TECHNICAL MANUAL

ProNex® Size-Selective Purification System

Instructions for Use of Products
NG2001, NG2002 and NG2003

Revised 2/18
TM508

ProNex® Size-Selective Purification 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 Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The ProNex® Size-Selective Purification System enables the rapid and efficient magnetic resin-based purification of double-stranded DNA (dsDNA) for next-generation sequencing (NGS), polymerase chain reaction (PCR) and general molecular biology applications. The ProNex® System allows users to select the desired size of purified dsDNA fragments, from 100bp to 750bp. The novel reagent formulation provides significantly improved selectivity, reproducibility and yield relative to traditional dsDNA purification methods. In addition, the ProNex® System can be used in both manual and automated high-throughput workflows. If interested in evaluating a high-throughput, automated method, please contact Promega Technical Services. Email: **techserv@promega.com**.

As the ratio of ProNex® Chemistry added to a sample is increased, smaller fragments of dsDNA are purified (Section 6). The ProNex® System can also be used to select a range of dsDNA fragments centered around a desired size in a dual size-selective approach (Section 7).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ProNex® Size-Selective Purification System	**10ml**	**NG2001**

Includes:

- 1 × 10ml ProNex® Size-Selective Chemistry
- 1 × 15ml Wash Buffer
- 1 × 10ml Elution Buffer (10mM Tris [pH 8.5])

PRODUCT	SIZE	CAT.#
ProNex® Size-Selective Purification System	**125ml**	**NG2002**

Includes:

- 1 × 125ml ProNex® Size-Selective Chemistry
- 1 × 180ml Wash Buffer
- 1 × 125ml Elution Buffer (10mM Tris [pH 8.5])

PRODUCT	SIZE	CAT.#
ProNex® Size-Selective Purification System	**500ml**	**NG2003**

Includes:

- 4 × 125ml ProNex® Size-Selective Chemistry
- 2 × 340ml Wash Buffer
- 4 × 125ml Elution Buffer (10mM Tris [pH 8.5])

Storage conditions: Upon receipt, remove the ProNex® Chemistry bottle(s) from the kit package and store at 2–10°C. Do not freeze. Do not allow ProNex® Chemistry to dry during storage. Store the remaining kit components at 15–30°C.

3. Materials Required

Materials to Be Supplied by the User

- 95–100% ethanol
- Magnetic stand (see Section 9.B, Related Products)
- Single or multichannel 20–200µl pipette and tips
- Single or multichannel 100–1000µl pipette and tips (optional, may be desired for some applications that require pipetting >200µl)
- Buffer trough (if using a multichannel pipette)
- 96-well plate capable of holding up to 5X the starting sample volume (depending on the protocol you are following) if working in a multichannel format

4. General Considerations

Desired size cutoffs can be achieved by varying the volume/volume ratio of ProNex® Size-Selective Chemistry added to the starting sample. The values provided in Sections 6 and 7 are guidelines, but they may require adjustment depending on specific purification conditions. Precise and reproducible size selection may require optimization. Different starting sample types can yield different size selection results. Samples with precipitants or crowding agents, such as polyethylene glycol (PEG), will require reduction of expected ratios to obtain desired size cutoffs. We strongly recommend performing pilot purifications with a generic dsDNA source (e.g., a DNA ladder) in the same sample buffer before attempting to purify a crucial sample. An alternative approach is to purify the sample using the 100bp cutoff example protocol in Section 6, eluting the population into Elution Buffer, and then proceeding with a size-selective purification using the recommended ratios from Section 6 or Section 7.

