TECHNICAL MANUAL

ReliaPrep™ DNA Clean-Up and Concentration System

Instructions for Use of Products
A2891, A2892 and A2893

6/18
TM540

ReliaPrep™ DNA Clean-Up and Concentration System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The ReliaPrep™ DNA Clean-Up and Concentration System is designed to quickly concentrate and purify dilute DNA solutions, extract and purify DNA fragments of 100bp–10kb from standard or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE) buffer, or to purify products directly from a PCR amplification. Up to 95% recovery is achieved depending upon the DNA fragment size. PCR products are commonly purified to remove excess nucleotides and primers. This membrane-based system, which can bind up to 60µg of DNA, concentrates as much as 300µl of dilute DNA, recovering isolated DNA fragments or PCR products in as little as 10 minutes, depending on the number of samples processed and the protocol used. A single reagent stream is used for all three procedures, making the system both fast and easy.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ReliaPrep™ DNA Clean-Up and Concentration System	**250 preps**	**A2893**

For Research Use. Each system contains sufficient reagents for 250 DNA purifications. Includes:

- 5 packs Reliaprep™ Minicolumns (50/pack)
- 1 pack Collection Tubes (250/pack)
- 5 packs Elution Tubes (50/pack)
- 2 × 20ml Membrane Binding Solution
- 24ml Column Wash Solution (CWE)
- 58.8ml Buffer B (BWB)
- 25ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
ReliaPrep™ DNA Clean-Up and Concentration System	**50 preps**	**A2892**

For Research Use. Each system contains sufficient reagents for 50 DNA purifications. Includes:

- 1 pack Reliaprep™ Minicolumns (50/pack)
- 1 pack Collection Tubes (50/pack)
- 2 packs Elution Tubes (25/pack)
- 20ml Membrane Binding Solution
- 5ml Column Wash Solution (CWE)
- 11.8ml Buffer B (BWB)
- 1.25ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
ReliaPrep™ DNA Clean-Up and Concentration System	**10 preps**	**A2891**

For Research Use. Each system contains sufficient reagents for 10 DNA purifications. Includes:

- 1 pack Reliaprep™ Minicolumns (10/pack)
- 1 pack Collection Tubes (10/pack)
- 2 packs Elution Tubes (5/pack)
- 4ml Membrane Binding Solution
- 1ml Column Wash Solution (CWE)
- 11.8ml Buffer B (BWB)
- 1.25ml Nuclease-Free Water

Storage Conditions: All components can be stored at 15–30°C.

3. General Considerations

Each column is capable of binding as much as 60µg of DNA. However, if concentrating or cleaning up DNA preparations of greater than 10µg, we recommend eluting twice or increasing eluate volume.

Agarose, a linear polymer extracted from seaweed, is commonly used for electrophoretic separation of nucleic acids. Standard agarose melts at 87–89°C and solidifies at 36–39°C. In low-melt agarose, hydroxyethyl groups have been introduced into the polysaccharide chain, resulting in an agarose that both melts and solidifies at much lower temperatures (65°C and 24–28°C, respectively). Low-melt agarose is often used for applications that require recovery of intact DNA fragments from the gel after electrophoresis. The ReliaPrep™ DNA Clean-Up and Concentration System can be used to recover DNA from either standard or low-melt agarose gels with no changes to the protocol or differences in recovery (Section 4).

Standard safety apparel should be worn, especially when handling ethidium bromide-stained agarose gels. This includes gloves and a UV-blocking face shield to protect the eyes and face from UV light. When excising the gel band, work quickly to minimize personal exposure to UV light and nicking of the DNA (1–4).

The ReliaPrep™ DNA Clean-Up and Concentration System is compatible with PCR products generated using a variety of amplification enzymes, buffers or PCR-enhancing additives.

Change pipette tips frequently to prevent sample cross-contamination.

Reagents contain irritants and should be properly disposed of as hazardous waste. Do not use products containing bleach for sanitation.

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4. DNA Purification and/or Concentration

For best results, do not stop the procedure once samples are loaded onto the column. After elution, store samples at –70°C. If desired, DNA can be eluted in TE buffer (not provided).

