Separation of up to 1000 bases on a modified A.L.F. DNA sequencer

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A modified version of the standard A.L.F DNA sequencer (Pharmacia, Uppsala) is described that allows the separation of up to 1000 bases per clone. Fragments were resolved at single peak resolution up to 700 bases. The raw data accuracy was >99.7%, resulting in an overall error rate of <0.01% for the final sequence after sequencing both DNA strands. The modifications include 60 cm thermostatic and notched glass plates of high planarity (separation distance 50 cm), a new hood that...
Sequencing reactions with either fluorescent labelled primers or fluorescein dATP as internal label were performed with the Pharmacia Autoread Sequencing Kit (Pharmacia, Uppsala) (1). A modified SequiTherm (Epicentre Technologies, Madison, WI) protocol was used for cycle sequencing reactions (2). Methods and techniques for the preparation, loading and handling of 250 to 350 μm thin thermostatically controlled slab gels for double stranded DNA sequencing were carried out as described previously (3). Plasmid DNA was isolated by alkaline lysis and purified on ion exchange columns (Qiagen, Diagen, Darmstadt; Nucleobond, Macherey-Nagel, Düren).

A Hydrolink gel matrix (AT Biochem, Malvern, PA) was used for the fast separation of DNA fragments with high resolution. The sequencing speed was increased by about 20% compared to a polyacrylamide gel matrix (3.3 % cross-linker) of the same gel concentration, allowing sequencing speeds of up to 90 bases per hour and clone (Table 1). Figures 1 and 2 show typical results obtained with the modified A.L.F. DNA sequencer. Both labelled primers and the fluorescein dATP as internal label resulted in similar sequencing performance. Table 1 summarises the results obtained with various Hydrolink gel concentrations:

<table>
<thead>
<tr>
<th>Hydrolink concentration</th>
<th>sequencing speed (%)</th>
<th>single base resolution (bases/h/clone)</th>
<th>reading length (bases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>80–90</td>
<td>500–600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>5</td>
<td>60–70</td>
<td>550–650</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>6</td>
<td>50–60</td>
<td>600–700</td>
<td>950</td>
</tr>
<tr>
<td>7</td>
<td>40–45</td>
<td>650–700</td>
<td>800</td>
</tr>
</tbody>
</table>

Table 1. Sequencing performance of the modified A.L.F. DNA sequencer at various Hydrolink gel concentrations.

About 10 experiments were performed for 4% and 7% Hydrolink gels, more than 70 experiments for gel concentrations in the range of 5% to 6%. Various double stranded DNA samples were used for these experiments, including S. cerevisiae Chr. XI and Chr XV DNA and Human Chr. 21 DNA. Field strength = 35 V/cm; Temperature = 50°C. Buffer: 89 mM Tris, 89 mM Boric Acid, 2 mM EDTA, pH 8.6.
length between 700 and 1000 bases with a single base resolution of up to 700 bases in unprocessed raw data were observed routinely. Hydrolink gel concentrations between 5% and 6% turned out to be the best compromise between sequencing speed, reading length and resolution. Parts of the experiments were performed in the course of the European Community (E.C.) *Saccharomyces cerevisiae* sequencing project at the EMBL (4). Readings between 500 and 1000 bases, with an average of about 750 bases, were observed with T7 DNA polymerase and the SequiTherm DNA sequencing kit using labelled primers on standard plasmid DNA. The same results were obtained for sequencing with unlabelled primers and fluorescein dATP with T7 DNA polymerase.

The increased reading length allowed a very efficient primer walking strategy. The number of sequencing reactions, walking primers and gels was reduced by approximately 40% compared to the standard sequencing device with 30 cm glass plates. The low error rate of the device was proven in a sequence verification project of the E.C. (4): No error was reported within 13 kb of *S.cerevisiae* sequence. In addition, the extended reading length makes it possible to determine correctly long repetitive DNA sequences of up to 700–800 bases. Repetitive sequences are present in many organisms and pose a great difficulty for any sequencing strategy. As an example, Figure 2 shows the determination of 700 bases of a repeat.

In summary, the modified device described in this report increases the reading length, accuracy and overall efficiency of sequencing projects and simplifies the analysis of long repeated sequences.

REFERENCES