Effective DNA Fragment Selection Using the Axygen® AxyPrep MAG PCR Clean-up Kit

Protocol

Introduction

The Axygen AxyPrep MAG PCR Clean-up kit utilizes solid-phase reversible immobilization technology, which involves the use of paramagnetic beads and polyethylene glycol (PEG)/NaCl buffer to selectively purify DNA fragments for a wide variety of research purposes and applications. According to DeAngelis, et al. (1995), microscopic beads composed of iron oxide can effectively eliminate primers and dimers after PCR to facilitate downstream processes. Furthermore, this kit has the ability to size-select DNA fragments within the 100 to 1,000 bp range. The size-selection protocol and clean-up process is combined into a single procedure, which is rapid and highly automatable. The protocol involves ratio selection, binding, separation, further binding, washing, and elution. Ultimately, the end product is free of contaminants, highly purified, and has a specific size range according to the needs of the scientist.

Product Highlights

- Efficient removal of <60 bp fragments and contaminants
- Manual or automation-compatible
- Fragment selection
- No centrifugation or filtration required
- Simple process completed within 30 minutes
- Compatible with several downstream applications

Application Areas

- PCR
- Gene library preparation
- Fragment selection
- Genotyping and SNP detection
- DNA purification
- Primer elimination

Sample Requirements

- Samples are limited to double-stranded DNA (dsDNA).
- DNA fragments must be dissolved in molecular biology-grade water or a buffer solution such as TE buffer (e.g., 10 mM Tris, 0.1 mM EDTA, pH 8.0). However, some contaminants will remain, such as enzymes and metal ions.
- The sample volume should be at least 50 µL. A smaller sample amount will lead to difficulty in achieving pipetting accuracy, which will ultimately cause poor selection.
- Although linear scale-up is theoretically possible, the abundance of beads in the suspension requires an extra powerful magnetic field to fully isolate the beads. Consequently, we recommend the initial sample volume does not exceed 500 µL.

How it Works

1. The basic principle of the Axygen AxyPrep MAG PCR Clean-up kit relies on the inverse relationship between the binding of DNA fragments of different sizes to the paramagnetic beads and the concentration of PEG in the binding buffer (i.e., for higher PEG concentrations, smaller dsDNA fragments will most effectively bind to the surface of the beads).
2. The reversible immobilization of dsDNA fragments is made possible via a salt bridge established between carboxyl groups on the surface of the beads and the phosphate backbone of the DNA molecules in the presence of cations.
3. Because the product contains a high concentration of salt, it must be washed thoroughly with 70% to 80% ethanol after the separation of beads from the suspension.
4. DNA fragment selection is conducted following a two-step, left- and right-hand selection procedure.
5. DNA purification or elimination of smaller fragments requires only a one-step process.

Axygen® AxyPrep MAG PCR Clean-up Kits

Corning Cat. No.	Description
MAG-PCR-CL-5	Axygen AxyPrep MAG PCR Clean-up kit, small, 5 mL
MAG-PCR-CL-50	Axygen AxyPrep MAG PCR Clean-up kit, medium, 50 mL
MAG-PCR-CL-250	Axygen AxyPrep MAG PCR Clean-up kit, large, 250 mL

Materials Supplied in the Kit

Axygen AxyPrep MAG PCR Clean-up paramagnetic bead solution

- Store at 4°C upon arrival for up to 12 months. Do not freeze.
- Visually, the solution should be homogenous throughout.
- The product remains viable after 12 months; however, the effectiveness may vary among lots after expiration.