Materials to be Supplied by the User

- 100% isopropanol, RNase-free
- 95–100% ethanol, RNase-free
- 1.5ml microcentrifuge tubes
- agarose gel (standard or low-melt; only for gel purification)
- 1X TAE or TBE electrophoresis buffer (only for gel purification)
- 50–65°C heating block (only for gel purification)
- microcentrifuge capable of maintaining at least 10,000 × *g* (14,000rpm)

4.A. Preparing Solutions

Solution	Preparation	Notes
Column Wash Solution (CWE)	**250 prep size** (Part# A254C): Add 36ml of 95–100% ethanol to the bottle containing 24ml of concentrate.	After adding ethanol, mark on the bottle label that this step has been performed. The reagent is stable at 15–30°C when tightly capped.
	50 prep size (Part# A254B): Add 7.5ml of 95–100% ethanol to the bottle containing 5ml of concentrate.	
	10 prep size (Part# A254A): Add 1.5ml of 95–100% ethanol to the bottle containing 1ml of concentrate.	
Buffer B (BWB)	**250 prep size** (Part# A288B): Add 100ml of 95–100% ethanol to the bottle containing 58.8ml of concentrate.	After adding ethanol, mark on the bottle label that this step has been performed. The reagent is stable at 15–30°C when tightly capped.
	50 prep size (Part# A288A): Add 20ml of 95–100% ethanol to the bottle containing 11.8ml of concentrate.	
	10 prep size (Part# A288A): Add 20ml of 95–100% ethanol to the bottle containing 11.8ml of concentrate.	
Membrane Binding Solution	Check that no precipitate is present in the bottle.	If there is precipitate, warm the contents in a 37°C waterbath, swirling until the precipitate redissolves.

4.B. Purifying and/or Concentrating DNA Samples

Notes:

1. This protocol requires 100% isopropanol (not provided).
2. Perform all centrifugation steps at 10,000 × *g* (14,000rpm).

Dilute DNA (µl)	Membrane Binding Solution (µl)	100% Isopropanol (µl)	Total Load (µl)
25	12.5	37.5	75
50	25	75	150
100	50	150	300
150	75	225	450
200	100	300	600
250	125	375	750
300	150	450	900

Note: Up to 300µl of a dilute stock of DNA can be processed in one load into a single ReliaPrep™ Minicolumn. If a dilute DNA sample exceeds 300µl, process the sample in multiple tubes in the manner described above and load the multiple centrifugations onto the same minicolumn. The wash protocol remains unchanged.

1. Pipet 25–300µl of dilute DNA into a 1.5ml microcentrifuge tube.
2. Add 1/2 volume Membrane Binding Solution and vortex 5 seconds.
3. Add 1 1/2 volumes of 100% isopropanol.
4. Load sample onto a ReliaPrep™ Minicolumn seated in a Collection Tube and centrifuge for 30 seconds.

   Note: This mixture is viscous so slowly transfer the sample to the column. Save the sample tubes after transfer to check that no significant volume remains.
5. Remove column, and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube.
6. Add 200µl of Column Wash Solution (CWE) and centrifuge for 15 seconds. Remove column, and discard the contents of the Collection Tube. Reseat the minicolumn into the same CollectionTube.
7. Wash with 300µl of Buffer B (BWB) and centrifuge for 15 seconds. Repeat wash with 300µl of Buffer B (BWB) and centrifuge again.
8. Remove column and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube and centrifuge for 1 minute to dry the columns; then transfer columns to Elution Tubes.
9. Pipet 15µl of Nuclease-Free Water or TE buffer (not provided) into the center of the ReliaPrep™ Minicolumn and then centrifuge for 30 seconds.

   Note: Touch the pipette tip to the column bed surface before dispensing Nuclease-Free Water or TE buffer to completely wet the column matrix. The color should change from light to dark tan.
10. For maximum recovery, repeat elution with an additional 15µl of Nuclease-Free Water or TE buffer.

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4.C. Purifying PCR Amplification Products

Note: Perform all centrifugation steps at 10,000 × *g* (14,000rpm).

1. Pipet 25–400µl of a PCR amplification or reaction pool into a 1.5ml microcentrifuge tube.
2. Add an equal volume of Membrane Binding Solution and vortex 5 seconds.

