

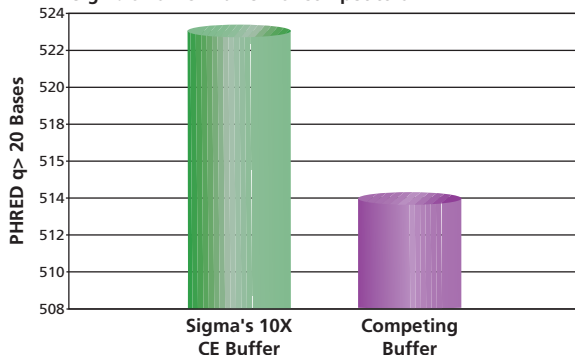
## Sequencing

## 10X Capillary Electrophoresis Running Buffer

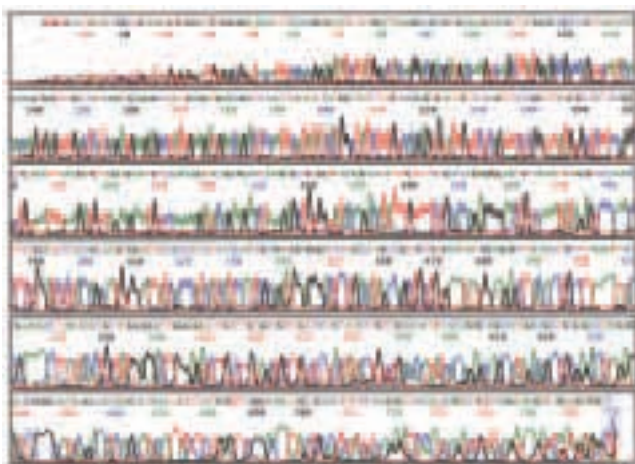
**B 4930** Sigma's 10x Capillary Electrophoresis Running Buffer was designed to meet the high quality and high throughput demands of today's sequencing labs. As a primary manufacturer and leading supplier of TBE to the gel-based sequencing community Sigma has the know how and large scale capacity to meet your buffer needs. Scientists at Sigma have taken this experience and joined it with today's advancing technologies to optimize a buffer for use with capillary electrophoresis sequencing instruments.

## Features and Benefits

- Cost efficient - high quality buffer at a great value
- Quality control testing of every lot ensures proper pH, conductivity and performance for Capillary Electrophoresis Sequencing
- Compatible with ABI Prism® 3700, 3100 and 310 DNA sequencers
- Room temperature stability for convenient shipping and storage
- Plastic bottles provide easy handling
- 1, 4 and 20 L as well as custom packaging options available

PHRED q> 20 Scores  
Sigma's 10X CE Buffer vs. Competitors

Data quality analysis based on PHRED q>20 scores. Average read length of both single-stranded and double-stranded DNA samples were analyzed on the ABI Prism® 3700 under standard run conditions of 3 hours at 1,000V, 50 °C. Samples were prepared using BigDye™ terminators with SigmaSpin™ post-reaction clean up.



Sequencing reaction was purified using a SigmaSpin 96-well plate. pFLAG™ MAC plasmid (Sigma, E 5644) was sequenced using BigDye™ Terminator v 3.0 chemistry (20 µl total reaction volume; BigDye premix diluted 1:1 with SeqSaver™ [Sigma, S 3938]). Data was generated on an ABI Prism® 3700 DNA Analyzer with POP-6™ polymer and 10X CE Buffer (Sigma, B 4930). Phred 20 > 600.

## SeqSaver™ Sequencing Premix Dilution Buffer

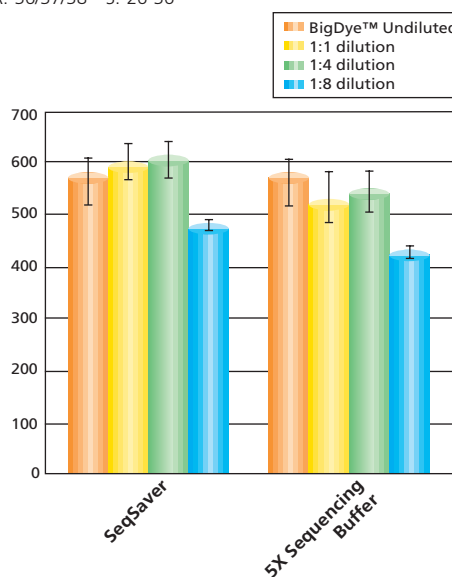
**S 3938** SeqSaver Sequencing Premix Dilution Buffer is designed to decrease the overall cost of sequencing by allowing the user to decrease the amount of reaction premix needed for each sequencing reaction. SeqSaver is a universal sequencing reaction diluent compatible with all commonly available reaction premixes including BigDye™ (v. 1.0, 2.0, and 3.0), DYEnamic™ ETs, and other standard terminator premixes.

