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Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: 5.310
Product: MPG® Streptavidin (10 mg/ml, 4-6 × 10^7 particles/ml)
Procedure: Isolation and purification of an immobilized single strand DNA template.
Storage: 4°C, DO NOT FREEZE

PRODUCT NUMBER	DESCRIPTION	VOLUME
MSTR0502	MPG® Streptavidin, 5 µm, 50 nm (500 Å) pore diameter	2 ml (20 mg)
MSTR0510		10 ml (100 mg)

General Procedure

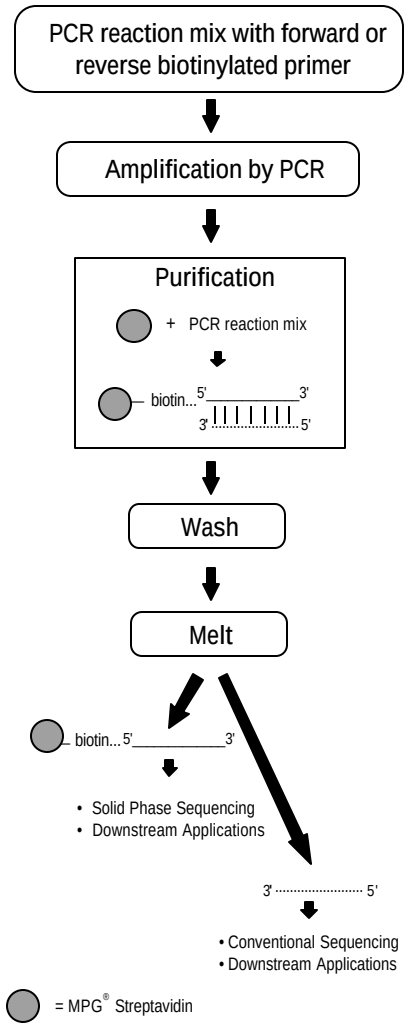
THIS PROTOCOL CAN BE OPTIMIZED FOR ISOLATION OF 1 TO > 3 KILOBASE PCR FRAGMENTS BY FOLLOWING THE MODIFICATIONS IN APPENDIX A.

Materials: (MPG® Streptavidin 10 mg/ml, suspended in PBS, pH 7.5, 0.1% BSA, 0.02% NaN_3)

Plasmid containing DNA of Interest
Forward PCR Primer
Reverse PCR Biotinylated Primer
Taq DNA Polymerase
2 -Deoxycytidine 5 -Triphosphate, Disodium Salt (dCTP)
2 -Deoxyadenosine 5 -Triphosphate, Disodium Salt (dATP)
2 -Deoxyguanosine 5 -Triphosphate, Disodium Salt (dGTP)
2 -Deoxythymidine 5 -Triphosphate, Disodium Salt (dTTP)
Tris Hydrochloride (Tris-HCl)
Potassium Chloride (KCl)
Sodium Chloride (NaCl)
EDTA, disodium ($C_{10}H_{14}N_2O_8Na_2$)
Mineral Oil
Magnesium Chloride, Hexahydrate ($MgCl_2$)
Tween 20
Sodium Hydroxide (NaOH)
Sterile, Nuclease-free, Deionized Water (dH_2O)
Magnetic Particle Separator Prod No. MPS0301 or MPS0001
PCR Tubes (Nuclease-free)
1.5 ml Nuclease-free Microcentrifuge Tubes
Vortex Mixer
Thermal Cycler
Nuclease-free Pipette and Pipette Tips
Low Speed Rotator

Solution	Preparation
PCR Buffer (20 mM Tris-HCl, 50 mM KCl, 0.1% Tween 20, 2.0 mM $MgCl_2$, 200 µM of each dNTP, pH 8.3)	Dissolve 3.16 mg Tris-HCl, 3.73 mg KCl, 410 µg $MgCl_2$, 1 µl Tween 20, 110 µg dATP, 100 µg dTTP, 100 µg dCTP, and 100 µg dGTP in 900 µl Nuclease-free Water. Adjust pH to 8.3 and bring volume to 1 ml with dH_2O. Store at -20°C.
Binding/Wash Buffer (2.0 M NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5)	Dissolve 158 mg Tris-HCl, 11.7 g NaCl, and 37 mg EDTA in 80 ml dH_2O. Adjust to pH 7.5 and bring volume to 100 ml with dH_2O.
Melting Solution (0.1 M NaOH)	Dissolve 400 mg NaOH in 99.6 ml dH_2O.
TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	Dissolve 158 mg Tris-HCl and 37 mg EDTA in 80 ml dH_2O. Adjust to pH 8.0 and bring volume to 100 ml with dH_2O.