Materials to be Supplied by the User

Consumables and Hardware

Description	Recommended Model	Corning Cat. No. (Description)
96-well microplate	96-well round bottom microplate. Microplate selection depends on the PCR volume.	3797 (96-well clear, round-bottom, 360 µL) 3957 (96-well clear, V-bottom, 0.5 mL) 3365 (96-well clear, round-bottom, 360 µL) 3959 (96-well round-bottom, 1 mL) 3961 (96-well V-bottom, 2 mL)
	96-well cycling microplate	PCR-96M2-HS-C (96-well polypropylene half-skirt)
384-well PCR microplate	384-well cycling microplate	PCR-384M2-C (384-well polypropylene, full skirt)
PCR microplate sealing foil	Easy-peel heat sealing foil	HS-300
Multichannel pipettor	Axygen AxyPrep Pipettor	AP-12-200

Reagents

Reagents	Application Step
70% to 80% ethanol	Wash solvent; should be prepared fresh each time
10 mM Tris-HCl, pH 8.0	DNA elution
Water, reagent-grade	
1 x TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)	

Axygen IMAG Handheld Magnetic Separation Devices Selection Guide

Axygen IMAG handheld magnetic devices have been designed and optimized for different Axygen AxyPrep MAG protocols. These magnets are applicable for different tube volumes and plate types, as shown below.

Tube-based Process

Protocol	Corning Cat. No.	Tube Description	Corning Cat. No.
Axygen AxyPrep MAG Kits	SCT-050-SS-C	0.5 mL self-standing, screw cap tube, polypropylene	IMAG-12T (Handheld magnetic separation device for screw cap tubes)
	SCT-150-SS-C	1.5 mL self-standing, screw cap tube, polypropylene	
	SCT-200-SS-C	2.0 mL self-standing, screw cap tube, polypropylene	

Plate-based Process

Protocol	Corning Cat. No.	Microplate Description	Material	Corning Cat. No.
Axygen AxyPrep MAG Kits	3365	96-well round-bottom, 360 µL	Polypropylene	IMAG-96P (Handheld magnetic separation device for 96-well microplates)
	3797	96-well round-bottom, 360 µL	Polystyrene	
	3957	96-well V-bottom, 0.5 mL	Polypropylene	
	3959	96-well round-bottom, 1 mL	Polypropylene	
	3961	96-well V-bottom, 2 mL	Polypropylene	
	PCR-96M2-HS-C	96-well PCR half skirt, 200 µL	Polypropylene	

Size Selection Protocol

1. Preparation

a. Determine the amount of Axygen® AxyPrep MAG PCR Clean-up solution needed for the desired selection range.

Refer to the table below for a general guideline:

Lane	Initial Ratio	Final Ratio	Selection Range	Peak (bp)
1	0.9x	1.1x	220 - 80 bp	232
2	0.8x	1.0x	250 - 320 bp	252
3	0.7x	0.9x	270 - 350 bp	304
4	0.6x	0.8x	300 - 500 bp	352
5	0.5x	0.7x	330 - 700 bp	457
6	0.4x	0.6x	500 - 1,500 bp	1,100

b. Gently shake the reagent bottle to fully resuspend the settled beads.

c. Prepare at least 20 µL of elution buffer and 400 µL of 70% to 80% ethanol solution (dissolved in biomolecular-grade water) for each reaction.

2. First Binding

a. Add the pre-determined amount of Axygen AxyPrep MAG PCR Clean-up solution to the microcentrifuge tube or 96-well microplate. Pipet-mix the solution 5 times and incubate at room temperature for 5 minutes. This process binds the larger DNA fragments to the beads so that the smaller fragments remain in the solution.

The addition ratio designates the volume of reagent by the following formula:

PCR Clean-up Solution volume added = _____ (*Initial ratio*) *x Initial input DNA sample volume*

Note: For example, if input sample volume is 50 µL and the initial ratio is 0.9, the added PCR Clean-up Solution volume = 0.9 x 50 µL = 45 µL.

Pulse-spin the solution to collect all the droplets.

Note: Do not vortex. Vortexing can lead to a poor binding ratio. Incubation at 50°C is optional but not necessary.

b. Place the sample on an Axygen IMAG or other magnetic device to separate the paramagnetic beads from the solution. Wait for 1 min. or until the solution is completely clear.

c. Transfer the supernatant into a fresh microcentrifuge tube or 96-well microplate; the desired DNA fragments are in the solution.

