# Application Note

## CleanSEQ® for use with the ABI PRISM 3100

*Removal of unincorporated dye-terminators is required prior to sequence delineation on capillary electrophoresis instrumentation. CleanSEQ has proven an efficient and highly effective dye-terminator removal system with the ABI 3700 and 3730xl sequencers. Samples run on the ABI 3100 are more prone to evaporation and exposure to air before machine injection which can have deleterious effects on sample integrity including the appearance of dye blobs. This application note will demonstrate the effective use of CleanSEQ for dye-terminator removal in combination with the ABI 3100.*

### Introduction

CleanSEQ has proven to outperform many other dye-terminator removal technologies when used in conjunction with the high throughput ABI PRISM® 3700 and 3730xl sequencers. The ABI 3100 is a lower throughput instrument that unlike the 3700 and 3730xl systems, has historically given more reproducible results with formamide elution and injection. Formamide acts to reduce sample evaporation which helps maintain sample integrity. Formamide, however, is well known to significantly reduce signal strengths.

CleanSEQ is a magnetic bead dye-terminator removal system based on SPRI® chemistry. Experimentation during CleanSEQ development indicated that samples eluted in water were of high quality and produced sequences with long Phred20 read lengths and much higher signal intensities than samples eluted in formamide. Our standard CleanSEQ protocol, therefore, recommends the elution of sequencing products in water (Figure 1). We believe the septa seals on the 3100 may not effectively prevent exposure to air, potentially subjecting samples eluted in water to evaporation, degradation and the appearance of dye blobs in sequence traces. We tested a number of CleanSEQ elution buffers and procedures in an effort to inhibit sample evaporation and prevent sample degradation, thereby improving sequence quality on the 3100 (data not shown). As result of these studies, we recommend the elution of samples from CleanSEQ microparticles using water followed by application of mineral oil.

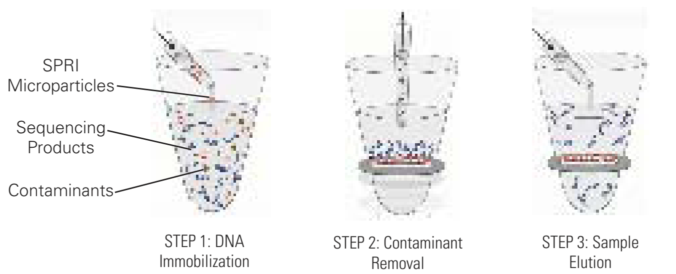


***Figure 1:*** *CleanSEQ Protocol. CleanSEQ is added directly to sequencing reactions. Sequencing products are immobilized onto paramagnetic particles leaving contaminants such as unincorporated dye-terminators, dNTPs, primers and salts in solution. Samples are placed onto SPRIPlate magnetic plates and contaminants removed. Sequencing products are eluted using an aqueous solution. For 3100 users, we suggest addition of mineral oil to samples following elution.*

We collectively ran over 1000 samples using our recommended oil overlay protocol and found significantly increased signal strengths and extended protection of samples from degradation compared with formamide elution. Importantly, no decrease in capillary performance was observed due to mineral oil usage. This simple procedure results in high quality sequence reads and eliminates the appearance of dye blobs (Figure 2).

### Experimental Methods

pGEM was sequenced using v3.1 BigDye® Terminator chemistry at 1/4x dilution. Sequencing reactions were purified using the standard CleanSEQ 96 protocol. Samples were eluted in either 50μl of 100% formamide, 50μl of water, or 50μl of water followed by application of 20μl of mineral oil. No sample transfers were required* from the time of sequencing set up through loading onto the 3100 with POP6 polymer. *Plate transfer required when using POP4 polymer.

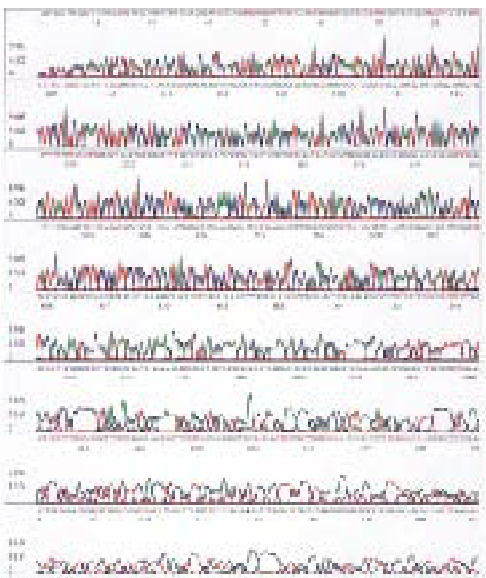

***Figure 2:*** *Sequencing using CleanSEQ and the 3100. Electropherogram of pGEM® sequenced and purified using methods described above. Template was eluted from SPRI microparticles in water and mineral oil overlaid prior to injection.*

