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Product Application

Automated High-Throughput dsDNA Size Selection or Purification using the ProNex® Size-Selective Purification System on the CyBio FeliX Liquid Handler from Analytik Jena

Purify up to 96 DNA samples simultaneously using the ProNex® Size-Selective Purification System on the CyBio FeliX Liquid Handler within 1 hour.

Kit: ProNex® Size-Selective DNA Purification System (Cat.# NG2002)

Analyses: TapeStation Analysis, qPCR

Sample Type(s): dsDNA

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM508, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- ProNex® Size-Selective DNA Purification System (Cat.# NG2002)
- CyBio FeliX Basic Unit with Enclosure (Analytik Jena, Cat.# OL5015-24-100)
- CyBio FeliX Extraction Set (Analytik Jena, Cat.# OL5015-25-120)
- MagnaBot® FLEX 96 Magnetic Separation Device (Promega, Cat.# VA1290)

Plastic Consumables Used per 96 samples:

- 5 - 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates (Thermo Fisher, Cat.# 278743) for single size selection (6 for dual size selection)
- 1 - Deep Well Reservoir, Four Columns (Analytical, Cat.# 96733)
- 1 - 1000µl CyBio TipRack 96/1000µl, PCR-certified (Analytik Jena, Cat.# OL3811-25-539-N) – *only 8 tips are required*
- 3 - CyBio RoboTipTray 96/250 µL DW (Analytik Jena, Cat.# OL3810-26-661)
- 1 - FrameStar® 96 Well Skirted PCR Plate (4titude®, Cat.# 4ti-0960/C)

CyBio FeliX Method: Pronex_SizeSelection_v1.0.bms

Method Run Time (for up to 96 samples): 46 minutes for single size selection, 57 minutes for dual size selection.

Preprocessing Time: 30 minutes to 1 hour for equilibration of the ProNex® Size-Selective Chemistry to room temperature, 10 minutes hands-on setup.

Protocol:

1. Allow the ProNex® Size-Selective Chemistry to equilibrate to room temperature for 30-60 minutes.
2. Load and run the CyBio FeliX method "*Pronex_SizeSelection_v1.0.bms"* and follow the on-screen instructions. The method is constituted of two parts separated by a break, the instrument and deck setups are illustrated in Figure 1 for the first part and in Figure 2 for the second part. (See details in the next section "Method details").

Note: It is strongly recommended that the conditions intended for use (e.g. input sample volume and ProNex® Size-Selective Chemistry-to-input sample ratios) with this method be tested ahead of processing crucial samples.

Figure 1. Deck setup for ProNex® Size-Selection Purification System on the CyBio FeliX Liquid Handler. Part 1: Addition of ProNex® Size-Selective Chemistry to the wells of the Bead Plate(s) - (2-5min). Schematic representation (left) and photo (right) of the deck setup for part 1. ProNex® Size-Selection Chemistry (paramagnetic beads) is added by the user to a 4-column Reservoir at deck position 8. The instrument then dispenses the selected volume of beads into the selected wells of a 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates at position 9 (first size selection) and at position 12 (for dual size selection only, this position can be left empty for single size selection).

