PRINCETON
SEPARATIONS

Electro-Sep™ 5kb-20kb
DNA Recovery Kit, ES-100

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Introduction

This protocol describes how to use the Princeton Separations **Electro-Sep™ 5kb-20kb DNA Recovery Kit** to perform size-selection of DNA fragments in the 5kb-20kb range and recover the fragments using Gene Stix™. It is intended to replace typical DNA purification methods for extracting a DNA band of interest after agarose gel electrophoresis separation. The kit uses a special capture and release device, called the Gene Stix™, along with a simple gel casting modification that enables the user to perform DNA size-selection from a complex mixture of DNA.

Utilize the gel electrophoresis system of your choice, but substitute the PSI Electrophoresis DNA Binding Buffer (working buffer) compatible with using Gene Stix™. The gel-casting DNA Binding Buffer enables the user to cast a SeaKem® Gold Agarose (Lonza Catalog #50152) gel with 0.8% agarose concentration and sample loading wells of your choice. The initial run, after gel casting and sample loading, involves standard electrophoretic separation of up to 4 individual DNA samples plus a DNA reference ladder in the presence of an approved DNA dye.

Part A. Standard Gel Electrophoresis and direct DNA Binding:

Electrophoresis is run under user optimized separation conditions (minimally 0.8% SeaKem® Gold Agarose; 100V, 45 min) (with or without DNA staining dye) depending on the resolution desired. Pulsed Field gel electrophoresis may be used and it will improve the resolution of large DNA fragments but it will take longer. Intercalating DNA stains (like SYBR® Gold and Greenview®) used with Blue LED transluminators are generally thought not to damage DNA and can be used if they are compatible with the user application. The kit can be used free of DNA dyes by calibrating the **DNA Migration Gauge** which comes with the kit.

Preparation of reagents:

Prepare 1 L of working DNA Binding Buffer from concentrate by diluting the **Electrophoresis (5kb - 20kb) DNA Binding Buffer, Concentrate** bottle into a 1 L volumetric flask. Rinse the bottle twice to transfer all of the buffer to the flask and then add reagent grade water to 1.0 L. Mix well.

Preparation of 0.8% agarose 14-Well gel (hand cast):

Prepare 0.8% SeaKem® Gold Agarose gel using the working DNA Binding Buffer (100 g) and 800 mg SeaKem® Gold Agarose (Lonza). Heat to boiling in a microwave (2 min on high), add DI water as needed, cool to 37 deg C before casting the gel. While the 0.8% agarose is still liquid and warm, cast the gel with a 14-well comb in place. Allow the gel to solidify at room temperature for at least 1 hour before loading a sample. Use additional buffer to fill the electrophoresis chambers and submerge the gel.

Running the electrophoresis gel:

Load samples (typically 30 µL) into wells, leaving empty wells between samples. Run the samples in an electric field (100V, 45 min). Longer times and lower voltage will provide better separation and improved resolution.

Calibrating the DNA Migration Gauge (using SYBR® Gold-DNA ladder):

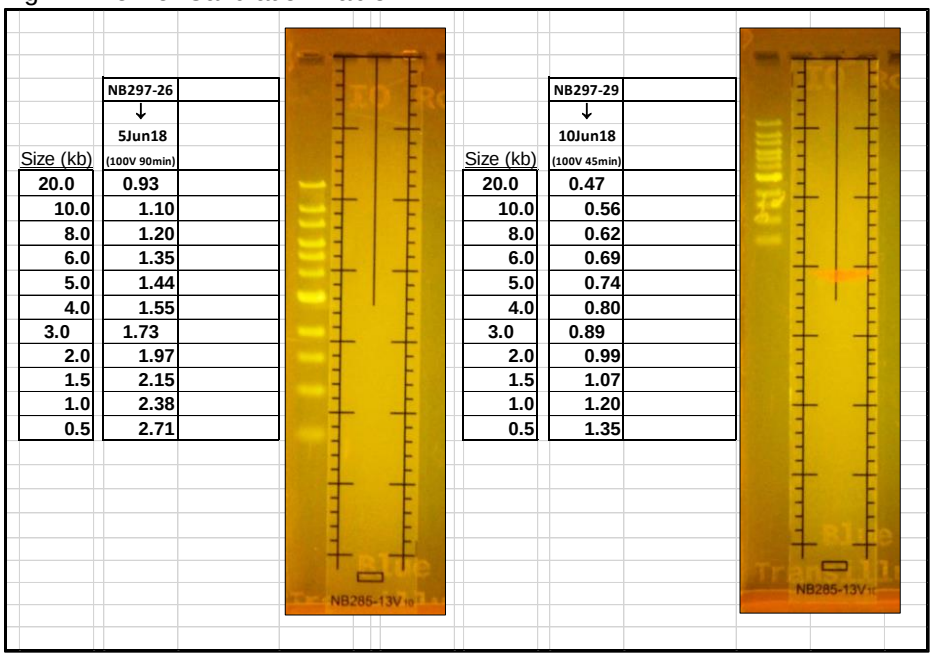
Mix 1kb DNA Ladder (500ng) with 0.5 µL (6X SYBR® Gold) and add buffer to 30 µL. Load this SYBR® Gold-DNA ladder into Lane 1(Ln1), along with other DNA samples in other lanes (Ln3,Ln5,…) and run in the electric field above (100V 45min). Place the gel onto a Blue LED transluminator with required Orange filter to observe the DNA pattern. Position the **DNA Migration Gauge** on top of the gel in Blue light above the DNA calibration pattern (align with the sample wells). Note the position of the DNA calibration pattern on the DNA Migration Gauge and/or photograph the gel for a permanent record. Create a table of DNA fragment size versus the measured distance (Fig. 1). The gauge can now be used as a guide to locate DNA Locator Tabs in unstained gel lanes as long as the electrophoresis conditions (Buffer, Voltage and Time are constant) are not changed.