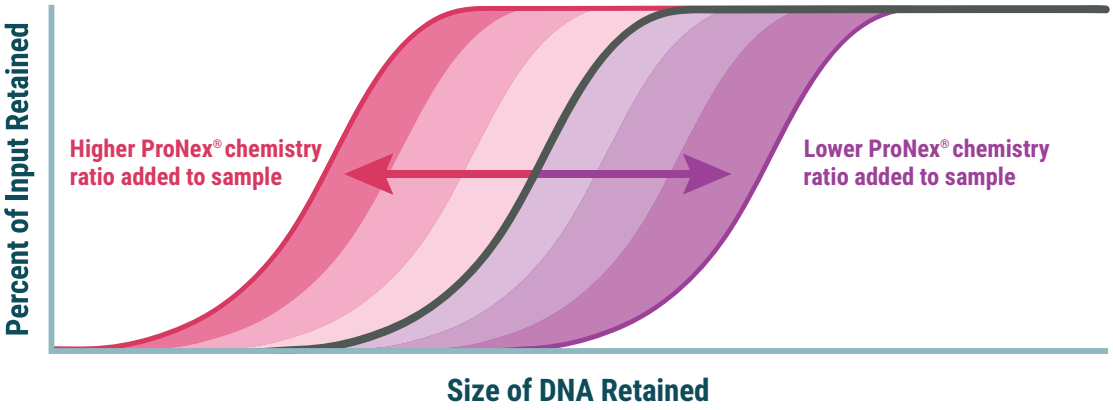

Figure 1. The effects of changing the ProNex® chemistry:sample ratio on size-selective purification.

5. Before You Begin

1. Reconstitute the Wash Buffer by adding 95–100% ethanol to the wash buffer provided in the kit. See Table 1 for amounts to add for each size container.

Table 1. Amounts of Ethanol to Add to Wash Buffer.

Cat.#	Part #	Wash Buffer Volume	Volume of Ethanol to Add (per Bottle)	Final Volume of Wash Buffer with Ethanol Added (per Bottle)
NG2001	NG105A	15ml	75ml	90ml
NG2002	NG105B	180ml	900ml	1,080ml
NG2003	NG1051	2 × 340ml	1,700ml (per bottle)	2,040ml (per bottle)

Aliquots of the Wash Buffer may be removed and reconstituted in smaller containers if desired. Add 5 volumes of ethanol for every volume of Wash Buffer.

Notes:

1. ProNex® Size-Selective Chemistry contains 0.02% sodium azide. The Wash Buffer, after addition of ethanol, contains approximately 80% ethanol, which is flammable. Please safely handle and dispose of the liquid and dry wastes generated by this product in accordance with your institutional policies.
2. All steps should be performed at room temperature (15–30˚C).
3. Wear safety glasses, a lab coat, and gloves while handling samples and kit components.

2. For maximum reproducibility, equilibrate the bottle of ProNex® Chemistry to ambient temperature for 30 minutes to 1 hour before beginning purification.
3. Ensure that the bottle cap is secure. Thoroughly resuspend the ProNex® Chemistry by vigorous vortexing for 10 seconds or longer.

6. Purification of PCR Amplicons, Sample Cleanup, or Removal of Small Fragments

The protocol described below is designed to purify DNA fragments above a desired size and remove lower molecular weight DNA below the desired size, while also removing contaminants (e.g., buffers, proteins, salts, etc.—see Figures 2 and 3). To achieve purification of a chosen size cutoff, refer to Table 2.

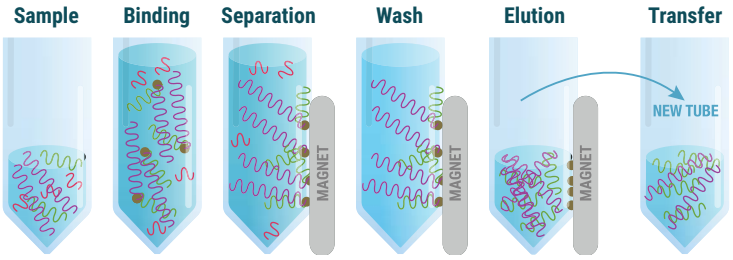

Figure 2. Size-selective purification of dsDNA fragments larger than a given size.

