



DNA SizeSelector™-I Kit Protocol

Catalog Numbers: Z-6001-5, Z-6001-50, Z-6001-250

Contents

Protocol Manual Revision 15.1.1

| | |
|-----------------------|---|
| Introduction..... | 2 |
| Process Overview..... | 2 |
| Specifications..... | 2 |
| Materials | 3 |
| Supplement | 6 |

Please refer to <http://www.alinebiosciences.com> Support section for updated protocols and MSDS when handling or shipping any chemical hazards. The information provided is subject to change without notice.



INTRODUCTION

The ALINE SizeSelector-I™ purification system utilizes ALINE’s unique paramagnetic bead technology for quick high-throughput DNA size selection in next generation sequencing library cleanup for all NGS platforms, e.g. Illumina, PacBio, SOLiD, etc.

This kit helps Users to effectively achieve a result of the desired size selection range. DNA fragment size larger or smaller than the DNA of interest is removed during the binding and rebinding steps.

The binding of DNA to magnetic beads is based on the amount of SizeSelector-I™ added into DNA solution. The more SizeSelector-I™ used in a reaction, the smaller size DNA selected. DNA fragment ranging from 200-700bp can be isolated from a population of DNA fragments directly use the procedures in this manual. Selection of other sizes can also be achieved using the information in the Supplement.

PROCESS OVERVIEW

1. Add DNA SizeSelector™ into DNA solution to allow binding of large size DNA onto magnetic beads (this step removes DNA smaller than target DNA).
2. Separate beads containing target DNA from clear supernatant.
3. Add additional beads to supernatant to recover target DNA onto beads.
4. Wash beads twice with 70-80% Ethanol to remove salt and contaminants.
5. Elute target DNA.

SPECIFICATIONS

The ALINE DNA SizeSelector-I™ kit can be performed in a tube and 96-well formats. The following table illustrates the number of reactions a DNA SizeSelector-I™ kit can perform depending on the volume of DNA solution.

Product catalog information

| DNA SizeSelector-I™ Products | P/N |
|------------------------------------|------------|
| DNA SizeSelector-I™ - Small 5 mL | Z-6001-5 |
| DNA SizeSelector-I™ - Medium 50 mL | Z-6001-50 |
| DNA SizeSelector-I™ - Large 250 mL | Z-6001-250 |

Number of reactions in each kit

| DNA Reaction Volume (96 well, µl) | Z-6001-5 (# reactions) | Z-6001-50 (# reactions) | Z-6001-250 (# reactions) |
|--------------------------------------|---------------------------|----------------------------|-----------------------------|
| 50 | 60 | 600 | 3000 |

MATERIALS

Supplied in the Kit:

ALINE DNA SizeSelector-I™ paramagnetic bead Solution

- ✓ **Store at 4°C upon arrival (do not freeze) for up to 12 months**
- ✓ **Mix the reagent well at room temperature** to completely resuspend the beads prior to use. It should show homogenous in visual appearance.

To be supplied by the User:

Apparatus

| Name | Recommended Model | Recommended Vendor and P/N |
|----------------------------|---------------------------------------|---|
| 96-well PCR reaction plate | 96-well round-bottom microtiter plate | Corning, Inc., # 3797, www.corning.com Fisher Scientific # 07-200-105, www.fishersci.com |
| | 96-well cycling plate | ABgene Limited, # AB-0800, AB-1000, AB-1400, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S. |
| | | ABgene product # AB-1111, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S. |
| Magnetic PCR plate | 96-well ring stand | Ambion Inc., (acquired by Applied Biosystems), # AM10050, www.appliedbiosystems.com |
| PCR Plate Seals | Easy Peel Heat Sealing Foil | Abgene Limited, # AB-3739 and AB-3739/s, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S. |
| 1.5mL Eppendorf tube | | |
| Liquid handling robotics | | |
| multichannel hand pipette | | |



ALINE DNA SizeSelector™-I Protocol v15.1.1

Reagents

| Reagents | Steps |
|--|-----------------|
| 80% ethanol, non-denaturing | Washing solvent |
| Nuclease –free water or standard Tris Buffer | DNA elution |

PROCEDURE - FOR DNA SIZE SELECTION RANGE OF 400-600bp

For other ranges, the bead volumes are indicated in the Supplement section.

1. To 50µl of DNA solution (Tube 1), add **70µl SizeSelector-I™** into DNA sample.

NOTE: The ratio of SizeSelector™ to DNA sample is 1.4X. If DNA volume other than 50ul is used, adjust the volume accordingly to maintain the ratio.

2. Pipette-mix for five times and incubate the solution at room temperature for 5 minutes.
3. Pellet beads on a magnet for 1 minute or until the solution is clear.
4. Transfer the supernatant to a fresh tube (Tube 2).
5. Add **20µl of SizeSelector-I™** to the supernatant (into Tube 2) from STEP 4.
6. Incubate at room temperature for 5 minutes to allow target DNA to bind to beads.
7. Separate beads on a magnet for 1 minute or until the solution is clear.
8. Remove the cleared supernatant and discard.
9. Wash the beads by adding 200µl of 80% ethanol and incubate the tube for 30 seconds while

the tube is on magnet. Alternatively, wash by pipetting ethanol five times while the Tube 2 is on magnet.

NOTE: Do not disturb the magnetic beads.

10. Remove 80% ethanol after the final wash and let the beads dry for 2 minutes.

NOTE: If running a submerged gel later, complete drying is recommended.

11. Remove the tube from the magnet and resuspend beads in 20µl of reagent grade water.

12. Place the tube on magnet to settle the beads from solution.

13. Transfer clear DNA solution to a fresh Tube.

14. Examine the DNA size distribution and concentration.

SUPPLEMENT

Selection of different size ranges with Aline DNA SizeSelector-I by using the recommended volume of SizeSelector-I in Steps 1 and 5. The other steps stay the same in all size range selection procedures.

| PCR Reaction Volume | Volume in Step 1 | Volume in Step 5 | Size Selection Range (bp) |
|---------------------|------------------|------------------|---------------------------|
| 50µl | 100 µl | 40 µl | 200-400 |
| 50µl | 80 µl | 20 µl | 300-500 |
| 50µl | 70 µl | 20 µl | 400-600 |
| 50µl | 60 µl | 20 µl | 500-700 |

NOTE: adjust the amount of SizeSelector-I™ accordingly if other DNA size is desired. Add more SizeSelector™ to increase the ratio of SizeSelector™ to DNA if smaller size DNA is desired. Decrease the ratio of SizeSelector™ to DNA if larger size DNA is desired.



This Procedure can also be used for 96-well plate.

Selected reference(s):

KH Wong et al., Multiplex Illumina Sequencing Using DNA Barcoding *Current Protocols in Molecular Biology*, 2013, 101:7.11 1-7.11.11; <http://www.ncbi.nlm.nih.gov/pubmed/23288465>

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