If your application cannot tolerate DNA dye, it is best to run 2 identical gels - one with dye and one without dye. The DNA dye gel can be used to calibrate the **DNA Migration Gauge** to help position DNA

Locator Tabs in the electrophoresis gel without DNA dye. Alternatively, the user can add SYBR® Gold to a DNA Ladder and include it in one electrophoresis lane.

Use the calibrated DNA Migration Gauge and the user list of DNA fragments to be recovered to determine the marking positions on the gauge. It is recommended that the user mark the gauge (Sharpie™) with the intended locations.

Fig.1 – View of Calibration Table



Size (kb)	NB297-26 ↓ 5Jun18 (100V 90min)	
20.0	0.93	
10.0	1.10	
8.0	1.20	
6.0	1.35	
5.0	1.44	
4.0	1.55	
3.0	1.73	
2.0	1.97	
1.5	2.15	
1.0	2.38	
0.5	2.71	

Size (kb)	NB297-29 ↓ 10Jun18 (100V 45min)	
20.0	0.47	
10.0	0.56	
8.0	0.62	
6.0	0.69	
5.0	0.74	
4.0	0.80	
3.0	0.89	
2.0	0.99	
1.5	1.07	
1.0	1.20	
0.5	1.35	

Using the DNA Migration Gauge:

After the initial size separation (typically 100V, 45 min) (Fig. 2), the gel tray is removed from the electrophoresis chamber and the calibrated DNA Migration Gauge (marked with the locations) is placed on top of the gel, aligning it with the sample well (see Fig. 3a). A DNA Locator Tab is inserted into the gel directly in front of the identified DNA band position. The positioning of the DNA Locator Tab is critical for good results since it is a placeholder for the Gene Stix™ device. Dispose of the DNA Locator Tabs after a single use to prevent cross contamination. (Extra DNA Locator Tabs are included in the kit, so that the user can cut the Tabs in a way to produce a sharp corner.) When inserting the DNA Locator Tab into the gel it is best to cut the surface of the gel with the corner of the Locator Tab and then work the rectangular end to the bottom of the gel until it rests on the bottom of the gel tray. Document the locations of the DNA bands in the gel (we suggest names based on the Lane number, the size of the DNA band of interest and Gauge distances). After all DNA Locator Tabs are inserted into the gel and locations documented, the Gene Stix™ are placed and the DNA Migration Gauge is removed (as described below see Fig. 3b).

Placing the Gene Stix™:

A serialized Gene Stix™ is inserted into the gel lane by positioning it behind the DNA Locator Tab (Fig. 4) and then sliding it down the back of the DNA Locator Tab until it stops at the bottom of the gel. Wetting the Gene Stix™ with Separation Buffer before inserting it into the gel is advised. While holding the Gene Stix™ in the gel, the DNA Locator Tab is pulled out of the gel leaving the Gene Stix™ in its place (Fig. 5). Discard the DNA Locator Tab. Repeat for each DNA band of interest, keeping track of the lane number and DNA band associated with each Gene Stix™. (Note: each Gene Stix™ has a unique serial number and lot number which can be used to keep track of the DNA band locations.)

Alternative Placement Procedure 1:
In the alternative, another way to use the Gene Stix™ is to place several Gene Stix™ into one lane, one behind the other which acts to block DNA of different sizes from migrating into the Gene Stix™ down field from it. DNA Locator Tabs are placed one behind the other based on DNA size, then slide the Gene Stix™ down the back of each Locator Tab and remove the Tabs.

