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Multiple separations of DNA sequencing fragments with a non-cross-linked polyacrylamide-filled capillary: capillary electrophoresis at 300 V/cm

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Abstract

The use of non-cross-linked polyacrylamide for multiple DNA sequencing runs on the same capillary is demonstrated. To minimize template loading, cycle sequencing was used to prepare fluorescently labeled samples. To minimize ion depletion, the current was reversed for several minutes between runs. To achieve stable operation, non-cross-linked polyacrylamide was aged for several days before use. This procedure allowed the successful generation of at least nineteen sequencing runs from the same capillary without replacement of the polyacrylamide and without trimming of the capillary tip. These separations were performed at an electric field of 300 V/cm.

1. Introduction

Non-cross-linked polyacrylamide is a useful medium for DNA sequencing by capillary electrophoresis. The material has been used to separate DNA sequencing fragments over 500 bases in length. An important issue in DNA sequencing is repeated separations in the same capillary; replacement of the capillary can require a tedious realignment step. While replacement of the separation medium is possible, it requires relatively high pressures, which is not convenient in certain situations [1].

The same capillary can be used for replicate analysis of synthetic DNA standards and restriction fragment digests in non-cross-linked polyacrylamide-filled capillaries [2]. However, replicate injections of DNA sequencing fragments have not proved successful; either the capillary performance degrades unacceptably or a portion of the capillary tip must be trimmed after each injection [3,4]. On the other hand, freshly prepared capillaries can separate sequencing fragment much longer than 500 bases in length when operated in a pristine condition [5,6].

It appears that two phenomena are important in limiting re-use of the capillary. First, depletion of ions from the injection tip of the capillary is caused by the mismatch in transport numbers across the buffer–polyacrylamide interface [7,8]. This ionic depletion leads to a large current drop at the injection tip, which leads to damage of the material. Second, the large template migrates with relatively low mobility. Conventional sequencing protocols generate at most one sequencing fragment per template molecule; as a result, the template is present in higher concentration than all sequencing fragments combined. This high concentration, along with the

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low mobility but high charge of the template, probably leads to gel damage at the injection tip of the capillary.

One simple solution to polyacrylamide degradation is found by replacing the separation medium after each run. Low-concentration non-cross-linked polyacrylamide can be replaced by pumping the material from the capillary. However, pumping the material requires high pressures, particularly when dealing with capillaries of small inner diameter [1]. Further, it is common to apply an electric field across the separation medium before operation to flush small ions from the capillary; this pre-electrophoresis run can account for a significant fraction of the time necessary for separation, leading to an undesirable extension of the analysis time.

Rather than replacing the separation medium, this paper reports repeated separations of DNA sequencing fragments without replacement of the separation medium. Successful re-use of a capillary relies on three steps. First, as noted in a companion paper [9], it is necessary to use polyacrylamide several days after polymerization; freshly prepared material leads to an unacceptable decrease in migration time reproducibility. Second, we rely on cycle sequencing for sample preparation. In cycle sequencing, thermal cycling allows repeated generation of sequencing fragments from the same template. This procedure is a variant of the polymerase chain reaction that produces a linear increase in sequencing template with the number of cycles. As a result, much less template is loaded on to the capillary. Third, we reverse the polarity of the electric field for a few minutes between each run. This field reversal decreases ionic depletion, yielding a homogeneous electric field in the capillary and more reproducible migration times for the sequencing fragments.

2. Experimental

The instrument and sample preparation method have been described in a companion paper [9]. The capillary used for this experiment had aged for 3 days before use.

3. Results and discussion

As noted [9], non-cross-linked polyacrylamide more than a few days old generates reproducible retention times for four subsequent injections. However, after more injections there is a gradual loss of current in the capillaries, presumably due to depletion of ions from the injection tip of the capillary. To decrease the effect of the depletion of ions, we reversed the electric field between each sequencing run. This voltage reversal allowed the depleted ions at the injection end to recover. The polarity was reversed for 10–20 min at +400 V/cm between each run. Up to 0.4 μA in current was recovered by this technique. Reversing the polarity was previously proposed by Swerdlow et al. [7] to eliminate the plugging of the gel pores by the DNA template.