Sequencing data obtained using SeqSaver in a 1:1 ratio with reaction premix yields resolution, read length and accuracy identical to unmodified reaction mixes. Strict QC and performance testing eliminates lot to lot variation resulting in consistent results every run. SeqSaver is suitable for analysis using ABI Prism® gel-based or capillary sequencing instruments and Amersham Biosciences MegaBACE™. With additional optimization some templates allow for further dilution and reduced total reaction volumes for even greater savings.

## Features and Benefits

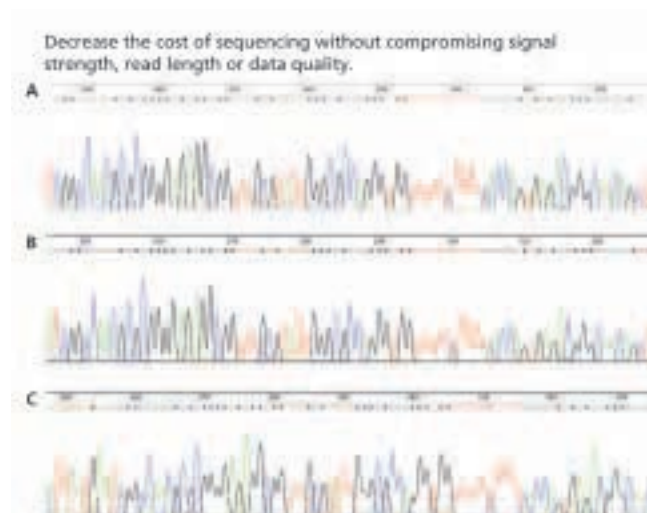
- Can be used with BigDye™ (v. 1.0, 2.0, and 3.0), DYEnamic™ ETs, ThermoSequenase™ II, and other standard terminator premixes
- Reduces the amount of costly reaction premix by 50% or more
- No compromise in read length, resolution, or signal strength
- SeqSaver can be mixed with individual reactions or directly to your stock premix
- Can be used with reaction volumes of 10 µl or less
- Custom packaging options available for large scale use

R: 36/37/38 S: 26-36



**Figure 1.** Data quality analysis based on PHRED q>20 scores. Comparison of sequencing reactions generated with dilutions of reaction premix using SeqSaver and a 5X Sequencing buffer. Data was generated from M13MP18 and pGEM templates using BigDye™ terminators and SigmaSpin post-reaction clean-up plates. Reaction premix was diluted 1:1, 1:4 and 1:8 in a 20 µl reaction and compared to undiluted reaction premix control.

## Sequencing

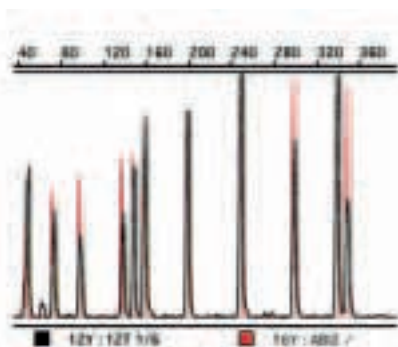


**Figure 2:** Sequencing results indicating signal strength and base resolution. BigDye™ terminator reaction premix diluted using SeqSaver. **A.** no dilution **B.** 1:1 dilution **C.** 1:4 dilution. Data was generated from M13MP18 and pGEM templates using BigDye™ terminators and SigmaSpin™ post-reaction clean-up plates.