Table 2. Ratios for Size-Selective Purification of DNA Fragments of Various Sizes.[a]

ProNex® Chemistry Ratio (v/v)[a]	Approximate Size Cutoff (bp)	Estimated Equivalent AMPure® XP Ratio[b]
3X	100	1.8X
2X	150	1.5X
1.5X	250	0.95X
1.3X	350	0.75X
1.2X	475	0.65X
1.15X	550	0.625X
1.1X	650	0.6X
1.05X	800	0.55X
1X	1,000	0.5X

[a]The presence of DNA precipitants, such as polyethylene glycol (PEG), in the sample can lead to the retention of lower molecular weight DNA fragments than intended. For proper size selection of samples with DNA precipitants present, the ratio required to achieve desired size cutoffs must be reduced. We strongly recommend performing a pilot purification assay with a disposable sample, such as a DNA ladder, in the sample matrix before attempting to purify an important sample.

[b]We do not recommend specific ratios to be used with other size-selective products, such as AMPure® XP. This information is provided as an approximate guide. Users should determine the optimal ratios empirically.

Note: The ratios of ProNex® Chemistry to sample are higher than those used in other methods with similar size-selective chemistries. If you have experience with another size-selective DNA purification product, such as AMPure® XP or SPRIselect®, do not use the same ratios of binding reagent to sample, as this will result in the loss of desired fragments.

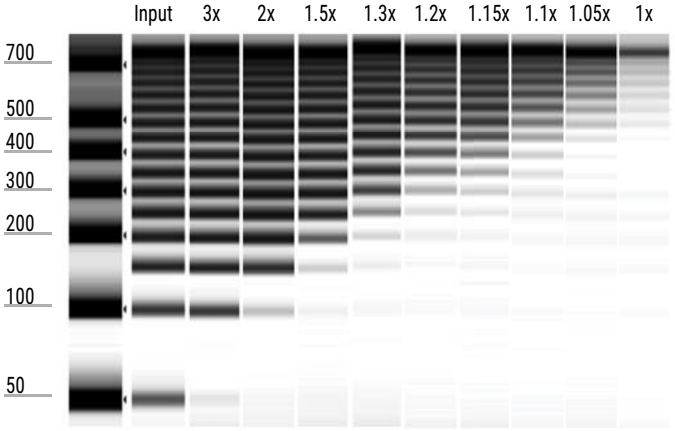

Figure 3. Size selection of DNA with varying ProNex® chemistry:sample ratios.

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6.A. Protocol

This is an example protocol, based on a 50µl input sample volume, that can be used to purify dsDNA fragments ≥100bp while removing contaminants (e.g., buffers, proteins, salts, etc.) and low molecular weight DNA (e.g., dsDNA adapters, ssDNA oligonucleotides and nucleotides) smaller than 50bp (Figures 2 and 3). For smaller or larger input sample volumes, adjust the volumes of the ProNex® Chemistry, Wash Buffer and Elution Buffer proportionally. To achieve purification of a chosen size cutoff other than ≥100bp, refer to Table 2.

1. Allow the ProNex® Chemistry to equilibrate to room temperature for 30 minutes to 1 hour.
2. Pipette a 50µl sample into a tube or well capable of holding up to 250µl.
3. Ensure that the ProNex® Chemistry bottle cap is tightened securely. Resuspend the resin by vigorous vortexing for 10 seconds or longer.
4. Mix 150µl of ProNex® Chemistry into the sample (i.e., a 3:1 v/v ratio of ProNex® Chemistry to sample) by pipetting 10 times.

   Note: For selection of sizes larger than 100bp, use lower ratios of ProNex® Chemistry to sample (v/v) as directed in Table 2.
5. Incubate the sample at room temperature for 10 minutes.
6. Place the sample on a magnetic stand for 2 minutes.
7. Carefully remove and discard the supernatant.
8. Leaving the sample on the magnetic stand, add 200µl of Wash Buffer to the sample and allow it to incubate for 30–60 seconds. Remove and discard the Wash Buffer. For larger samples, increase the volume of Wash Buffer proportionally to the total volume of sample and ProNex® Chemistry.
9. Repeat step 8 (for a total of 2 washes).
10. Allow the sample to air-dry for 5 minutes.