PCR Volume (µl)	Membrane Binding Solution (µl)	Total Load (µl)
25	25	50
50	50	100
100	100	200
150	150	300
200	200	400
250	250	500
300	300	600
350	350	700
400	400	800

3. Load sample onto a ReliaPrep™ Minicolumn seated in a Collection Tube and centrifuge for 30 seconds.
4. Remove column and discard the contents of the Collection Tube. Reseat the minicolumn into the same Collection Tube.
5. Add 200µl of Column Wash Solution (CWE) and centrifuge for 15 seconds. Remove column and discard the contents of the Collection Tube. Reseat the minicolumn into the same Collection Tube.
6. Wash with 300µl of Buffer B (BWB) and centrifuge for 15 seconds. Repeat wash with 300µl of Buffer B (BWB) and centrifuge again.
7. Remove minicolumn, and discard the contents of the Collection Tube. Reseat the minicolumn into the same Collection Tube and centrifuge for 1 minute to dry the minicolumn; then transfer minicolumn to an Elution Tube.
8. Pipet 15µl of Nuclease-Free Water or TE buffer (not provided) to the center of the minicolumn, then centrifuge for 30 seconds.

   Note: Touch the pipette tip to the column bed surface before dispensing Nuclease-Free Water or TE buffer to completely wet the column matrix. The color should change from light to dark tan.
9. For maximum recovery, repeat elution with an additional 15µl of Nuclease-Free Water or TE buffer.

4.D. Purifying DNA from Gel Slices

Note: Perform all centrifugation steps at 10,000 × *g* (14,000rpm).

1. Load and run your gel using an established protocol. DNA can be extracted from standard or low-melt agarose gels run with either TAE or TBE buffer.
2. Weigh a 1.5ml microcentrifuge tube for each DNA fragment to be isolated, and record the weight.
3. Visualize and photograph the DNA using a long-wavelength UV lamp and an intercalating dye such as ethidium bromide. To reduce nicking, irradiate the gel for the absolute minimum time possible (1–4). Excise the DNA fragment of interest in a minimal volume of agarose using a clean scalpel or razor blade. Transfer the gel slice to the weighed microcentrifuge tube, and record the weight. Subtract the weight of the empty tube from the total weight to obtain the weight of the gel slice (see Notes 1–3).

   Note: The gel slice may be stored at 4°C or at –20°C for up to 1 week in a tightly closed tube under nuclease-free conditions before purification.
4. Add Membrane Binding Solution at a ratio of 10µl of solution per 10mg of agarose gel slice.

Gel Slice (mg)	Membrane Binding Solution (µl)	Approximate Total Load (µl)
25	25	50
50	50	100
100	100	200
150	150	300
200	200	400
250	250	500
300	300	600
350	350	700

4.D. Purifying DNA from Gel Slices (continued)

5. Vortex the mixture (see Note 4) and incubate at 50–65°C for 10 minutes or until the gel slice is completely dissolved. Vortex the tube every few minutes to increase the rate of agarose gel melting. Centrifuge the tube briefly at room temperature to ensure the contents are at the bottom of the tube. Once the agarose gel is melted, the gel will not resolidify at room temperature.

Notes:

1. Recovery from 1% high-melting-point agarose is comparable to that from 1–2% low-melting-point agarose. High-melting-point agarose concentrations of up to 3% have been tested. Gel slices with higher agarose concentrations (2–3%) may require a longer time to melt completely than a 1% agarose gel slice and may show reduced yields.
2. The maximum capacity of the ReliaPrep™ Minicolumn is 350mg of gel mass dissolved in 350µl of Membrane Binding Solution per column pass. For gel slices >350mg, continue to pass additional sample through the ReliaPrep™ Minicolumn until all of the sample has been processed.
3. The maximum binding capacity of the ReliaPrep™ Minicolumn is approximately 60µg per column, and as little as 10ng has been successfully purified.
4. DNA fragments that are larger than 5kb should be mixed gently to prevent shearing. Do not vortex if DNA fragment is larger than 5kb; mix by inversion.

6. Load sample onto a ReliaPrep™ Minicolumn, centrifuge for 1 minute and move minicolumn to a clean collection tube.
7. Wash with 200µl of Column Wash Solution and centrifuge for 15 seconds. Remove column and discard the contents of the Collection Tube. Reseat the minicolumn into the same Collection Tube.
8. Wash with 300µl of Buffer B (BWB) and centrifuge for 15 seconds. Repeat wash with 300µl of Buffer B (BWB) and centrifuge again.
9. Move the minicolumns to empty Collection Tubes, centrifuge for 1 minute and transfer minicolumns to Elution Tubes.
10. Pipet 15µl of Nuclease-Free Water or TE buffer to the center of the minicolumn and centrifuge for 30 seconds.