## genSIZE T350 Genotyping Marker

**G 0164** 11 Fragments, 50–350 bp 0.4 mL  
 -20°C R: 36/37/38 S: 26-36

◆  
 DRY ICE

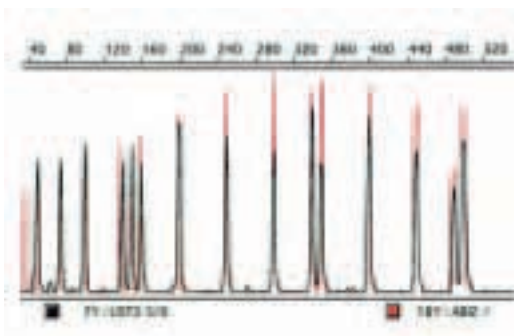


**Figure 1.** genSIZE T350 analyzed using ABI Genescan software compared with ABI Genescan 350-TAMRA

## genSIZE T500 Genotyping Marker

**G 9913** 15 Fragments, 50–500 bp 0.4 mL  
 -20°C R: 36/37/38 S: 26-36

◆  
 DRY ICE



**Figure 2.** genSIZE T500 analyzed using ABI Genescan software compared with ABI Genescan 500-TAMRA

## SigmaSpin™ Sequencing Reaction Clean-Up

## SigmaSpin Post-Reaction Clean-Up Columns

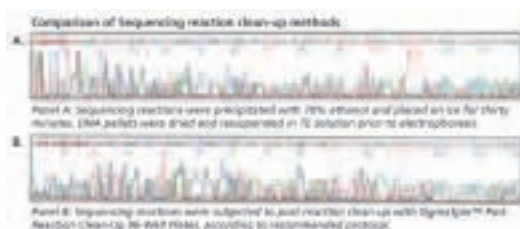
SigmaSpin Post-Reaction Clean-Up Columns are ideal for lower throughput applications, such as clean-up of probe labeling reactions or small numbers of sequencing reactions. These columns can accept sample volumes up to 100 µl. Each column comes with a collection tube to collect the DNA during centrifugation.

## SigmaSpin 96-Well Post-Reaction Clean-Up Plates

SigmaSpin 96-Well Post-Reaction Clean-Up plates provide a fast, simple, and highly efficient method for removing unincorporated dyes, excess salts, and other interfering reaction components (Fig. 1). Each plate is packed with a pre-hydrated size-exclusion resin, equilibrated with molecular biology grade water, and supplied in our unique plate design with long drip directors to minimize contamination between samples. The plate design also includes a snap-cap bottom seal and a foil seal top to ensure that the resin remains hydrated. SigmaSpin has been tested in high-throughput genome centers and core facilities with ABI Prism® 3700, 3100, 310 and 377. Each well can accept sample volumes up to 20 µl.

## Features and Benefits

- Validated with many common automated sequencers and dye terminator chemistries
- Pre-qualified size-exclusion resin guarantees optimum performance
- Unique drip directors prevent cross-contamination between samples during collection
- Plates are sealed to eliminate leakage or drying during shipping or storage
- Suitable for use with multi-channel pipettes and automated workstations



**Figure 1.** Single stranded M13MP18 plamid was sequenced with a -21M13 forward sequencing primer using ABI BigDye™ terminator chemistry. Sequencing reactions were resolved on an ABI Prism® 377 XL instrument with a 48 cm gel cassette containing 4.5% AutoPAGE™ Plus acrylamide at 2.88kV for 7 hrs.