Note: Do not transfer the beads along with the cleared supernatant. Incomplete elimination of beads in the first step results in selection variability.

3. Second Binding

a. Add more PCR Clean-up solution to the new microcentrifuge tube or 96-well microplate and gently pipet-mix 5 times.

PCR Clean-up solution volume added = [___ (*Final ratio*) – ___ (*Initial ratio)*] *x Initial input DNA sample volume*

Note: For example, if input sample volume is 50 µL, the initial ratio is 0.9, and the final ratio is 1.1, the added PCR Clean-up solution volume = (1.1 - 0.9) x 50 µL = 10 µL. So, for second binding step, only 10 µL solution is needed.

b. Pulse-spin the tube or 96-well microplate to collect all droplets, then incubate at room temperature for 5 min. The solution should appear homogenous.

c. Place the tube or 96-well microplate back onto the magnetic device and wait until the solution clears.

d. While the tube or 96-well microplate is still on the magnet, gently aspirate off the supernatant. Be extra cautious not to disturb the beads settled on the sides of the tube; loss of beads at this point is associated with a poor recovery rate.

Note: The target DNA fragments are on the beads.

4. Wash

While the reaction microplate or microcentrifuge tube is still on the magnet, dispense 200 µL of 70% to 80% ethanol into each well. Incubate for 30 seconds at room temperature, gently pipet-mix 5 times, then aspirate off the ethanol. Repeat for a total of two washes.

Elimination of the settled beads will reduce the recovery rate of the final product. After the wash step, remove as much of the ethanol as possible. Residual ethanol may interfere with downstream applications.

Note: Allowing the microplate or microcentrifuge tube to stand for 5 min. at room temperature will enable the remaining ethanol to evaporate completely. Be careful not to dry the beads completely (the layer of settled beads will appear cracked if this happens), as this will reduce the elution efficiency and, consequently, the recovery of DNA fragments.

5. Elution

a. Once the ethanol is fully eliminated, remove the reaction microplate or tube from the magnetic device and add 20 µL of elution buffer [reagent-grade water, Tris-HCl (pH 8.0), or 1 x TE] to each 96-well microplate or tube. Pipet-mix the solution 5 times and incubate at room temperature for 5 min. If the beads remain settled, gently pipet-mix or use a larger elution volume.

Note: The DNA is rapidly eluted off the magnetic beads, and it is not necessary for the beads to go back into solution for elution to occur. More than 20 µL of elution buffer can be used if a larger end-product volume is required, but this will result in a more diluted final product. Use of less than 40 µL of elution buffer will require extra mixing to ensure that the liquid comes into contact with the beads.

b. To separate the paramagnetic beads from the supernatant, place the reaction microplate or microcentrifuge tube onto the magnetic device for 1 min. or until the solution becomes clear. The supernatant now contains the eluted DNA, and the beads may be discarded.

c. Transfer the supernatant to a fresh microplate or microcentrifuge tube for storage and downstream processes.

Reference

DeAngelis MM, Wang DG, and Hawkins TL (1995). Solid-phase reversible immobilization for the isolation of PCR products. Nucleic Acids Research, 23(22):4742-4743.

Appendix: Troubleshooting Guide

Problems of low recovery and incomplete purification are addressed in this troubleshooting guide.

