One-Step Sequencing Cleanup Kit

Sanger sequencing, also known as "chain terminator sequencing," is a method of DNA sequencing that relies on DNA polymerase's selective incorporation of fluorescent-labeled (bigdye terminator) dideoxy nucleotide chain terminators during in vitro DNA replication for use in a single sequencing reaction. The chain termination PCR produces DNA fragments of varying lengths, each of which ends with a fluorescently labeled dideoxynucleotide. It functions similarly to standard PCR, with one key exception. In contrast to regular PCR, a low ratio of modified nucleotides is introduced along with normal dNTPs. These modified dNTPs are known as dideoxynucleotides (ddNTPs) and have a fluorescent label known as Dye. It quickly became the most used sequencing method for a variety of applications, including de novo sequencing, mutation discovery and confirmation, and resequencing.

After the cycle sequencing reaction, it is necessary to remove contaminants from DNA extension products (e.g., unincorporated fluorescent dyes, residual salts, dNTPs, primers, and enzymes). These impurities, such as dye peaks or "dye blobs," can frequently interfere with the quality and signal intensity of sequencing data, obscuring sections of the sequencing chromatogram and interfering with the base-calling accuracy of sequencing analysis tools. To clean and purify the sequencing reaction extension items on the market, a number of dye-terminator removal (sequencing clean up or sequencing purification) products are available. However, those sequencing purification protocols are either time-consuming (for example, using a spin column, ethanol precipitation, and SPRI paramagnetic beads) or result in sample loss due to protein precipitation, several transfer stages, and a lengthy process.

BcMag™ One-Step Sequencing Cleanup Kit is specifically designed for fast and efficient purification of the post-Sanger Sequencing reaction. The entire protocol takes only one tube and is complete in less than 5minutes (Fig 1). The magnetic beads are added directly to the finished sequencing reactions and vortexed to capture the impurities (e.g., unincorporated dyes, dNTPs, residual salts, and other interfering components). After vortexing, the beads are magnetically captured, while the clean supernatant can be directly loaded onto a capillary sequencer.

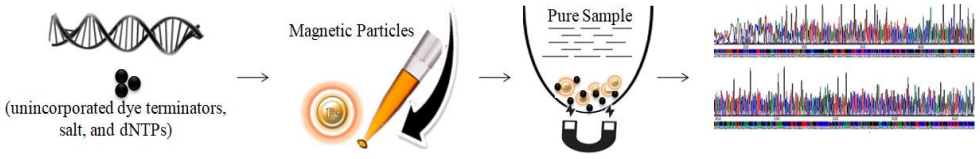

Fig.1 Workflow of sequencing cleanup

Features and Advantages:

- One tube, 3 min protocol, No sample loss
- Reliable results: excellent Long and short fragment recovery, Q20 read length > 800 bases
- Cost-Effective: Tremendously reduced labor costs and other consumed material such as columns, filters, laborious repeat pipetting, and ethanol.
- High-throughput: Compatible with many different automated liquid handling systems.
- Compatible with BigDye XTerminator run modules, e.g., unnecessary to remove the magnetic beads from the tube, the supernatant can be directly loaded onto the capillary sequencer.
- Efficient removal of any dye terminator

Handling and Storage

- Store at 4°C upon arrival for up to 6 months.

Components	Storage	100 preps, Cat # AI-101	250 preps, Cat # AI-102
BcMag™ One-Step Sequencing Cleanup Kit	4°C	1ml	5 ml

PROTOCOL

IMPORTANT!

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The following protocol is optimized for the efficient purification of sequencing reactions containing 2 µl (1:4 dilution of the terminator) or less of BigDye Terminator v3.1 and v1.1 or other dye terminators in a total reaction volume of 10 µl. If an alternative reaction scale or dye terminator is used, the procedure may need to be optimized.

There are two methods of purifying the DNA sequencing reaction products.

1. Using 96-well PCR plates/tubes
2. Using 96-Well Microplates.

Materials Required by the User

Item	Source
18.2 MΩ.cm, DNase/RNase-Free Ultrapure Water	
BcMag™ 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible racks.	Bioclone, Cat#: MS-06
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and Speed should be optimized, and the mixer should be Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
PCR plates/tubes ** ***IMPORTANT!*** If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates has to be ≥2.5mm.	
0.2 ml 96-well PCR Plates	Applied Biosystems, Cat #: 4306737
Olympus 0.2 ml 96-Well PCR Plate, Non-Skirted	Genesee Scientific, Cat #: 24-300
0.2 mL Olympus 8-Strip PCR Tubes	Genesee Scientific, Cat #: 24-706)
0.2mL Axygen™ 8-Strip PCR Tubes	Fisher, Cat #: 14-222-252)
Addition items are required if using 96-well microplates	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and Speed should be optimized, and the mixer should be: Orbit ≥3 mm-4 mm, Speed≥ 800 rpm	
Fisher Scientific™ Microplate Advanced Vortex Mixers	Fisher, Cat#:02-216-101
OHAUS Microplate Vortex Mixers	OHAUS, Cat#:30392160
IMPORTANT! If using 96-well microplates, choose clear Flat-bottom Non-Binding Assay Microplates.	
Thermo Scientific™ 96-Well Microtiter™ Microplates	Fisher, Cat #: 14-245-142, or 14-245-71
Greiner Bio-One 96-Well Non-Binding Microplates	Fisher, Cat #: 07-000-090
Eppendorf™ MTP 96 Microplates	Eppendorf Cat #: 951040048

Procedure

A. Prepare the Premix Beads solution

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IMPORTANT!