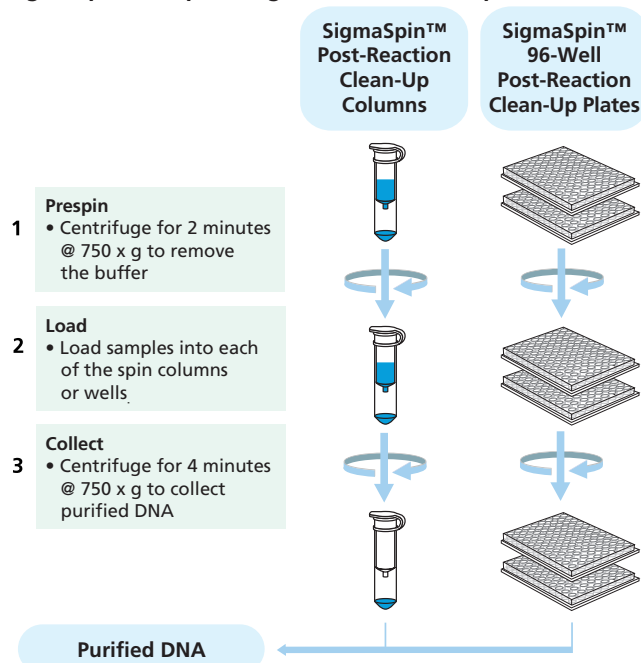


**Figure 2.** Sequencing reaction was purified using a SigmaSpin 96-well plate. pFLAG™ MAC plamid (Product Code **E 5644**) was sequenced using BigDye™ Terminator v 3.0 chemistry (20 µl total reaction volume; BigDye premix diluted 1:1 with SeqSaver™ [Product **S 3938**]). Data was generated on an ABI Prism® 3700 DNA Analyzer with POP-6™ polymer and 10X CE Buffer (Product Code **B 4930**). PHRED q20 >600.

## Sequencing

(Continuation of)

### SigmaSpin™ Sequencing Reaction Clean-Up



SigmaSpin™ Post-Reaction Clean-Up technology comes in two convenient forms, 96-well plates and single spin columns. Each format comes ready for immediate use.

**S 5059** SigmaSpin™ Post-Reaction Clean-Up Columns 50 each  
 2-8°C 70 each

**S 4309** SigmaSpin™ 2 Post-Reaction Clean-Up Plates 2 each  
 2-8°C

**S 4434** SigmaSpin™ 10 Post-Reaction Clean-Up Plates 10 each  
 2-8°C

**S 4559** SigmaSpin™ 50 Post-Reaction Clean-Up Plates 25 each  
 2-8°C 2 x 25 each

## Cloning

### Directional Cloning System

#### Director Universal Cloning System

**RDC-1** The Director Universal Cloning System 1 kit  
 -20°C provides a simple, rapid and universal method to directionally clone PCR products into a vector cleaved with 5' overhang-producing restriction endonucleases.  
 ◆ WET ICE Directionality is achieved by pairing directionally designed PCR primers (e.g., containing restriction sites) with any appropriately digested plasmid. The kit contains an optimized nucleotide triphosphate mix, containing dATP $\alpha$ S and dGTP $\alpha$ S, that is used for the PCR step. After PCR, the cohesive 5' termini of the amplicon are generated by Exonuclease III digestion instead of being generated by traditional restriction enzyme digestions. The dA/GTP $\alpha$ S that was incorporated into the amplicon during PCR protects it from over-digestion by Exonuclease III. The nucleotide mix in the kit is specially formulated so that the amplicon terminates at a statistically determined array of 3' dA/G $\alpha$ S sites. PCR primers are designed such that the 5' termini compliment the 5' overhangs of the predigested plasmid. The simple three step procedure (PCR, Exonuclease III digestion and rapid ligation/transformation) can be completed in one day. The typical cloning efficiency using this method is greater than 80%.

#### Features and Benefits

- Universal - PCR amplicon can be cloned into any expression vector
- High Cloning Efficiency - Typically >80%
- High Expression Efficiency - Typically >66%
- High Fidelity - Long and accurate, hotstart enzyme generates amplicons up to 20 kb with fidelity up to 6.5x greater than standard *Taq* DNA polymerase
- Fast - Simple three-step procedure allows completion in less than one day sufficient for 25 PCR reactions

#### Components:

10x AccuTaq™ LA DNA Polymerase Buffer, 250  $\mu$ l  
 Control PCR Template, 1ng/ $\mu$ l, 10  $\mu$ l  
 Control RDC primer-R (with 5' phosphorylation), 25  $\mu$ l  
 Control RDC primer-F (with 5' phosphorylation), 25  $\mu$ l  
 Exo-Deoxynucleotide Mix (20x), 62.5  $\mu$ l  
 ExoNuclease III, 100 units/ $\mu$ l, 25  $\mu$ l  
 JumpStart™ REDAccuTaq™ LA DNA Polymerase, 1 unit/ $\mu$ l, 62.5  $\mu$ l  
 Molecular biology grade water, 500  $\mu$ l

This product is sold under license from Roche Molecular Systems, Inc. and Applied Biosystems and the sale and use of this product are expressly limited and governed by a limited license - the details of which appear in full on the inside back cover of this product guide.

JumpStart Taq antibody is licensed under U.S. Patent No. 5,338,671 and 5,587,287 and corresponding patents in other countries.

Director is a trademark of Sigma-Aldrich.

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