To generate samples with low amounts of template, cycle sequencing was used. Fig. 1

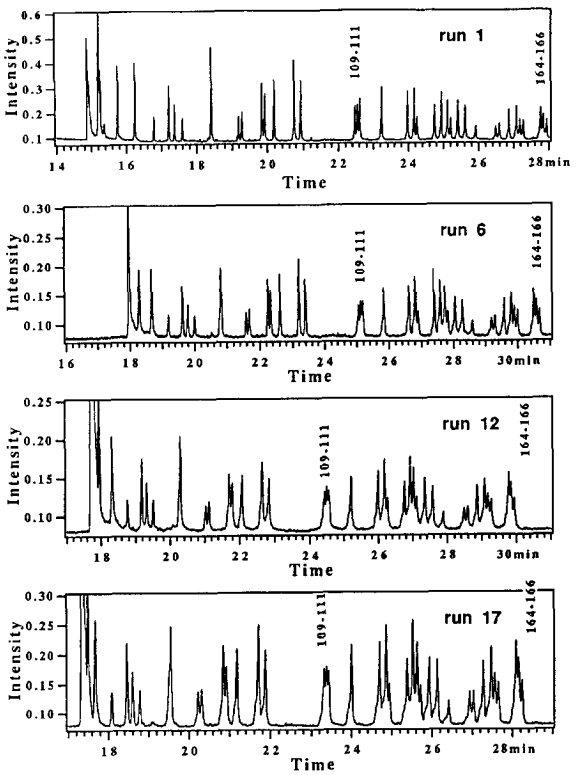

Fig. 1. Multiple runs of DNA sequencing samples on the same capillary at −3000 V/cm. The samples were prepared from M13mp18 and terminated with ddATP.

shows the sequence from the primer up to base 166 for runs 1, 6, 12 and 17 on the same capillary. By reversing the polarity between runs, a total of nineteen sequential runs were obtained on the same capillary without refilling the capillary. The mobility increases and the resolution decreases from the first to the last run; however, acceptable resolution remains even after nineteen runs.

Fig. 2 shows the change in current versus the run number. There is still a slight decrease in current (from 1.49 to 1.29 μA), which is not compensated for by reversing the polarity. This current drop could be due to incomplete deplugging of the pore. These experiments were performed over a 3-day period; an increase in current from run 7 to 8 and run 13 to 14 occurred during the overnight rest between these runs.

Fig. 3 shows the change in mobility versus the run number. The relative standard deviation of the mobility ranges from 3% to 6% for base 91 to base 350. Thus, the mobility of the sequencing samples is fairly stable within nineteen runs.

The peaks for bases 91, 142, 252 and 350 were

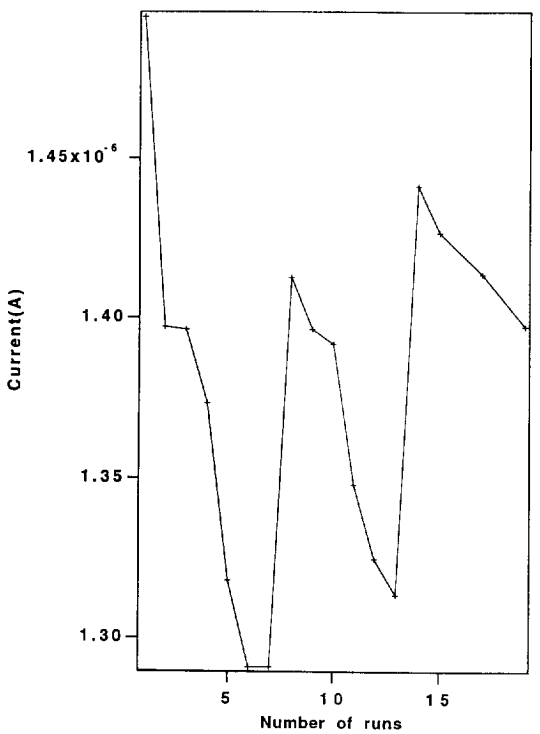

Fig. 2. Current versus number of runs.

