

# SEQUENCING

## SigmaSpin™ Post-Reaction Clean-Up

For removal of unincorporated dyes, excess salts and other interfering components from sequencing reactions.

### 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, 3101 and 377. Each well can accept sample volumes up to 20 µl.

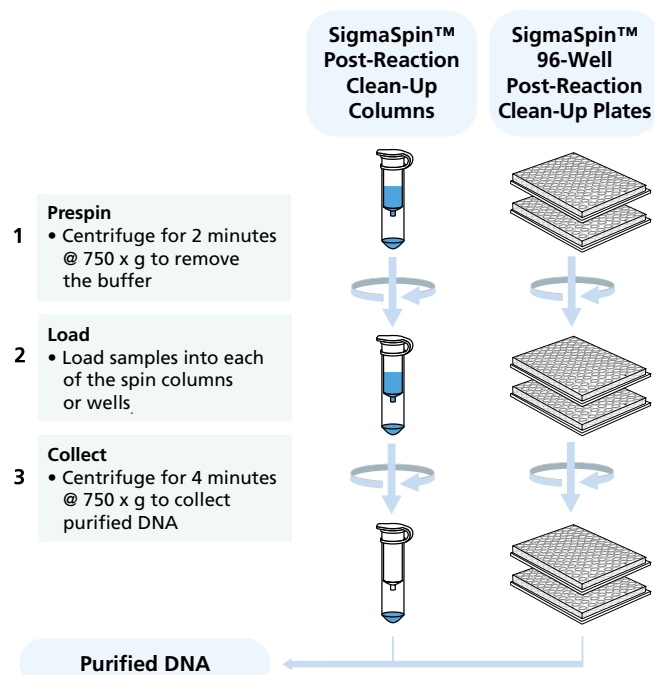
### Ideal for removing

- Dye-terminator nucleotides and primers from sequencing reactions
- Radiolabeled nucleotides, primers, and fluorescent dyes from nucleic acid probe labeling reactions

### Features and Benefits

- Validated with all automated sequencers and all dye terminators including BigDye™ v. 3.0 (Fig. 2)
- 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

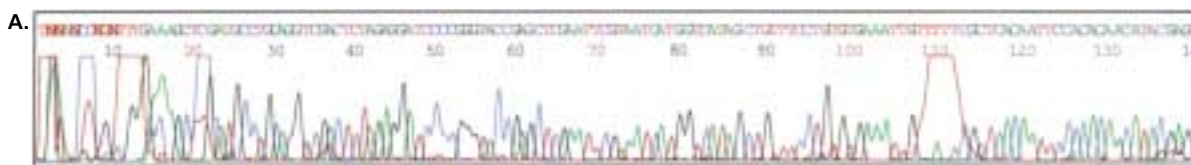
**Storage:** 2-8 °C



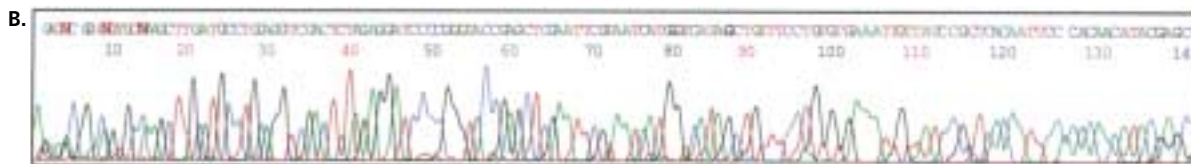
SigmaSpin™ Post-Reaction Clean-Up technology comes in two convenient forms, 96-well plates and single spin columns. Each format comes ready for immediate use.