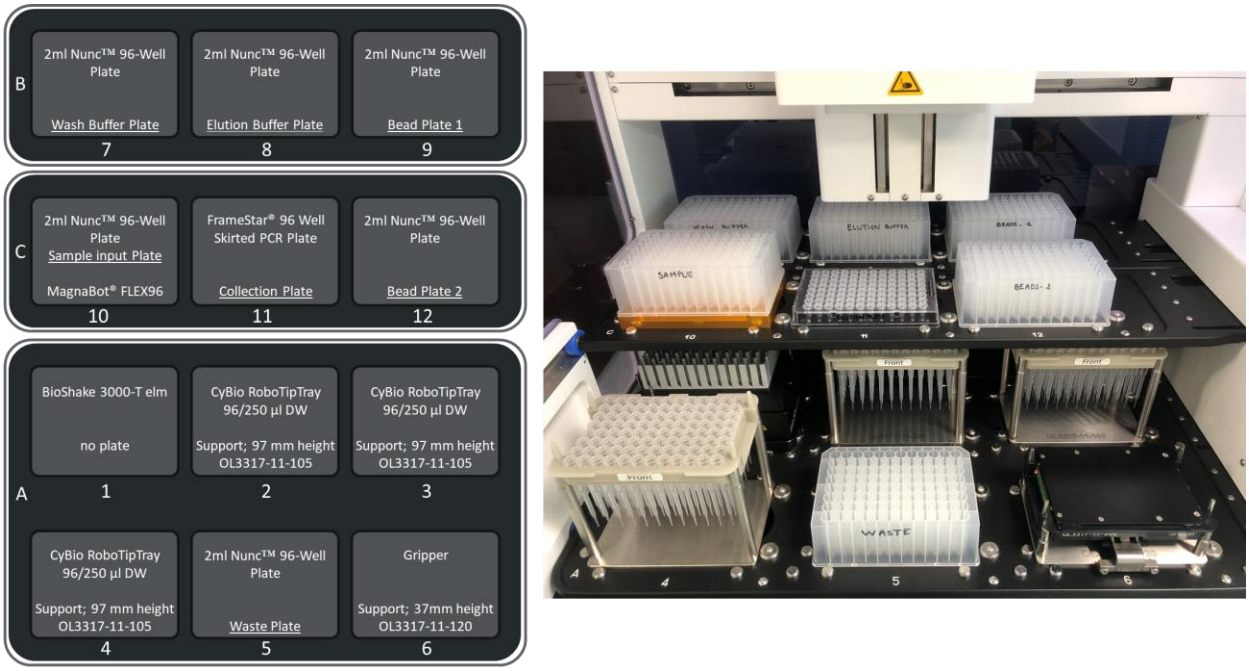

Figure 2. Deck setup for ProNex® Size-Selection Purification System on the CyBio FeliX Liquid Handler. Part 2: Addition of input samples and size selection - (46-57min). Schematic representation (left) and photo (right) of the deck setup for the part 2. The following reagents are dispensed by the user in the selected working wells of 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates: Wash Buffer at position 7, Elution Buffer at position 8, Sample input at position 10 on the MagnaBot® FLEX 96 Magnetic Separation Device. Position 12 can be left empty for single size selection.

Method details:

This automated method allows for single-sided or dual-sided size selection. It is completed in two parts separated by a pause to update the CyBio FeliX deck.

Part I: Bead Plate(s) Preparation

User Selections:

1. The user is instructed to:
   - Determine the number of columns of DNA samples to purify (1-12);
   - Choose between Single or Dual size selection;
   - Select the Bead dispense range (entire columns can be selected);
   - Indicate the column number of the 4-column Reservoir where ProNex® Size-Selective Chemistry will be dispensed from.
   - Indicate the volume of beads for Single (and Dual) size selection(s).
2. The user is then instructed to fill the selected column of the 4-column Reservoir with the calculated amount of ProNex® Size-Selective Chemistry to be used based on the number of columns being processed and the selected bead volume plus dead volume (automated calculation).The user is then instructed on how to setup the deck (Figure 1).

Automated method:

3. The instrument thoroughly tip mixes then transfers the selected volume of ProNex® Size-Selective Chemistry to the selected wells of a processing plate (Bead Plate 1). If Dual size selection was selected, the beads are mixed again and dispensed to the selected wells of a second processing plate (Bead Plate 2).

Part II: Size Selection

User Selections:

1. The user is instructed to determine the desired volumes (in µl): Starting sample volume, Wash Buffer volume per wash (Note: recommended Wash Buffer volume per wash = 200µl; valid input range: 150-250µl), Elution Buffer addition volume, Elution Buffer recovery volume.
2. The user is then instructed to fill the selected working wells of the Sample input Plate, Wash Buffer Plate, and Elution Buffer Plate with the appropriate volumes of sample input, Wash Buffer, and Elution Buffer, respectively. The user is then instructed on how to setup the deck (Figure 2).

Automated method:

3. Sample is transferred from the Sample input Plate to Bead Plate 1.
4. Bead Plate 1 is moved onto the BioShake, and binding is carried out for 3 minutes at 1500rpm followed by 10 minutes without shaking.
5. Bead Plate 1 is moved onto the magnet, incubated for 2 minutes, and the entire supernatant is aspirated and transferred either to the Waste Plate (for single size selection) or to Bead Plate 2 (for dual size selection).
6. **For dual size selection only**: Steps 4 and 5 are repeated with Bead Plate 2 and supernatant is removed and transferred to the Waste Plate.
   At this stage, Bead Plate 1 or 2 is on the magnet and the supernatant has been removed. The active plate is now designated as Bead Plate.
7. Wash Buffer is dispensed to the Bead Plate (without contact) and incubated for 60 seconds.
8. Wash Buffer is aspirated from the Bead Plate and discarded in the Waste Plate.
9. Steps 7 and 8 are repeated for a total of 3 washes.
10. The Bead Plate is incubated for an additional 60 seconds, then any residual Wash Buffer is aspirated and discarded in the Waste Plate. The Bead Plate is incubated for 5 minutes to dry the beads.
11. The Bead Plate is moved on to the BioShake, and the selected volume of Elution Buffer is dispensed. Elution is carried out by tip mixing and shaking (2000rpm for 30 seconds) followed by a 5 minute incubation without shaking.
12. The Bead Plate is moved on to the magnet and incubated for 2 minutes.
13. The specified volume of eluate is aspirated from the Bead Plate and dispensed in the Collection Plate.