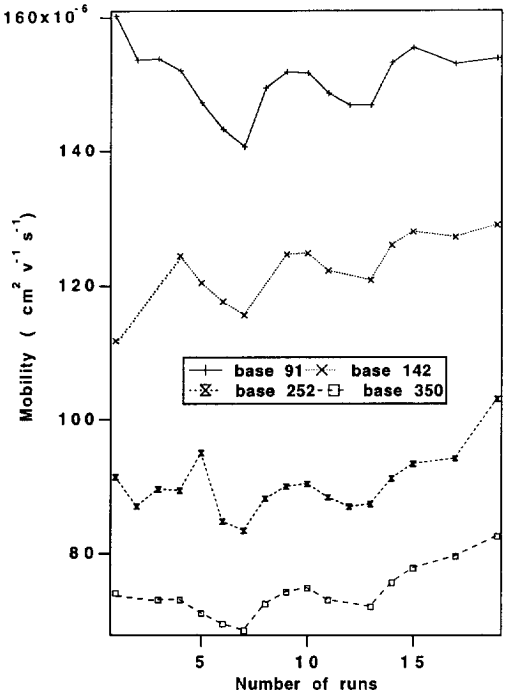

Fig. 3. Mobility versus number of runs for different peaks.

fitted with a Gaussian model. Fig. 4 shows the resolution for adjacent bases versus number of runs. The main decrease in the resolution occurs in the first four runs; however, after nineteen runs, the resolution had dropped by only a factor of two compared with a pristine capillary. We believe that the general decrease in resolution with increasing fragment length is associated with the effects of biased reptation [10]; operation of the capillary at lower electric field reduces this effect, generating longer sequencing reads [5]. The general loss of resolution with replicate runs must be associated with a change in the physical make-up of the non-cross-linked polyacrylamide. In principle, damage to the matrix could result in the formation of channels within the polymer, which leads to a band broadening mechanism that is similar to eddy diffusion in chromatography.

4. Conclusion

We have shown that it is possible to perform multiple separations of DNA sequencing samples

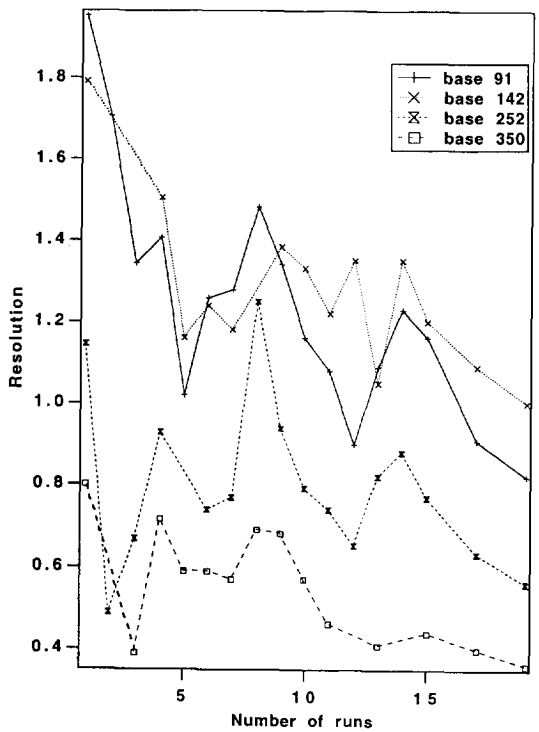

Fig. 4. Resolution versus number of runs for different peaks.

on the same capillary without replacing the separation medium. Up to nineteen sequential runs were obtained on the same capillary; this number of replicate analyses was limited by the operator's patience and not by failure of the separation medium; it appears that non-cross-linked polyacrylamide may be re-used virtually indefinitely. As a result, the tedious replacement of separation medium between runs may be eliminated, which will speed up large-scale sequencing efforts.

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