automated fashion. For a given set of electrophoresis settings, the eluted fragment size can be determined from elution module position. An example of SageELF DNA fractionation is shown below.

Overview of SageELF workflow for RE screening

<table>
<thead>
<tr>
<th>Blood or tissue sample</th>
<th>Isolate HMW DNA</th>
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</thead>
<tbody>
<tr>
<td>Restriction digest – cut single-copy sequences on both sides of repeat region.</td>
<td>1.5 hrs</td>
</tr>
<tr>
<td>Electrophoretic size-selection (separate expanded and expanded repeat fragments).</td>
<td>2-3 hrs</td>
</tr>
<tr>
<td>Isolate of DNA from 12 sequential sections of gel lane.</td>
<td></td>
</tr>
<tr>
<td>qPCR using single copy target on fragment containing repeat region (qPCR target lies on one side of repeat – not across repeat).</td>
<td></td>
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<tr>
<td>Determine presence/size of repeat expansion from position of qPCR signal in elution fractions.</td>
<td></td>
</tr>
</tbody>
</table>

Confirmation of expansion status by repeat-primed PCR

By changing gel concentrations and other electrophoresis conditions, the SageELF can also resolve smaller DNA fragments, and therefore could be used for other diseases such as Huntington’s Disease, Fragile X syndrome, Friedrich’s ataxia, etc., which have smaller repeat expansions. An example of an ELF separation on sheared genomic DNA using a 2% agarose gel is shown below.

Applications to other repeat expansion diseases

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