Precipio, Inc.

Sanger Sequencing Protocol

Sanger Sequencing Protocol (ABI 3730XL) for ICE COLD-PCR Product Including PCR Purification and Cycle Sequencing/Cleanup

Table of Contents

Notes for this Assay

This procedure reflects the Precipio Standard Operating Procedures for Purification of PCR Products which includes the AMPure XP® PCR Purification Kit, Cycle Sequencing of AMPure XP Purified PCR Products, and Dye-Terminator Removal from Cycle Sequencing Reactions using the CleanSEQ® Dye-Terminator Removal Kit.

Reagents and Equipment Required but not Supplied

Equipment:

1. Calibrated Pipettes, P-1000, P-200, P-100, P-20, P-10, and P-2 or equivalent.
2. Multichannel Pipettes, 1-10 µL, 5 µL – 50 µL and 50 µL – 300 µL or equivalent
3. Repeat Pipettor
4. Plate Spinner
5. SPRIPlate 96-well magnetic plate, Agencourt Product #: A29164 or equivalent
6. NIST Traceable Digital Timer, FisherBrand Model 14-649-11 or equivalent
7. Lab-Line Instruments, Incubator Model 203 or equivalent
8. Thermal Cycler, GeneAmp PCR System 9700 or equivalent
9. Fragment Analysis System, ABI 3730XL or equivalent

Reagents and Consumables:

1. Nuclease-free, aerosol resistant pipette tips
2. DistriTips Syringe, 125 µL volume
3. DistriTips Syringe, 1250 µL volume
4. 0.2 mL, nuclease-free, thin-walled PCR tubes and caps
5. 96-Well PCR Plate
6. Cap Strips for PCR plates
7. Sterile Reservoirs
8. Sterile 1.7 mL tube
9. 15 mL Sterile Conical Tubes
10. Sequencing Plate Raised Skirt
11. AMPure XP Reagent (Beckman Coulter, Brea, CA)
12. Ethanol, 200 Proof
13. Molecular biology grade water
14. Big Dye v3.1 (Thermo Fisher Scientific, Waltham, MA)
15. Better Buffer (Gel Company, San Francisco, CA)
16. Sequencing Primer
17. CleanSEQ Reagent (Beckman Coulter, Brea, CA)
18. 0.5M EDTA

Workplace Preparation Suggestions

1. Wipe down the surfaces 10% bleach, followed by DNA Away.
2. Wipe down the pipettes with 10% bleach, followed by DNA Away.

Sanger Sequencing Protocol – Preparing ICE COLD-PCR Products for Sanger Sequencing Analysis

1. Purification of ICE COLD-PCR Products

NOTE: Please use an appropriate pipetteman, multichannel pipetteman or repeat pipettor for all steps below.

a. If not already done, prepare, in a sterile bottle, a 70% ethanol solution using 200 proof ethanol and molecular biology grade water (e.g. 700 mL 200 proof ethanol and 300 mL molecular biology grade water).
b. Mix the AMPure XP reagent thoroughly before use by gently shaking the bottle to re-suspend the magnetic particles. It should appear homogeneous in color.
c. PCR product and AMPure XP volumes:
    i. For ICE COLD-PCR product, use 10 µL PCR product to 18 µL AMPure XP.
d. Dispense the appropriate volume of PCR Product into a 96-well PCR plate.
e. Dispense the appropriate volume of AMPure XP according to 1.c. into the 96-well PCR plate containing the PCR product and pipette mix 10 times creating a homogenous mixture. A multichannel pipetteman and sterile reservoir for the AMPure XP may be used with large sample numbers. Centrifuge briefly as needed and let incubate for 3 - 5 minutes at room temperature.
f. Place the sample plate onto the SPRIPlate for 2 minutes to separate beads from solution.
    i. Ensure the solution is clear before proceeding to the next step. If the solution is not clear, repeat step 1.f. until solution is clear.
g. With the sample plate situated on the SPRIPlate, aspirate the cleared solution and discard. Do not disturb the ring or pellet of separated magnetic beads. Check pipette tips before discarding solution. If magnetic beads are seen in the pipette tip dispense the solution back in appropriate well, repeat step 1.f. and attempt to extract cleared solution again. Repeat until clear solution is obtained then discard clear solution.
h. With the sample plate situated on the SPRIPlate dispense 200 µL of 70% ethanol to each reaction well and incubate for approximately 30 seconds at room temperature. A multichannel pipetteman and sterile reservoir for the ethanol may be used with large sample numbers. Do not disturb the separated magnetic beads during this process.
    i. Aspirate out and discard the ethanol. If magnetic beads are seen in the pipette tip, place solution back in appropriate well, allow the solution to clear and attempt again. Repeat until clear solution is aspirated and only then discard.
    ii. Repeat wash step for a total of two washes.
    iii. Be sure to remove all ethanol from the bottom of the well, as it may contain residual contaminants.
i. Evaporate the residual ethanol in the samples by placing the sample plate uncovered in the incubator for approximately three minutes.
    i. The incubator should be set to approximately 60 °C.
    ii. After three minutes, inspect the sample wells to ensure all residual ethanol is removed. If ethanol is still present, repeat step 1.i. until residual ethanol has evaporated. Take care not to over dry the bead ring or pellet (bead ring or pellet appears cracked) as this will significantly decrease elution efficiency.