    Notes:

    1. If working with a multichannel pipette and buffer trough, return the unused Wash Buffer to the Wash Buffer bottle at this point. Tighten the bottle cap to prevent ethanol evaporation.
    2. The resin may be allowed to air-dry for longer than 5 minutes. Depending on the sensitivity of downstream applications to ethanol, drying times up to 1 hour can be used. ProNex® Chemistry does not suffer from the loss of high molecular weight DNA upon extended drying.
11. Remove the sample from the magnetic stand.
12. Add 50µl of Elution Buffer and resuspend the resin by pipetting and/or shaking on a plate mixer. Incubate the samples at room temperature for 5 minutes to elute the DNA.

    Note: Depending on the downstream application, you can add more or less Elution Buffer to elute the sample. Higher elution volumes do not result in significant yield increases. However, elution volumes <25% of the starting sample volume can be difficult to work with and may result in some yield loss due to the resin void volume.
13. Return the sample to the magnetic stand for 1 minute, then carefully transfer the eluted DNA to a clean tube or well.
14. Return the ProNex® Chemistry bottle to storage at 2–10°C.

7. Dual Size-Selection of DNA Fragments

The protocol described below is designed to produce a population of dsDNA fragments centered on a desired size (see Figures 4 and 5), removing fragments above and below a chosen size range. The size range and center point of the population can be adjusted based on user needs (see Table 3).

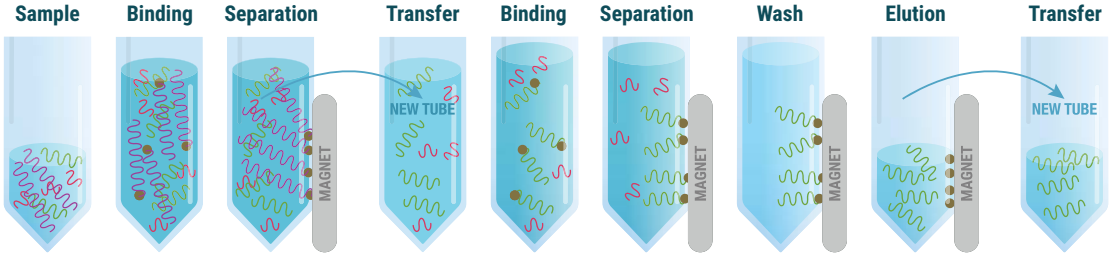

Figure 4. Size-selective purification of dsDNA fragments centered around a desired size.

Table 3. Volumes and Ratios for Dual Size-Selective Purification of DNA Populations of Various Sizes, Based on a 50µl Sample Volume.[a]

Average Insert Size	Target Library Size (Insert + 2 Adapters)[b]	First ProNex® Chemistry Selection		Second ProNex® Chemistry Selection	
		Volume	Ratio	Volume	Ratio
150bp	275bp	62µl	1.24X	20µl	0.4X (1.6X total)
225bp	350bp	55µl	1.1X	17.5µl	0.35X (1.45X total)
325bp	450bp	52.5µl	1.05X	17.5µl	0.35X (1.4X total)
425bp	550bp	50µl	1.0X	15µl	0.3X (1.3X total)
475bp	600bp	47.5µl	0.95X	15µl	0.3X (1.25X total)
575bp	700bp	45µl	0.9X	12.5µl	0.25X (1.15X total)

[a]The presence of DNA precipitants, such as PEG, in the sample can lead to the retention of lower molecular weight DNA fragments than intended. For proper size selection of samples containing DNA precipitants, the ratio required to achieve desired size cutoffs must be reduced. We strongly recommend performing a pilot purification with a disposable sample, such as a DNA ladder, in the sample matrix before attempting to purify an important sample.

[b]Target library size assumes average total basepair length of 125 bases added to insert size.