    Note: Touch the pipette tip to the column bed surface before dispensing Nuclease-Free Water or TE buffer to completely wet the column matrix. The color should change from light to dark tan.
11. For maximum recovery, repeat elution with an additional 15µl of Nuclease-Free Water or TE buffer.

5. Determining DNA Yield and Purity

The ReliaPrep™ DNA Clean-Up and Concentration System can be used to purify and concentrate DNA sourced from dilute solutions, PCR or gel slices. The total DNA yield may be determined spectrophotometrically at 260nM, where 1 absorbance unit (A_{260}) equals approximately 50µg of double-stranded DNA/ml. The purity also may be estimated using the relative absorbances at 230nm, 260nm and 280nm (i.e., A_{260}/A_{280} and A_{260}/A_{230} ratios). If overall expected yield is less than 1µg, spectrophotometric analysis will not produce accurate results due to the lack of absorbance sensitivity for low-concentration nucleic acids. Alternatively, low DNA yield can be determined with good accuracy using the QuantiFluor® dsDNA System (Cat.# E2670).

DNA purified using the ReliaPrep™ DNA Clean-Up and Concentration System is substantially free of contaminating protein. Pure DNA should exhibit an A_{260}/A_{280} ratio of 2.0; however, variations between individual starting materials and skill in performing the procedure may reduce this ratio to the 1.7–2.1 range. This is not necessarily problematic for downstream applications. DNA will usually exhibit an A_{260}/A_{230} ratio of 1.8–2. Results from agarose gel slice purification may be slightly lower. A low A_{260}/A_{230} ratio can indicate guanidine contamination, which may interfere with performance or accuracy of results in downstream applications. A low A_{260}/A_{230} ratio may also indicate ethanol contamination. Be sure to follow the centrifugation recommendations in the protocol.

A.

Starting DNA	A_{260}/A_{280}	A_{260}/A_{230}	Yield (µg)	Recovery	CV
1µg	1.97	2.09	0.96	96%	7.9%
3µg	1.93	2.22	2.86	95%	8.7%
5µg	1.91	2.19	4.43	89%	5.1%
10µg	1.88	2.11	8.36	84%	1.4%

B.

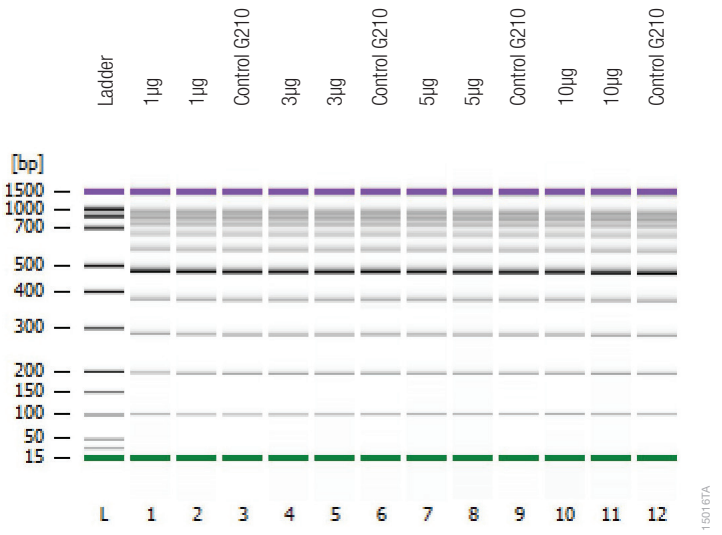

Figure 1. Recovery and concentration of DNA from dilute solution using the ReliaPrep™ DNA Clean-Up and Concentration System. DNA (1, 3, 5 and 10µg of the Promega 100bp DNA Ladder [Cat.# G2101]) was diluted in triplicate into a final 100µl of water, repurified using the standard protocol and eluted in 15µl of Nuclease-Free Water. **Panel A.** Average yield and purity was 90.8%. **Panel B.** Samples were further analyzed on an Agilent Bioanalyzer® 2100 using a DNA 1000 chip and protocol.

5. Determining DNA Yield and Purity (continued)

A.