Binding Electrophoresis:
After all Gene Stix™ are positioned in front of the DNA bands of interest, the gel tray is returned to the electrophoresis chamber. Electrophoresis is continued, causing the DNA band(s) of interest to electrokinetically migrate into the Gene Stix™ (Fig. 6). The amount of time needed to bind the DNA molecules to the Gene Stix™ depends on the size of the DNA, its migration rate in the electric field and the distance to the Gene Stix™. Occasionally gas bubbles will build up in the Gene Stix™ forming a thin film which will occlude the DNA from migrating into the Gene Stix™. To prevent this from affecting the DNA binding, stop the electrophoresis about halfway through and shake the Gene Stix so any bubbles which formed will dissipate, then continue the electrophoresis. It is possible to calculate the time needed based on the distances from the calibrated DNA Migration Gauge. (Example: **you run electrophoresis for 90 min, the 10kb band moves 1.10 Gauge units; while 5kb moves 1.44 Gauge units (Calibration Table, Fig1). You want to capture 8kb thru 10kb DNA. The leading edge of 8kb is 1.2 GU, the trailing edge of 10kb is 1.07 GU. You decide to place the Gene Stix at 1.25 GU. You run binding electrophoresis for 15.1 min. [(1.25-1.07)/(1.07/90) = (migration distance of the trailing edge)/(rate of migration of the trailing edge) = binding time]** We recommend at least 20 minutes to complete DNA band binding to Gene Stix™.

Leave the Gene Stix™ in the gel until ready to recover the DNA. The Gene Stix™ are removed from the gel (Fig. 7-9) and the bound DNA is cleaned (washed with water). The DNA is released in a series of Elutions and centrifugation steps, resulting in a highly pure, size-selected DNA sample. The recovered DNA is ready for downstream sequencing or any PCR application desired.

Part B. DNA Recovery Steps:
Gene Stix™ removal, water washing and Elution:

Mark and tare Collection Tubes as needed. Remove the Gene Stix™ from the gel after DNA has bound to it. Wash the Gene Stix™ membrane under running DI water to remove electrophoresis buffer and any agarose gel clinging to the Gene Stix™. Blot the excess water from the Gene Stix™ on a paper towel (do not touch the membrane with your fingers!). Carefully curl (bend) the Gene Stix™ handle using your index finger and thumb and place the curled Gene Stix™ (membrane first) into a marked Spinfunnel™ so that the curved handle just sticks out of the Spinfunnel™. Using the orientation tab on the Spinfunnel™, position the Gene Stix™ so that the curve of the Gene Stix™ handle is under the orientation tab.

Place the Spinfunnel™ into a marked and tared Collection Tube. Elute the DNA from the Gene Stix™ membrane using multiple elutions of 50 µL each of Elution Buffer. (Use regular Elution Buffer or Elution Buffer containing 0.1% Tween - the choice is based on your application). Apply the 50 µL of Elution Buffer evenly to the top edge of the membrane in the Spinfunnel™. Rocking the Spinfunnel™ side to side while adding Elution Buffer to the top of the membrane helps to distribute Elution Buffer evenly across the Gene Stix™ membrane. Place the Collection Tube into the rotor of a table top centrifuge. Orient the Spinfunnel™ so that the curved Gene Stix™ faces the inside of the rotor and the orientation tab faces the edge of the rotor. Be sure to balance the rotor properly. Put the lid of the rotor in place to prevent sample evaporation while spinning. Spin for 2 min at 770 rcf.

Repeat the 50 µL Elution and centrifugation steps. For maximum recovery, elute the membrane with 6X50 µL using Elution Buffer. Each time spin for 2 min at 770 rcf (with the lid in place).

Mix the eluted sample in the Collection Tube by gently pipetting it up and down using a pipette tip (minimize shearing - no mixing by mechanical vortex!). Measure the final weight of the marked Collection Tube and calculate the weight of the DNA sample solution (about 300 mg – 6X50 µL).

Save the Gene Stix™ (membrane and handle) and all the tubes (Spinfunnel™ and Collection Tube) in a PE bag (mark it with lot number and serial number of the Gene Stix™).