# SEQUENCING

## Comparison of Sequencing reaction clean-up methods



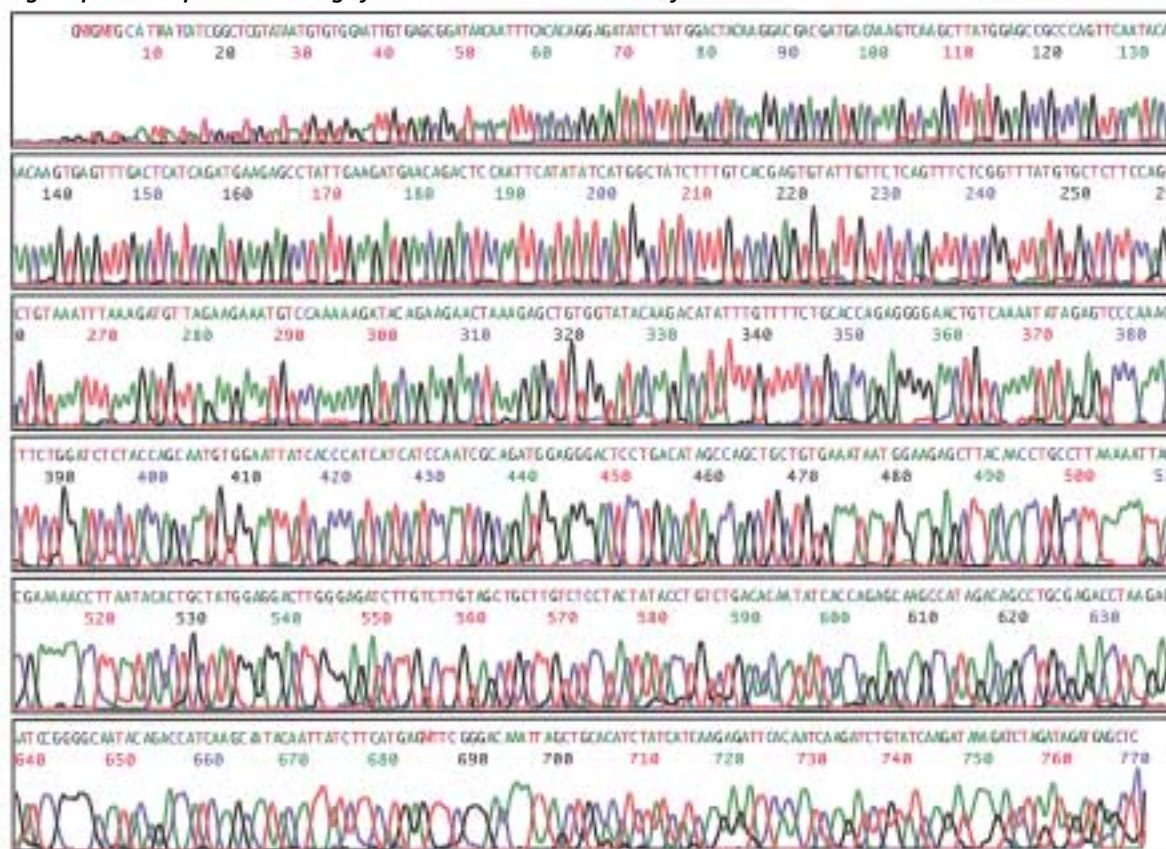
**Panel A:** Sequencing reactions were precipitated with 70% ethanol and placed on ice for thirty minutes. DNA pellets were dried and resuspended in TE solution prior to electrophoresis.



**Panel B:** Sequencing reactions were subjected to post-reaction clean-up with SigmaSpin™ Post-Reaction Clean-Up 96-Well Plates, according to recommended protocol.

**Figure 1.** Single stranded M13MP18 plasmid 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.

### SigmaSpin is compatible with BigDye™ v. 3.0 terminator chemistry



**Figure 2.** 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.

## ORDERING INFORMATION

Product	Product Description	Quantity
<a href="#">S 5059</a>	SigmaSpin™ Post-Reaction Clean-Up Columns (with collection tubes)	70/pkg
<a href="#">S 4309</a>	SigmaSpin™ 96-Well Post-Reaction Clean-Up Plates*	2 each
<a href="#">S 4434</a>	SigmaSpin™ 96-Well Post-Reaction Clean-Up Plates*	10 each
<a href="#">S 4559</a>	SigmaSpin™ 96-Well Post-Reaction Clean-Up Plates*	50 each
<a href="#">M 1905</a>	96-Well U-bottom Wash Plates	100 each
<a href="#">Z37,212-9</a>	96-Well V-bottom Collection Plates	50 each

\*Wash and collection plates included in 2- and 10-each package sizes

# SEQUENCING

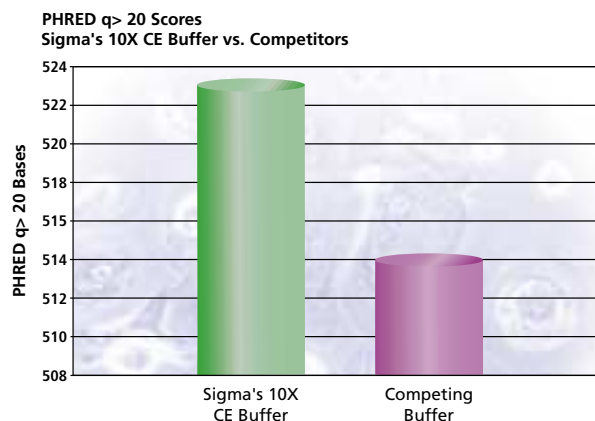
## 10X CE Buffer

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 & 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

**Storage:** Room Temperature



**Figure 1.** 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 3 hours at 1,000V, 50° C. Samples were prepared using BigDye™ terminators with SigmaSpin™ post-reaction clean up.