	Reaction Concentration (ng/µl)	A_{260}/A_{280}	A_{260}/A_{230}	Yield (µg)
1	304.2	1.9	2.24	4.56
2	296.91	1.89	2.06	4.45
3	309.07	1.9	1.99	4.64
4	281.48	1.91	2.26	4.22
5	287.28	1.9	2.17	4.31
6	327.4	1.89	2.25	4.91
7	280.72	1.91	2.25	4.21
8	313.38	1.88	2.25	4.7

Average A_{260}/A_{280}	1.9
Average A_{260}/A_{230}	2.18
Average Yield (µg)	4.5
Standard Deviation	0.25
CV	5.5%

B.

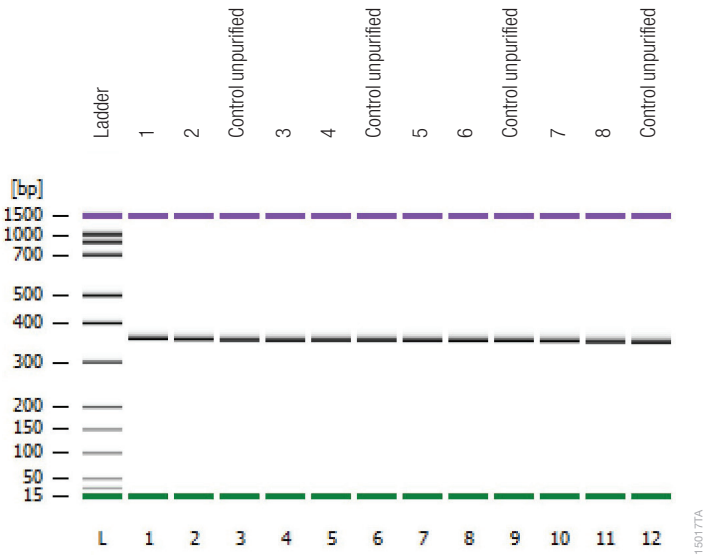

Figure 2. Recovery and concentration of PCR products using the ReliaPrep™ DNA Clean-Up and Concentration System. Multiple 50µl amplification reactions using a DNA control sequence that produces a 360bp amplicon were pooled. **Panel A.** Eight purifications using 50µl/minicolumn were performed using the standard protocol, and eluted in 15µl of Nuclease-Free Water. Nearly equivalent yields of target DNA amplicon resulted. **Panel B.** Sample purity was compared for all eight purifications using an Agilent Bioanalyzer® 2100 and DNA 1000 chip.

A.

	Sample Concentration (ng/µl)	A_{260}/A_{280}	A_{260}/A_{230}	Yield (µg)	DNA Recovered
Control	128.52	1.9	2.07	2.97	n.a.
1	135.46	1.88	1.76	2.03	68.4%
2	158.2	1.93	1.77	2.37	79.9%
3	164.73	1.89	1.79	2.47	83.2%
4	161.9	1.9	2.05	2.43	81.8%
5	162.78	1.91	1.72	2.44	82.2%
6	175.18	1.89	1.79	2.63	88.5%
7	169.74	1.93	1.62	2.55	85.8%
8	152.24	1.9	1.72	2.28	76.9%

Average A_{260}/A_{280}	1.9
Average A_{260}/A_{230}	1.78
Average Yield (µg)	2.4
Standard Deviation	0.18
CV	7.6%

B.