Results:

5-fold diluted 100bp DNA ladder (Cat.# G2101) was subjected to single (Figure 3A) or dual (Figure 3B) size selection with the ProNex® Size-Selective Purification System using the *Pronex_SizeSelection_v1.0.bms* method on the CyBio FeliX Liquid Handler as described above (input sample volume = 50µl). The resulting eluates were then analyzed using the TapeStation 4200 System. Additionally, cross-contamination studies were carried out using the *Pronex_SizeSelection_v1.0.bms* method, and no cross-contamination was observed (Figure 4).

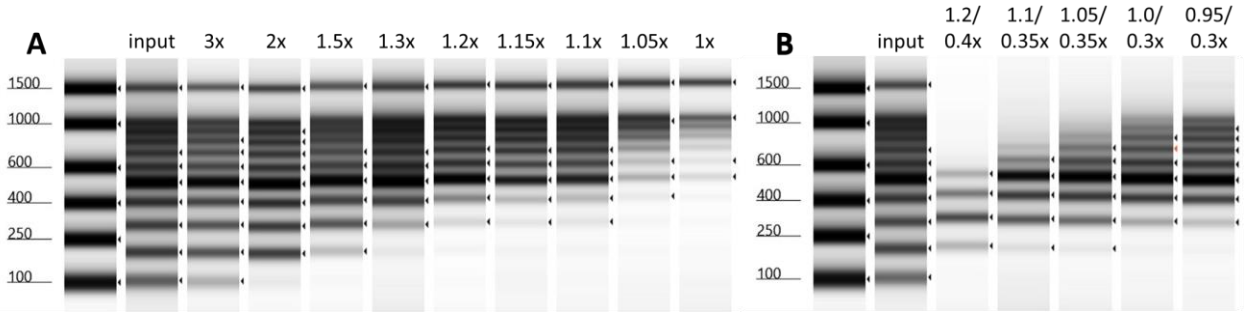

Figure 3. Automated processing of 100bp DNA ladder using the ProNex® Size-Selection Purification System on the CyBio FeliX Liquid Handler. 100bp DNA ladder (Cat.# G2101) was diluted 5-fold in Nuclease-Free Water and processed using the automated method described above with a single size-selective approach (A) or a dual size-selective approach (B). The resulting eluates were analyzed using the TapeStation 4200 System with D5000 ScreenTape and Reagents. Lane 1: input, corresponds to the unprocessed input sample. Other lanes correspond to processed samples; the ProNex® Size-Selection Chemistry-to-input sample ratios are indicated above each lane (example: 3x corresponds to 50µl of input sample and 3x50=150µl of ProNex® Size-Selection Chemistry).

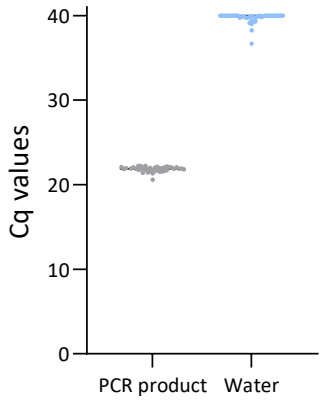

Figure 4. Cross-contamination Study: ProNex® Size-Selective Purification System on the CyBio FeliX Liquid Handler. The input sample was either a 650bp amplicon (grey) or Nuclease-Free Water (blue) arrayed in a checkerboard pattern across the first 10 columns of the plate. The ProNex® Size-Selective Chemistry-to-input sample ratio used was 1.5x. Eluates (n = 40 for each condition) were diluted 10^4-fold in Nuclease-Free Water and then amplified by qPCR using the GoTaq® qPCR Master Mix (Cat.# A6002) and primers targeting the 650bp PCR amplicon. The mean ± standard deviation Cq values were 21.93 ± 0.23 for amplicon samples and 39.84 ± 0.40 for Nuclease-Free Water samples, illustrating the absence of cross contamination. (Cq values for the Negative Template Control: 39.76 and 38.99).