Schematic diagram:



THIS PROTOCOL CAN BE OPTIMIZED FOR ISOLATION OF 1 TO > 3 KILOBASE PCR FRAGMENTS BY FOLLOWING THE MODIFICATIONS IN APPENDIX A.

PCR Amplification of DNA Sample

1. Combine in a PCR tube: Plasmid containing DNA fragment to be amplified, 1 µl (15 pmole) Forward PCR Primer, 1 µl (5 pmole) Reverse PCR Biotinylated Primer, 2.5 units *Taq* DNA Polymerase, 50 µl PCR Buffer, and enough Mineral Oil to cover the reaction.

2. Program the Thermal Cycler for a 25 cycle reaction using the following parameters:

Number of Cycles	Time	Temperature
1	2 minutes	94°C
25	1 minute	94°C
	1 minute	50°C
	2 minutes	72°C
Hold	Indefinite	4°C

Note: Increase temperature to 65°C, if non-specific bands are seen.

3. Mix 5 µl of the reaction with loading dye and electrophorese in a 1% agarose gel with molecular weight markers. Sequencing should only be performed if the insert fragment is the primary band in the gel. Yield should be approximately 2 to 4 µg of dsDNA insert. Store at -20°C prior to sequencing.

Preparation of MPG® Streptavidin for DNA Template Isolation

1. Warm the MPG® Streptavidin to room temperature.

2. Vortex to resuspend the MPG® Streptavidin particles and transfer 10 to 60 µl (typically, 20 µl (200 µg)) into a microcentrifuge tube. Add 20 µl Binding/Wash Buffer per 200 µg MPG® Streptavidin particles and mix well. Magnetically separate using a Magnetic Particle Separator and carefully remove the supernatant. Repeat one more time.

3. Resuspend the 200 µg MPG® Streptavidin in 40 µl of Binding/Wash Buffer.

Isolation of Amplified DNA Template with MPG® Streptavidin

1. Thaw the pooled, amplified dsDNA (from *PCR Amplification of DNA Sample* Section, Step 3).

2. Add 40 µl of amplified dsDNA to the MPG® Streptavidin in Binding/Wash Buffer. Gently rotate or vortex at intervals of 30 seconds for 15 minutes. Magnetically separate and remove the supernatant.

3. Resuspend the dsDNA-bound MPG® Streptavidin particles in 40 µl of Binding/Wash Buffer. Magnetically separate and remove the supernatant.

4. To denature the dsDNA resuspend in 10 µl of Melting Solution and incubate at room temperature for 10 minutes. Magnetically separate and carefully remove the supernatant.

5. Resuspend the ssDNA-bound MPG® Streptavidin particles in 50 µl of Melting Solution. Magnetically separate and immediately remove the supernatant.

6. Resuspend the ssDNA-bound MPG® Streptavidin particles in 40 µl Binding/Wash Buffer. Magnetically separate and remove the supernatant.

7. Resuspend the ssDNA-bound MPG® Streptavidin particles in 50 µl TE Buffer. Magnetically separate and remove the supernatant.

8. Resuspend the ssDNA-bound MPG® Streptavidin particles in 5-10 µl of dH_2O .

Sequencing of Purified Template Strand

1. The above sample is ready to be used in a manual or automated sequencing protocol of your choice. Sequencing Primer is annealed to the ssDNA-bound MPG® Streptavidin particles, followed by extension and termination reactions.

