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TECHNICAL NOTE

DNA Sequencing of GC rich Templates

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Abstract

Most samples sent by customers to Geneservice Ltd are straightforward to sequence. However, in a small percentage of cases high GC rich areas within the sample cause the sequencing reaction to be truncated. This is due to the presence of a secondary structure, which inhibits the passage of the DNA polymerase along the remainder of the length of the DNA sample. It was found in experiments using an in-house cocktail of Betaine, dGTP and DMSO in the sequencing reaction, that 83% of sequence reactions showed a significant improvement in read quality, all of which sequenced through the problem area.

Introduction

Geneservice Ltd is one of the UK's leading service providers for DNA sequencing, with over 30 Institutional customers throughout UK and Europe. We use the most up-to date DNA Analyzer, the ABI 3730*xl*, along with Data Collection 2.0 (ABI) and Sequencing Analysis 5.1 (ABI) software to obtain clear, well resolved sequences of up to 900bp after quality clipping.

DNA samples received by the service are sequenced using Big Dye v3.1 (ABI) and can be processed in less than 2 days. During the course of our service we discovered that Big Dye reagent alone, was unable to sequence through highly GC rich areas. We decided to investigate the effect of using increased Betaine, dGTP and DMSO levels in the sequencing reaction. The method was tested on highly GC rich sequences, that had bonded together, forming a hairpin loop secondary structure, and also on other problematic DNA which contained poly-A and dinucleotide repeats.

Materials and Methods

Samples DNA samples were obtained from Geneservice Ltd customers that had previously been found to produce truncated sequencing results, potentially due to the presence of a secondary structure, or where a poly-A region or dinucleotide repeat was visibly causing the sequencing to fail..

Secondary Structure Solution Reagent (SSS) Our reagent is an optimised and proprietary mix of Betaine (Sigma), Sequencing Buffer (ABI), Big Dye v3.1 (ABI), dGTP Big Dye (ABI) and DMSO (Sigma). This was added to 5µl of prepared plasmid DNA and suitable volume of the appropriate primer. This was then sequenced using MJ thermal cyclers (GRI Ltd) using standard conditions.

Clean Up and Analysis In house clean up of the DNA samples was then performed using standard ethanol precipitation. DNA analysis was carried out using the ABI 3730*xl* DNA Analyser (ABI) and Sequencing Analysis 5.1 (ABI) software was used to interpret the results.

Results and Discussion

If a DNA sample provides a sharply truncated sequence result (figures 2a and b) then we assume the presence of a problem area of DNA. Secondary structures occur when there are a high percentage of the nucleotide bases G and C in a small area of DNA. Each GC base pair forms three hydrogen bonds and so is physically more stable than an AT base pair, as a result hairpin loop occurs (figure 1). The presence of this leads to inefficient primer-template annealing and incomplete extension by the DNA polymerase (Tarpinian 2004).

Figure 1. Example of a hairpin loop structure in plasmid DNA caused by high GC content.

A different problem occurs with DNA that contains poly-A regions and dinucleotide repeats. The repeating area of DNA means the DNA polymerase is no longer able to bind to the DNA, resulting in enzymic slippage. The result of a sample containing any of these problems is often considered a failure and is unusable by the customer.

We experimented using samples of DNA from various customers, all of which we believed to either be GC rich or suffering from a poly-A region or a dinucleotide repeat. All of the samples with suspected secondary structures showed a significant improvement when repeated with SSS and produced strong reads with high quality well-resolved base peaks and with read lengths of approximately 800bp (figures 2c and d). The samples suspected of containing Poly-A or dinucleotide repeats showed no significant improvement when using the SSS reagent.

To summarise, we suggest that it is straightforward to resolve sequences where secondary structure has previously proved obstructive. We have now introduced the SSS reagent into the Geneservice Ltd portfolio and can cost effectively sequence these loci. For further details on sequencing service provision please go to: www.geneservice.co.uk/service/sequencing/

Reference

Tarpinian S. Using the TripleMaster™ PCR system for Robust Amplification of GC Rich DNA Templates. http://www.eppendorf.com/script/cms-newspic.php?id=4479&inline=1&col=DOWNLOADFILE

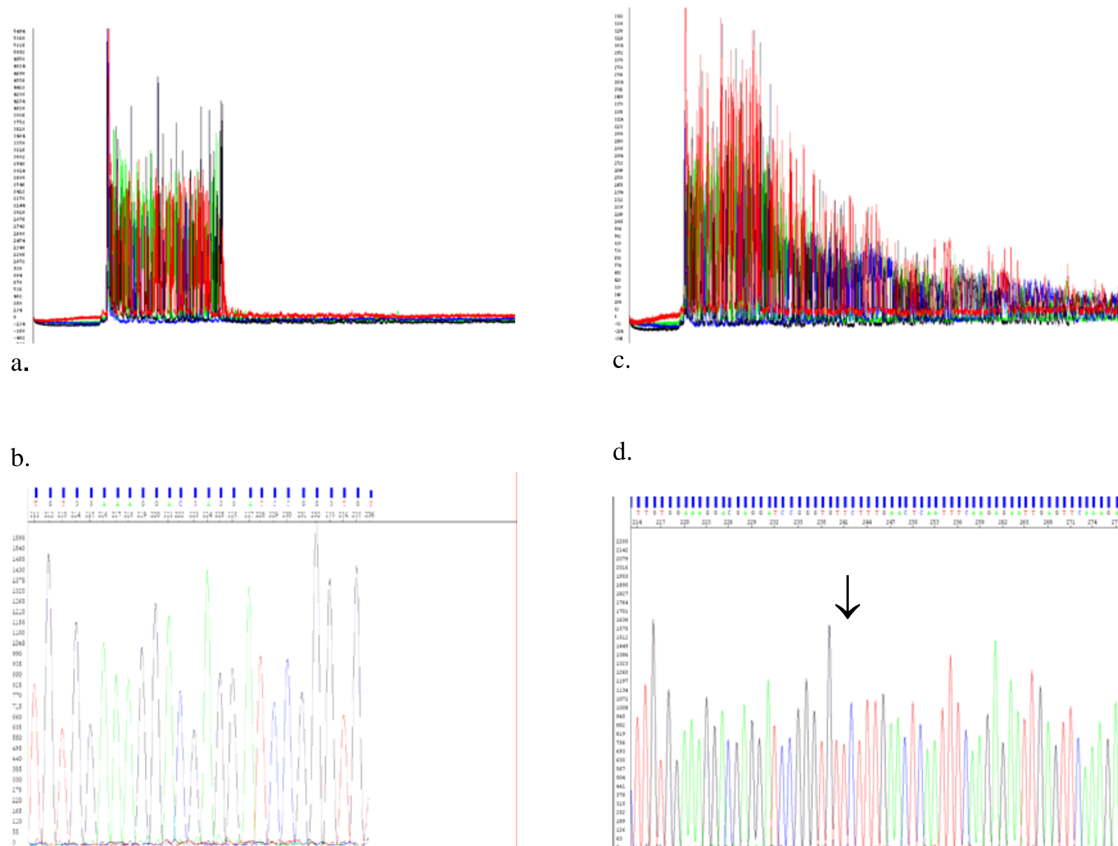

Figure 2. a) Raw control data using sample A and sequenced with ABI Big Dye v3.1, b) zoomed in electrophreogram of control sequence, shows point at which secondary structure causes truncation of the sequence read c) Sample A sequenced using SSS reagent, note raw data no longer shows a truncated read d) zoomed in electrophreogram of sample A sequenced with SSS reagent at same locus as in 2b, arrow indicates point at which truncation had previously occurred.