- The magnetic beads and water can be added as a premix or sequentially.
- Do not allow the magnetic beads to sit for more than 2 minutes before dispensing**.**
- Before pipetting, shake or vortex the bottle to completely resuspend the Sequencing Magnetic Beads

1. Prepare a fresh Master Mix following Table1 for the number of samples to be processed, plus 10% more (e.g., if you have 10 samples, prepare Master Mix for 11). Add the following components to the reservoir.
2. Add the following components to each well/or reagent reservoir based on table 1.
3. Add 52 µL premix the beads solution to each well of PCR plate or PCR tube (using 96-well PCR plates/tubes method) or each well of the microplate (using 96-Well Microplates).

IMPORTANT!

- Pipet up and down premix beads solution in a reagent reservoir until the solution is homogeneous before dispensing.
- Do not allow the magnetic beads to sit for more than 5 minutes before dispensing.

Table 1

Component	One well (10 µL reaction volume)
Sequencing Clean Beads	7 µL
Ultrapure Water	45 µL
Total	52 µL

B. Sample Processing

1. After cycle sequencing is complete, remove the seal or cap and add 75 µL **Ultrapure Water to each well/tube** and mix well to make a sequencing solution.
2. Aspirate 10 µL sequencing solution to each well of plate or PCR tube in premix beads solution in step A3. (** if necessary, centrifuge at 2500 rpm for 30 seconds to bring all contents to the bottom)
3. Vortex according to table 2 or table 3

Table 2

96-well PCR plates/PCR tubes		
Vortexer	Speed	Time
Eppendorf MixMate	2000 rpm	10 minutes
Mix-3000 Smart Mixer	2000 rpm	5minutes
IMPORTANT! The vortex step is critical to get the best results. We strongly recommend the use of the above vertex mixers and vortex conditions. Other compatible vortex mixers may be used. However, the vortex mixer must meet the following specificities: Orbit ≥1.5mm and minimum speed ≥2000 rpm) and the vortex condition such as Speed and time has to be optimized.		

Table 3

96-Well Microplates		
Vortexer	Speed	Time
Fisher Scientific™ Microplate Advanced Vortex Mixers	800 rpm	3 minutes

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OHAUS Microplate Vortex Mixers	800 rpm	3 minutes
IMPORTANT! The user can use other compatible vortex mixers. However, the mixing condition should be optimized, such as time and Speed, and the mixer should be Orbit ≥3 mm-4 mm, Speed ≥ 800 rpm		

4. After vortexing, place the sample plate or PCR tube on the magnetic separation plate for 30 seconds or until the solution is clear.
5. Centrifuge at 2500 rpm for 30 seconds to remove bubbles
6. Place the sample plate or PCR tube on the magnetic separation plate for 30 seconds or until the solution is clear.

C. Prepare the reaction plate for the capillary sequencing instruments. (***IMPORTANT!*** Do not heat or use formamide with the sample.***)***

96-well PCR plates/PCR tubes	Direct injection	1. Remove the plate from the magnetic rack and place a septum on the plate. 2. Place a septum on the plate 3. and load the reaction plate in the sequencer. 4. Choose a BDX run module specified in the BigDye XTerminator™ Purification Kit User Guide (Pub. No. 4374408). 5. Modify the run module by : • adjusting injection to 25 seconds • adjusting injection voltage to 1200 v The original Bigdye X-terminator Purification Kit run modules are available at www.thermofisher.com/sangerpatches. 6. Start the electrophoresis run
	Indirect injection	1. Transfer 20-40 µL of the supernatant to a new plate, then place a septum on the plate. (** Make sure there is no bubble in the well. If necessary, centrifuge at 2500 rpm for 30 seconds to remove the bubble.) 2. Place a septum on the plate 3. load the reaction plate in the sequencer. 4. Choose an appropriate run module 5. Modify the run module by : • adjusting injection to 25 seconds • adjusting injection voltage to 1200 v 6. Start the electrophoresis run
96-Well Microplates	Indirect injection	1. Transfer 20-40 µL of the supernatant to a new plate, then place a septum on the plate. (** Make sure there is no bubble in the well. If necessary, centrifuge at 2500 rpm for 30 seconds to remove the bubble.) 2. Place a septum on the plate 3. load the reaction plate in the sequencer. 4. Choose an appropriate run module 5. Modify the run module by : • adjusting injection to 25 seconds • adjusting injection voltage to 1200 v 6. Start the electrophoresis run

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D. Troubleshooting

Problem	Probable cause	Suggestion
Dye Blobs (Dye artifacts)	Too much Dye	The protocol is optimized for 2 µl or less of ABI BigDye Terminator v3.1. If possible, use 2 µl or less. or increase the amount of the magnetic beads.
	Insufficient DNA template in the reaction	Increase DNA template concentration.
	Incomplete removal of Dye	• Optimize vortex time and Speed. • Use the right vortex mixer. • Use the right PCR tube or PCR plate
	The magnetic beads are not properly suspended during dispensing	Thoroughly resuspend the magnetic beads before using them.
Weak signal	Improper reaction conditions	Ensure a control sequencing reaction is performed during each thermocycling procedure and optimize reaction conditions if necessary.
	Injection time too short	Increase injection time to 35-40 seconds.
	Extension product concentration is too low.	Increase DNA template concentration.
	Vortex time is too long, or vortex speed is too fast	• Optimize vortex time and Speed. • Use the right vortex mixer. • Use the right PCR tube or PCR plate

Related Products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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