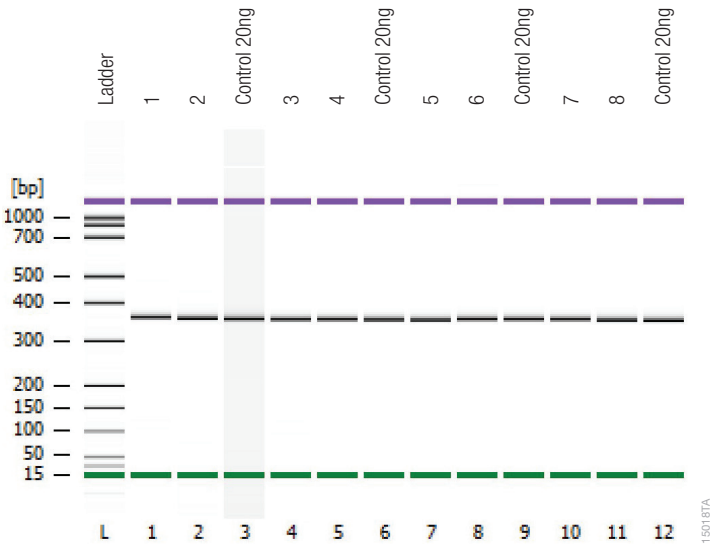

Figure 3. DNA recovery from agarose using the ReliaPrep™ DNA Clean-Up and Concentration System. Each of eight 3µg aliquots of a 360bp amplicon was suspended in 350µl of molten 1% TBE-buffered agarose and allowed to solidify. **Panel A.** The amplicons embedded in agarose were used as starting material for purification according to standard protocol, and eluted in 15µl of Nuclease-Free Water. Nearly equivalent yields of target DNA amplicon resulted. **Panel B.** Sample purity was compared for all eight recoveries using an Agilent Bioanalyzer® 2100 and DNA 1000 chip.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Low DNA yield	Verify that an equal volume of Membrane Binding Solution was added to the gel slice or PCR (10µl per 10mg gel slice or 10µl PCR).
	Make certain that the gel slice is completely melted before proceeding with the purification. Incubate at 50–65°C to completely melt the gel slice.
	If the amount of DNA purified is too small to quantitate by spectrophotometry, quantitate using QuantiFluor® dsDNA System (Cat.# E2670).
	Be sure to centrifuge at 10,000 × *g* (14,000rpm).
	Verify that ethanol was added to both wash solutions (see Section 4.A), and repeat the purification.
DNA yields on gel look low compared to spectrophotometer readings	Trace contaminants in eluted DNA can artificially inflate spectrophotometric readings. Use agarose gel electrophoresis followed by ethidium bromide or PicoGreen® staining to determine DNA yields.
Calculated yield lower than expected	DNA may be retained on minicolumn if expected yield is >10µg. Elute a second time with 15µl of Nuclease-Free Water.
Low A_{260}/A_{230} ratios	Typically due to guanidine isothiocyanate contamination. Low ratios do not necessarily indicate that the DNA will function poorly in downstream applications. If a low A_{260}/A_{230} ratio is a concern, ethanol-precipitate the DNA.
Clogged spin basket	Increase the length of the 50–65°C incubation to ensure the gel slice is completely melted.
Purified DNA floats out of the well when loaded on a gel	Ethanol carryover. Be certain that the DNA Wash Solution is not carried over from the wash steps.
	Ethanol not removed during centrifugation. Centrifuge empty minicolumn for 1 minute to remove any residual Buffer B (BWB).

Symptoms	Causes and Comments
Purified DNA bands are not sharp	DNA may be sheared. Mix the agarose gel slice gently with the Membrane Binding Solution.
	Nuclease contamination may be an issue. Autoclave the gel running buffer before use.
	Store the gel slice at 4°C or –20°C for no more than 1 week under nuclease-free conditions.

7. References

1. Zimmermann, M., Veeck, J. and Wolf, K. (1998) Minimizing the exposure to UV light when extracting DNA from agarose gels. *BioTechniques* **25**, 586.
2. Hengen, P. (1997) Methods and reagents. Protecting vector DNA from UV light. *Trends Biochem. Sci.* **22**, 182–3.
3. Grundemann, D. and Schomig, E. (1996) Protection of DNA during preparative agarose gel electrophoresis against damage induced by ultraviolet light. *BioTechniques* **21**, 898–903.
4. Cariello, N.F. *et al.* (1988) DNA damage produced by ethidium bromide staining and exposure to ultraviolet light. *Nucleic Acids Res.* **16**, 4157.

8. Related Products

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	100 preps	A1223
	250 preps	A1222
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
	300 preps	A2496
PureYield™ Plasmid Maxiprep System	10 preps	A2392
	25 preps	A2393
pGEM®-T Vector Systems	20 reactions	A3600
pGEM®-T Vector Systems + JM109 Competent Cells	20 reactions	A3610
pGEM®-T Easy Vector Systems	20 reactions	A1360
pGEM®-T Easy Vector Systems + JM109 Competent Cells	20 reactions	A1380
LigaFast™ Rapid DNA Ligation System	30 reactions	M8221
	150 reactions	M8225
QuantiFluor® dsDNA System	100µl	E2671
	1ml	E2670
QuantiFluor® ONE dsDNA System	100 reactions	E4871
	500 reactions	E4870

Note: You can use the CloneWeaver™ Workflow Builder to design a cloning workflow for your construct: **www.promega.com/resources/tools/cloneweaver/**