**Table 1. 10X CE Buffer Savings**

Number of Instruments	Runs/day	Total Runs/week	Savings per run using Sigma's 10X CE Buffer	Annual runs	Annual Savings
5	4	100	\$6.25	4,800	\$ 30,000
5	8	200	\$6.25	9,600	\$ 60,000
10	4	200	\$6.25	9,600	\$ 60,000
10	8	400	\$6.25	19,200	\$120,000
20	4	400	\$6.25	19,200	\$120,000
20	8	800	\$6.25	38,400	\$240,000
30	4	600	\$6.25	28,800	\$180,000
30	8	1200	\$6.25	57,600	\$360,000

Calculations: Based on 2002 list price for ABI and Sigma 4 L package size.

Assumptions: Volumes based on 1X buffer per run. Run is defined as 1 x 96-well plate.

**Table 2. 10X CE Buffer Performance**

	Run Time	Average PHRED q>20 bases	Average total read length
ABI Prism® 3700	3.0 (h)	520	800
MegaBACE®	2.75 (h)	495	760

Data quality analysis based on PHRED q>20 scores and total readable bases. Data was generated from M13, pGEM and pbluescript templates using BigDye™ Terminators and standard run conditions.

## ORDERING INFORMATION

Product Number	Product Description	Quantity
<a href="#">B 4930</a>	10X Capillary Electrophoresis Running Buffer	1 liter 4 liter 20 liter

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## QuickComb™-96

Sample loading and storage all in one convenient comb.

QuickComb™-96 is a sturdy and reliable porous membrane comb that allows for easy bench-top loading (Fig. 1). The sequencing reactions are spotted on individual teeth, the comb is inserted into the gel and electrophoresed. Samples are drawn from the porous teeth resulting in maximum signal strength and easier, more consistent lane tracking (Fig. 2).

With QuickComb™-96 you can load your samples and store them directly on the comb for up to 2 weeks (Fig. 3). For long-term storage, samples are stable on QuickComb™-96 for up to 6 months at -20 °C.

### Features and Benefits

- Bench-top loading eliminates the need for expensive syringe loaders and reduces user fatigue
- Increased data quality when compared to standard loading protocols
- Superior lane tracking capabilities
- No well to well leaching or loss in signal strength
- Spot and use your comb immediately or store spotted samples up to 6 months with no compromise in data quality
- Compatible with ABI Prism® 377 standard or beveled "step-glass" plates
- Laminated to avoid tearing and bending while inserting the comb

**Storage:** Room Temperature

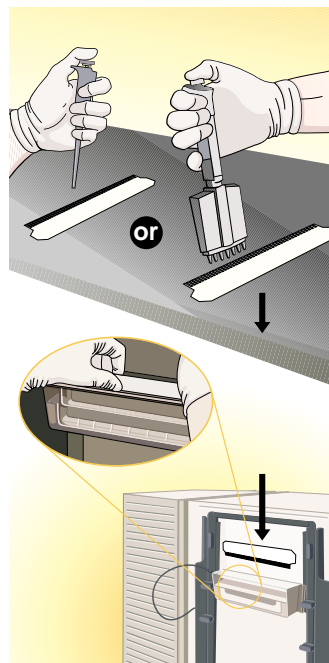


Figure 1. Use a single or multi-channel pipette to spot sequencing reactions on individual teeth. Insert the comb into the gel and electrophoresed.

Figure 2. QuickComb™-96 eliminates lane-to-lane leaching and allows for easier lane tracking.



**Panel A.** Samples loaded on adjacent teeth of QuickComb™-96. Both single- and double-stranded DNA templates were loaded. All samples were run on AutoPAGE™ Plus 4.5% gels using standard ABI 36E-1200 Run Module.



**Panel B.** Samples loaded directly onto cells in alternating lanes to combat lane-to-lane leakage. Both single- and double-stranded DNA templates were run on AutoPAGE™ Plus 4.5% gels using standard ABI 36E-1200 Run Module.