7. Dual Size-Selection of DNA Fragments (continued)

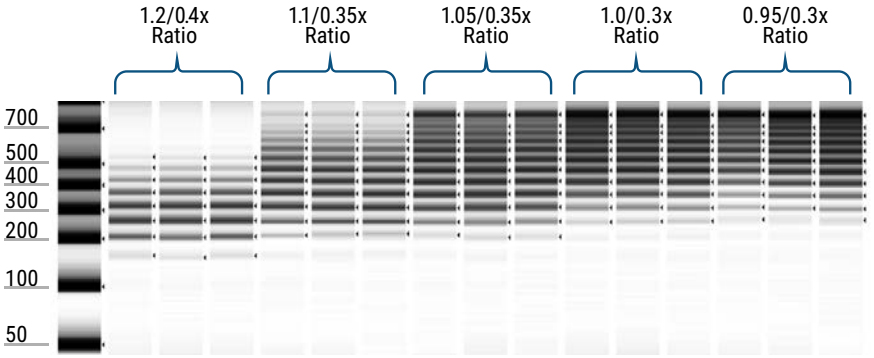

Figure 5. Size-selective purification of DNA with varying ratios of ProNex® Chemistry.

7.A. Protocol

This is an example protocol, based on a 50µl input sample volume, that can be used to produce a population of dsDNA fragments with a peak at 325 bp (Figures 4 and 5). For smaller or larger input sample volumes, adjust the volumes of the ProNex® Chemistry, Wash Buffer and Elution Buffer proportionally. To adjust the size range and center point of the final population, refer to Table 3.

1. Allow the ProNex® Chemistry to equilibrate to room temperature for 30 minutes to 1 hour.
2. Pipette a 50µl sample into a tube or plate well capable of holding up to 250µl.
3. Ensure that the ProNex® Chemistry bottle cap is tightened securely. Resuspend the resin by vigorous vortexing for 10 seconds or longer.
4. Mix 55µl of ProNex® Chemistry into the sample (i.e., a 1.1:1 (v/v) ratio of ProNex® Chemistry to sample volume) by pipetting 10 times.

   Note: For a size-selective cutoff other than 325bp, refer to Table 3.
5. Incubate the sample at room temperature for 10 minutes.
6. Place the sample on a magnetic stand for 2 minutes.
7. Carefully transfer the ***supernatant*** to a clean tube or well.

Caution: Do not discard the supernatant. The desired DNA fragments are in the supernatant at this point and the undesired, high molecular weight DNA fragments will be left bound to the resin.

8. Remove the sample from the magnetic stand.
9. Mix an additional 17.5µl of ProNex® Chemistry into the supernatant sample from step 6 (i.e., a 0.35:1 (v/v) ratio of ProNex® Chemistry to starting sample volume) by pipetting 10 times.

   Note: For a size-selective cutoff other than 325bp, refer to Table 3.
10. Incubate the sample at room temperature for 10 minutes.
11. Place the sample on a magnetic stand for 2 minutes.

12. Carefully remove and discard the supernatant. The undesired, low molecular weight DNA fragments are in the supernatant. The desired, high molecular weight DNA fragments will be left bound to the resin.
13. Leaving the sample on the magnetic stand, add 200µl of Wash Buffer to the sample and allow it to incubate for 30–60 seconds. Remove and discard the Wash Buffer. For larger samples, increase the volume of Wash Buffer proportionally to the total volume of sample and ProNex® Chemistry.
14. Repeat step 13 (for a total of 2 washes).
15. Allow the sample to air-dry for 5 minutes.

    Notes:

    1. If working with a multichannel pipette and buffer trough, return the unused Wash Buffer to the Wash Buffer bottle at this point. Tighten the bottle cap to prevent ethanol evaporation.
    2. The resin may be allowed to air-dry for longer than 5 minutes. Depending on the sensitivity of downstream applications to ethanol, drying times up to 1 hour can be used. ProNex® Chemistry does not suffer from the loss of high molecular weight DNA upon extended drying.
16. Remove the sample from the magnetic stand.
17. Add 50µl of Elution Buffer (or your preferred volume of Elution Buffer, see note below) and resuspend the resin by pipetting and/or shaking on a plate mixer. Incubate the samples at room temperature for 5 minutes to elute the DNA.