Fig. 2 – View of 45 min EP Pattern

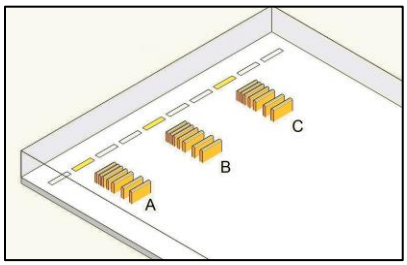


Fig. 3 – Placement of Locator Tabs using a calibrated DNA Migration Gauge

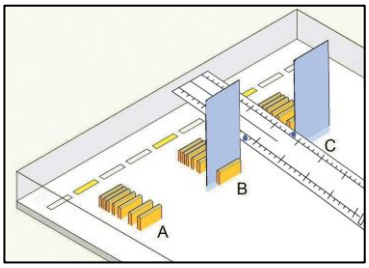


Fig. 3a

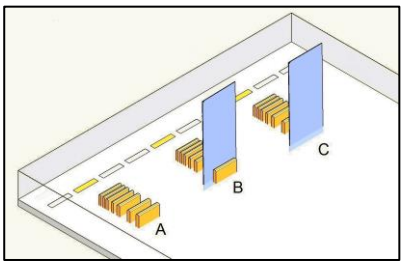


Fig. 3b

Fig. 4 – Placement of Gene Stix™

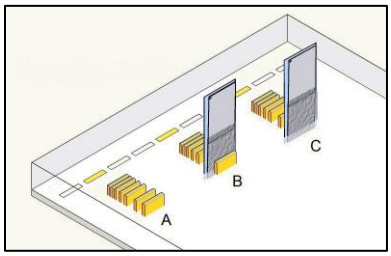


Fig. 5 – Removal of Locator Tabs

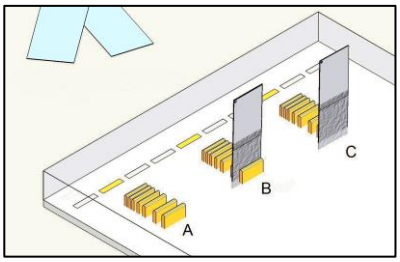


Fig. 6 – View of 55 min EP Pattern with 2 Gene Stix in place

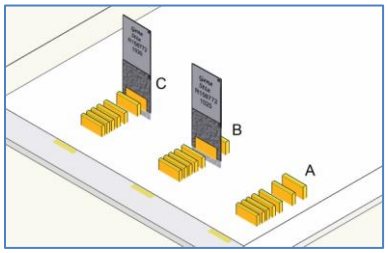


Fig. 7 – View of 55 min EP Pattern and removal of 1st Gene Stix™

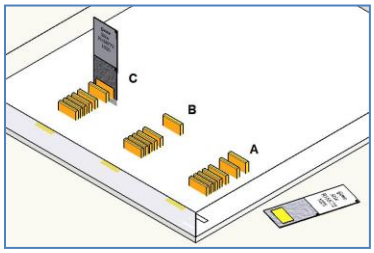


Fig. 8 – View of 55 min EP Pattern after removing a 2nd Gene Stix™

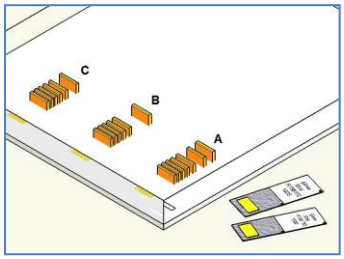
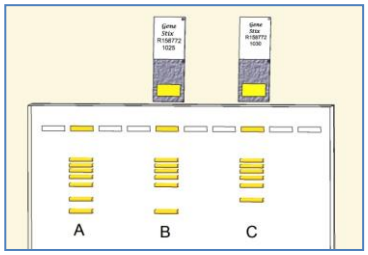


Fig. 9 –Top down view of 55 min EP after removal of the 2nd Gene Stix™



Electro-Sep™ 5kb-20kb DNA Recovery Kit, ES-100, Kit Contents:

- Electrophoresis (5kb - 20kb) DNA Binding Buffer, Concentrate (for 1L working buffer) (3X1L)
- DNA Migration Gauge, (1 per pack)
- DNA Locator Tabs (2X10 per pack)
- Gene Stix™ (10 per pack)
- Spinfunnels™ (10 per pack)
- (5kb-20kb) DNA Elution Buffer (3.1 mL)
- (5kb-20kb) DNA Elution Buffer containing 0.1% Tween (3.1 mL)
- Collection Tube with cap, 2 mL (10 per pack)

Equipment Checklist (items are required but are user provided)

- OWL Easy Cast Electrophoresis System
- SeaKem® Gold Agarose (Lonza Catalog #50152)
- Benchtop Microcentrifuge
- Forceps / Curling device equivalent
- DNA stains (SYBR® Gold, Greenview®, etc) – at user's option

References:

1. Lee, P. Y., Costumbrado, J., Hsu, C. Y., Kim, Y. H.
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   J. Vis. Exp. (62), e3923, doi:10.3791/3923 (2012)
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2. Dretzen, G., Bellard, M., Sassone-Corsi, P., Chambon, P.
   A reliable method for the recovery of DNA fragments from agarose and acrylamide gels.
   Anal. Biochem., 112: 295 (1981)

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