Problem	Possible Cause and Solutions
LOW RECOVERY	**Cause:** Measuring concentration using a spectrophotometer. dNTPs, unincorporated primers and other reaction components can interfere with the O.D. 260 measurement causing higher concentration prior to size selection. **Solution:** Use a different method to determine concentration of starting materials such as Pico Green assay or using the Qubit system.
	Cause: Loss of beads during the selection process. **Solution:** ◗ Axygen® AxyPrep MAG PCR Clean-up solution is viscous and can adhere to the outside as well as inside of the tip. Careful pipetting and the use of low retention tips can help to mitigate this issue. ◗ Care should be taken to prevent accidental aspiration of the beads as well as touching the beads during aspiration and wash steps. If this should occur, return contents back into well, allow beads to reattach to the side of the well and aspirate again. Using a finer tip or aspirating at a slower rate can also assist in preventing bead loss. ◗ Low sample volumes are more likely to have issues with bead loss. Keep the total volume such that the level of liquid is above the level of the magnet in the magnetic separation device.
	Cause: Incompatible fragment size **Solution:** Ensure that an adequate amount of the desired size is present in starting DNA.
	Cause: Insufficient mixing during binding and elution **Solution:** ◗ Mixing during each of the binding steps and elution step is critical to successful size selection. Mix at a volume slightly less (≤10 µL) from the total reaction volume and pipet up and down at least 10 times. ◗ A second mix step is included in the protocol and can assist in maximum DNA binding and recovery. ◗ Incubation times are also important so that the nucleic acid has sufficient time to bind or to dissociate from the beads.
	Cause: Large reaction volume **Solution:** ◗ When working with larger volumes (≥100 µL), it is recommended that the incubation time increase both for binding and elution. ◗ Also recommend increasing time on the magnetic separation device to ensure all beads have been removed from the supernatant prior to aspiration or transfer.
	Cause: Ethanol **Solution:** ◗ The concentration of ethanol used for washes must be at least 70%. ◗ It is recommended that the stock of 100% ethanol be tightly capped when not in use to minimize the amount of water the ethanol absorbs from the atmosphere overtime. ◗ Preparing a stock of 80% ethanol can be used to ensure the concentration of ethanol in the wash solution does not go below the recommended 70%. ◗ Carryover of ethanol can affect downstream applications so care must be taken to ensure the ethanol has evaporated from tubes/wells. After the final wash step, elution buffer can be added to the tubes/wells and incubated at room temperature for a few minutes to allow any excess ethanol to evaporate. The ethanol will continue to evaporate during the elution step.

Appendix: Troubleshooting Guide (continued)

Problem	Possible Cause and Solutions
LOW RECOVERY (continued)	**Cause:** Low elution volume **Solution:** Low elution volume can decrease recovery since the beads retain a small portion of the elution buffer. Depending on the magnetic separation device used, elution buffer can be added to the decrease in recovery. It is recommended that a minimum volume of ≥20 µL be used.
	Cause: DNA fragment dries onto the beads **Solution:** When drying the beads after the final wash, make sure the beads do not dry out completely. Once the DNA has dried completely on the beads, it is difficult to elute them off. One way of preventing this is to add the elution buffer immediately after removing all of the wash buffer from the final wash – allow the elution buffer to incubate in the well for longer period of time to allow any residual ethanol to evaporate.
INCOMPLETE PURIFICATION	**Cause:** Poor performance in downstream applications **Solution:** ◗ Certain applications are more sensitive to trace amounts of ethanol. Adding an additional drying step after elution can be helpful. ◗ Bead carryover is unlikely, but can happen. This can hinder downstream applications by binding to the DNA, thus preventing manipulation of DNA. • During the elution step, it is important to let the microplate/tube sit on the magnetic separation device for a minimum of 2 minutes to ensure all beads are bound. • Care must be taken when pipetting to not disturb the beads during elution. If this occurs, pipet solution back into the well/tube and incubate an additional 2 minutes.
	Cause: No primer dimer removal **Solution:** The correct volume was not used in the purification process. Equal volume of DNA and Axygen® AxyPrep MAG PCR Clean-up Kit are required for primer dimer removal.
	Cause: Primer carryover **Solution:** The correct volume was not used in the purification process. Equal volume of DNA and Axygen AxyPrep MAG PCR Clean-up Kit are required for primer removal.

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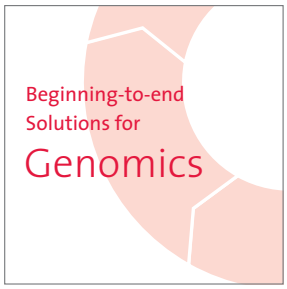

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