    Note: Depending on the downstream application, you can add more or less Elution Buffer to elute the sample. Higher elution volumes do not result in significant yield increases. However, elution volumes <25% of the ProNex® Chemistry volume used in step 9 (the second addition of ProNex® Chemistry) can be difficult to work with and may result in some yield loss due to the resin void volume.
18. Return the sample to the magnetic stand for 1 minute, then carefully transfer the eluted DNA to a clean tube or well.
19. Return the ProNex® Chemistry bottle to storage at 2–10°C.

8. Troubleshooting

Problem	Possible Cause and Recommended Solutions
Yield lower than expected	Improper quantification of purifiable dsDNA. For example, PCR samples contain ultraviolet (UV)-absorbing components, such as nucleotides and oligonucleotides, that contribute to the estimation of DNA quantity but will not be purified by ProNex® Chemistry. It is important to account for only dsDNA fragments that can be purified.
	No addition, addition of the incorrect volume, or addition of the incorrect concentration of ethanol to the Wash Buffer concentrate bottle. (Section 5, step 1)
	Ratio of ProNex® Chemistry to sample lower than recommended. The ProNex® procedure requires higher ratios of ProNex® Chemistry to sample than competitor products. (Section 6, step 4; Section 7, steps 4, 9)
	Insufficient mixing of ProNex® Chemistry into the sample. Ensure that the binding reagent is thoroughly mixed with the sample during the binding step. (Section 6, step 4; Section 7, steps 4, 9)
	Time allowed for binding was too short. Allow the sample and ProNex® Chemistry to incubate for 10 minutes. (Section 6, step 5; Section 7, steps 5, 10)
	Time allowed for magnetic resin attraction was too short. Allow sufficient time for the sample to clear. (Section 6, step 6; Section 7, steps 6, 11)
	Resin pellet was disrupted when the supernatant was removed. If the resin pellet is disrupted during supernatant removal, return the supernatant to the sample well on the magnetic stand, allow the resin to fully clear, then carefully remove the supernatant. (Section 6, step 7; Section 7, step 12)
	Excessive washing steps were employed. Two washes are optimal and additional washes can be detrimental. (Section 6, step 9; Section 7, step 14)
	Ethanol evaporated from the Wash Buffer. Close the bottle tightly to prevent ethanol evaporation and quickly return the Wash Buffer to the bottle when finished with a purification, if using a reagent trough. (Section 6, step 10; Section 7, step 15)
	Elution interval was too brief. Allow sufficient time for the DNA to elute. (Section 6, step 12; Section 7, step 17)

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Problem	Possible Cause and Recommended Solutions
Poor sample-to-sample reproducibility	ProNex® Chemistry was not equilibrated to room temperature before mixing with the sample. Using cold ProNex® Chemistry can increase sample-to-sample variability. (Section 5, step 2; Section 6, step 1; Section 7, step 1)
	Volume of the starting sample was not measured accurately. Be sure to measure the volume of the starting sample accurately. If desired, add Elution Buffer to samples or process a smaller sample volume in order to ensure greater sample-to-sample consistency. (Section 6, steps 2, 4; Section 7, steps 2, 4, 9)
Eluate population average size is smaller or larger than desired	Size-selection was not properly optimized with representative conditions prior to working with important samples. Optimize the purification ratios before attempting purification of important samples. (Section 6, step 4; Section 7, steps 4, 9)
	Ratio of ProNex® Chemistry to sample was lower than recommended. The ProNex® Size-Selective Purification procedure requires higher ratios of ProNex® Chemistry to sample than competitor products. (Section 6, step 4; Section 7, steps 4, 9)
	Volume of the starting sample was not measured accurately. Be sure to measure the volume of the starting sample accurately. If desired, add Elution Buffer to samples or process a smaller sample volume in order to ensure greater sample-to-sample consistency. (Section 6, step 2; Section 7, step 2)
Resin present in eluate	Eluate removed from ProNex® Chemistry too quickly or insufficient time allowed to collect resin with magnetic stand. Allow sufficient time to collect the resin with the magnetic stand and carefully transfer the eluate to a new container. It is possible to repeat the resin collection steps in order to remove residual resin. (Section 6, step 13; Section 7, step 18)
	Eluate removed from ProNex® Chemistry too aggressively. Remove a smaller volume from the eluate or increase the volume of Elution Buffer added to elute the final samples to provide the necessary volume for your downstream applications. (Section 6, step 13; Section 7, step 18)
Ethanol present in eluate	Insufficient drying time. Drying samples for longer than 5 minutes is not detrimental and does not result in loss of high molecular weight DNA. Extend the sample drying time up to 1 hour. (Section 6, step 10; Section 7, step 15)