### Higher Signal Strengths using Water Elution

We compared signal strengths and signal to noise ratios for samples eluted in water, water with mineral oil application and formamide. Signal strengths are significantly higher using water elution compared to those observed using formamide and even higher if water elution is followed by the addition of mineral oil (Table 1). We propose that mineral oil prevents evaporation and blocks oxygen exchange with the sample, thereby slowing dye degradation and maintaining high signal to noise ratios. The increased signal to noise ratios produced using CleanSEQ with water elution allow extensive dilution of BigDye Terminators without affecting Phred20 read lengths (data not shown).

| Injection Buffer | Signal:Noise |
|---|---|
| Water | 73.5 |
| Water + oil | 140.5 |
| 100% Formamide | 13.2 |

***Table 1:*** *Signal to Noise Ratios. Samples were eluted using indicated buffer and injected immediately. Background signals were similar for all samples, ranging from 1 to 3 RFU.*

### Extended Sample Stability

We assessed the stability of samples eluted in water and overlaid with mineral oil by comparing sequencing results from plates run immediately following CleanSEQ elution (fresh) versus samples reinjected 6 days later. Samples eluted in formamide were used as references, because they are known to be refractory to sample degradation. Both signal strength and Phred20 read length were unaffected by prior injection regardless of elution buffer (Figure 3). Signal to noise ratios were, however, far greater for samples eluted in water. These results clearly demonstrate that overlaying mineral oil stabilizes samples as well as formamide and allows flexible injection schedules.

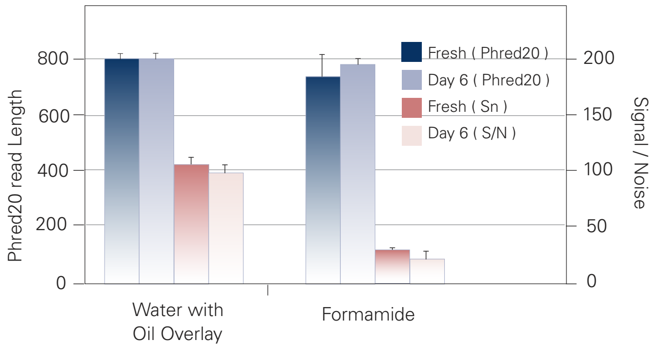


***Figure 3:*** *Sample Stability. Samples were eluted in the indicated buffer and directly injected (fresh) and subsequently reinjected after 6 days at room temperature. S/N = Signal:Noise*

### Instrument Compatibility

Because much is proprietary about the manufacturing of ABI capillaries, Agencourt conducted thorough testing to confirm the compatibility of mineral oil and the 3100 capillary array. We injected samples eluted with water and overlaid with mineral oil in the same capillaries for 99 consecutive runs. Formamide samples were run in parallel in alternate capillaries as a base line of capillary function. Samples loaded in formamide or in water overlaid with mineral oil had identical patterns of Phred20 read lengths over time (Figure 4). Thus, mineral oil had no detrimental effects on capillary performance.

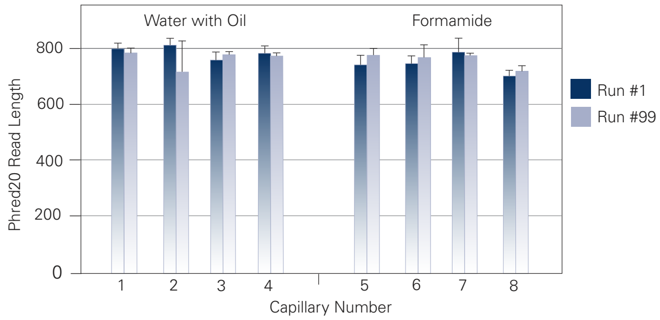


***Figure 4:*** *Capillary Integrity. A set of four capillaries was injected with samples eluted in the indicated buffers. A total of 99 samples was run through each capillary using a 50 cm array with POP6 polymer. Injection time = 22 sec at 1 kV.*

### Conclusions

- We recommend eluting samples from CleanSEQ beads with 50µl of water followed by application of 20µl of mineral oil for use with ABI 3100 sequencers.
- CleanSEQ samples eluted in water and overlaid with oil show no sample degradation after 6 days at room temperature, providing injection flexibility and eliminating the appearance of dye blobs.
- Increased signal strengths using CleanSEQ and water elution allow increased dilution of BigDye Terminators resulting in substantial cost savings.
- Injection of samples containing mineral oil does not adversely effect either short or long-term capillary performance.

### Available For Use In Your Laboratory

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