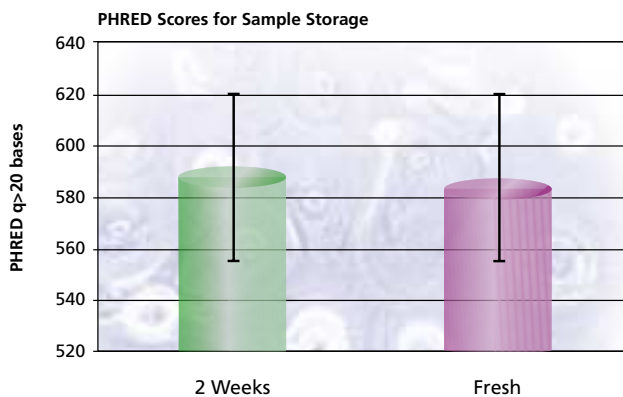


Figure 3. Analysis for samples loaded and stored for 2 weeks versus freshly loaded samples based on PHRED q>20 scores. Control samples were single-stranded DNA sequenced with ABI BigDye™ Terminators. Samples were run on AutoPAGE™ Plus 4.5% gels using BigDye™ standard ABI 36E-1200 Run Module.

### ORDERING INFORMATION

Product	Product Description	Quantity
C 3226	QuickComb™-96 Porous membrane loading and storage comb	10 each 50 each

# SEQUENCING

## SeqSaver™

*A universal dilution buffer for all common reaction premix chemistries – including BigDye™ v. 3.0.*

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), ThermoSequenase™ II, and standard terminator premixes.

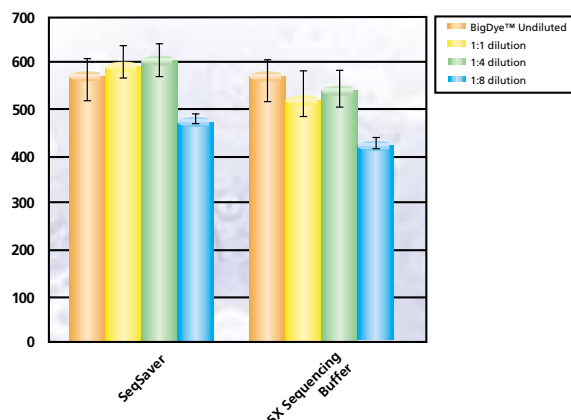
Sequencing data obtained using SeqSaver in a 1:1 ratio with reaction premix yields resolution, read length and accuracy identical to unmodified reaction mixes. Strict QC and performance testing eliminates lot to lot variation resulting in consistent results every run. SeqSaver is suitable for analysis 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), ThermoSequenase™ II, DYEnamic™ ETs, 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

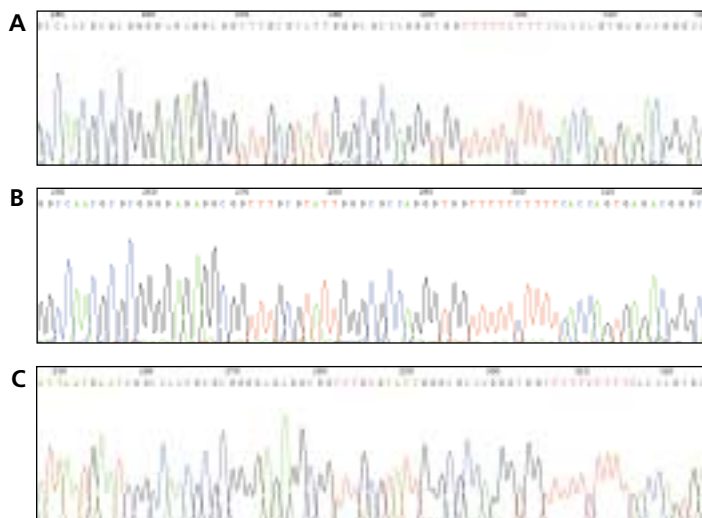
**Storage:** -20 °C

*Sequencing data obtained using SeqSaver™ in a 1:1 ratio with reaction premix yields resolution, read length and accuracy identical to unmodified reaction mixes.*



**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.

**Decrease the cost of sequencing without compromising signal strength, read length or data quality!**



**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.

### ORDERING INFORMATION

Product Code	Product Description	Reactions (20 µl rxn)	Reactions (10 µl rxn)	Quantity
<a href="#">S_3938</a>	SeqSaver™ Sequencing Premix Dilution Buffer	100	200	0.4 ml
		500	1000	2 x 1 ml
		1000	2000	4 x 1 ml
		5000	10000	20 ml

Custom packaging options are available – inquire for more information.