8. Troubleshooting (continued)

Problem	Possible Cause and Recommended Solutions
Retention of undesired low molecular weight DNA fragments	Presence of PEG or precipitants in starting sample. Many molecular biology sample buffers have PEG and other DNA precipitants, which can cause low molecular weight DNA fragments to bind to the resin. Optimize size-selective purification with a generic dsDNA source (e.g., a DNA ladder) in the same sample buffer before attempting to purify crucial samples. (Section 6, step 4; Section 7, steps 4, 9)
	Incomplete removal of supernatant prior to washing. Carefully and completely remove the supernatant from the resin before washing. (Section 6, step 7; Section 7, step 12)
Retention of undesired high molecular weight fragments	First step of dual size selection was not properly optimized. Increase the ratio of ProNex® Chemistry to sample and allow adequate time for binding to occur. Optimize size-selective purification with a generic dsDNA source (e.g., a DNA ladder) in the same sample buffer before attempting to purify crucial samples. Complete removal of high molecular weight fragments may not be possible when selecting for large populations. (Section 7, steps 4, 5)
	Accidental transfer of resin with supernatant in two-step binding protocol. Allow sufficient time to collect the resin with the magnetic stand and carefully transfer the eluate to a new container without disturbing the resin pellet. If the resin pellet is disrupted during supernatant removal, return the supernatant to the sample well on the magnetic stand, allow the resin to fully clear, then carefully remove the supernatant. (Section 7, steps 6, 7)
Resin clumping in the first binding step of a two-step purification	High molecular weight DNA fragments (e.g., intact genomic DNA) can bind to multiple resin particles and cause the resin to clump. Mix the samples on an orbital plate mixer, by pulse-vortexing, or by repeated pipetting during binding and continue purification according to the protocol. The ProNex® procedure is capable of removing >99% of intact genomic DNA at initial levels up to 200ng/µl. (Section 7, step 4)

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**

9. Appendix

9.A. Composition of Buffers and Solutions

Elution Buffer, Cat.# NG116

10mM Tris (pH 8.5)

9.B. Related Products

Product	Size	Cat.#
ProNex® DNA QC Assay Calibration Kit, 7500	1 kit	NG1001
ProNex® DNA QC Assay ABI 7500/7500FAST	200 reactions	NG1002
	800 reactions	NG1003
ProNex® DNA QC Assay BioRad CFX96™	200 reactions	NG1004
	800 reactions	NG1005
ProNex® DNA QC Assay Software		7002422
MagnaBot® 96 Magnetic Separation Device	1 each	V8151
Deep Well MagnaBot® 96 Magnetic Separation Device	1 each	V3031
MagneSphere® Technology Magnetic Separation Stand, 2 position, 1.5ml	1 each	Z5332
MagneSphere® Technology Magnetic Separation Stand, 12 position, 1.5ml	1 each	Z5342

10. Summary of Changes

The following changes were made to the 2/18 revision of this document:

1. Corrected information about magnetic stand (Materials Required, Section 3) and Deep Well MagnaBot® 96 Magnetic Separation Device (Related Products, Section 9.B).