Technical Notes

A. The most common cause of faint or absent PCR product in the agarose gel is excess primer in the PCR reactions. Unused primer binds more easily to streptavidin than does a biotinylated PCR product and can occupy most of the biotin binding sites on the streptavidin. The excess primer and unincorporated nucleotides can be removed from the PCR product with a gel filtration spin column designed for DNA. While this may be an expensive routine procedure, it is a good way to test the PCR reaction, the primers and the MPG® Streptavidin.

B. Always, at least while evaluating this protocol, analyze the PCR product in an agarose gel following gel filtration. Some gel filtration media irreversibly binds DNA. Running an agarose gel is strongly recommended in PCR Amplification of DNA Sample, Step 3.

C. Biotinylated primers can be tested by electrophoresis of the primers on a 20% polyacrylamide gel with markers and staining with Stains-All or, perhaps, by UV shadowing. The biotin label

will make the oligonucleotide appear 3 to 4 bases larger (e.g., a 17-mer will run at the molecular weight of a 20-mer or 21-mer).

D. *MPG® Streptavidin can be tested by binding a biotinylated reporter molecule. One method is to biotinylate one end of a restricted, cloned insert with Klenow fragment and biotin-dNTP. Restrict the other end and label with ^{32}P-dNTP using Klenow fragment; low specific radioactivity should be adequate. Bind the restriction fragment to the beads and wash with Binding/Wash Buffer, monitoring radioactivity at each step in the procedure. If the fragment binds to the beads, the beads will be radioactive.*

APPENDIX A[a]: Isolation of 1 to > 3 kb PCR Fragments Protocol Modification

High Salt Binding/Wash Buffer

1. Replace the Binding/Wash Buffer with High Salt Binding/Wash Buffer: 5.0 M NaCl, 1 mM EDTA, 10 mM Tris-HCL, pH 7.5.

Preparation of MPG® Streptavidin for DNA Template Isolation

1. Warm the MPG® Streptavidin to room temperature.
2. Vortex to resuspend the particles and transfer 60 µl into a microcentrifuge tube. Add 100 µl High Salt Binding/Wash Buffer and mix well. Magnetically separate using a Magnetic Particle Separator and carefully remove the supernatant with a pipette. Repeat one more time.
3. Resuspend the MPG® Streptavidin in 100 µl High Salt Binding/Wash Buffer.

Isolation of Amplified DNA Template with MPG® Streptavidin

1. Thaw the pooled, amplified dsDNA.
2. Add 100 µl of the amplified dsDNA to the MPG® Streptavidin in High Salt Binding/Wash Buffer. Gently rotate or vortex at intervals of 30 seconds at 50°C for 30 minutes. Magnetically separate and remove the clear supernatant.
3. Resuspend in 100 µl of High Salt Binding/Wash Buffer. Magnetically separate and remove the supernatant.
4. Resuspend in 20 µl of Melting Solution to denature the dsDNA. Incubate at room temperature for 10 minutes. Magnetically separate and carefully remove the supernatant.
5. Resuspend the ssDNA-bound MPG® Streptavidin particles in 100 µl of Melting Solution. Magnetically separate and immediately remove the supernatant.
6. Resuspend the ssDNA-bound MPG® Streptavidin particles in 200 µl of TE Buffer. Magnetically separate and remove the supernatant.
7. Resuspend the ssDNA-bound MPG® Streptavidin particles in 10 µl to 15 µl of dH_2O.

[a]Based on the following reference: Kotsopoulos, S.K. and Shuber, A.P. (1996) Isolation of 3.5-kb Fragments on Magnetic solid Supports. *Biotechniques* 20,198-200.

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RELATED PRODUCTS

STAQ0250	Taq-*FORCE*™ Amplification System 250 U
STAQ1000	Taq-*FORCE*™ Amplification System 4 x 250 U
STAQ050H	Taq-*FORCE*™ Amplification System (High Concentration) 500 U
STAQ200H	Taq-*FORCE*™ Amplification System (High Concentration) 4 x 500 U
SSP5001	SOLID*script*™ Solid Phase cDNA Synthesis Kit
MTIK1010	MPG® DNA TempPrep™ Kit