

Production Protocol for the EXO/SAP of Mutational Profiling PCR Products Prior to Sequencing

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Revision Date: 06/27/05

Version 1.2

PURPOSE: This protocol describes how to add EXO/SAP cocktail to PCR products on the Biomek FX to prepare them for sequencing.

MATERIALS AND EQUIPMENT:

Biomek FX with 384 head and tips
EXO I enzyme at 10Units/uL
SAP enzyme at 1Unit/uL
Sigma Water
ddH₂O
384-well V-Groove reservoir
Jouan GR4 22 Centrifuge
MJ Thermalcycler with 384 blocks
384-well Dental Dams
Refrigerator
Aluminum Foil
Tape
Permanent Marker

PROCEDURE:

1. Preparing Reagents

- 1.1. Obtain MP PCR products in 384-well MJ hard shell plates with dental dams firmly in place.
- 1.2. Store plates at 4°C in a refrigerator until ready to EXO/SAP.
- 1.3. Obtain fresh EXO/SAP mix once a week from Materials Core. **Note:** *The EXO/SAP mix is good for seven days and must be stored in a foil-wrapped conical at the back of the refrigerator except while in use on the Biomek. After a week, any remaining mix must be thrown away.*
- 1.4. 2uL of EXO/SAP mix is added to each well. Each 2uL addition of EXO/SAP mix consists of:
 - 1.45uL Sigma Water
 - 0.37uL EXO I @ 10Units/uL
 - 0.18uL SAP @ 1Unit/uL

2. Adding EXO/SAP mix on the Biomek FX

- 2.1. Open the Biomek FX software by double-clicking on the “Shortcut to Biomek FX” icon on the desktop.
- 2.2. Click on the “File” tab at the top of the page and highlight “Open”.
- 2.3. Select the appropriate program for the number of trays to be processed using the following pathway: C: Program Files/BiomekFX/Methods/Mutational Profiling/Exo_sap/ /Exo_sap_#trays_vgroove_MIX.bmt
- 2.4. Quick spin all 384 well MJ plates that are to have EXO/SAP mix added at 1500rpm for 30 seconds.
- 2.5. Remove the dental dams and place trays on the deck of the Biomek as indicated in the “Instrument Set-Up” window.
- 2.6. Empty the waste bucket and fill the water bucket with fresh dH₂O if necessary.
- 2.7. According to the “Instrument Set-Up” window, place a 384 head V-Groove reservoir on the deck in the correct position for the EXO/SAP mix.

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- 2.8. Retrieve the EXO/SAP mix from the back of the refrigerator and carefully pour the mixture into the 384 head V-Groove reservoir. Be sure the mix thoroughly covers the bottom of the reservoir.
- 2.9. Double-check the deck set-up and click the green arrow to run the program.
- 2.10. Carefully, watch the aspiration of EXO/SAP mix to be sure there is enough in the reservoir.
- 2.11. When EXO/SAP mix has been added to all trays, immediately pour the remaining EXO/SAP mix back into the foil-wrapped conical tube and place it in the back of the refrigerator.

3. Incubating the EXO/SAP Reactions

- 3.1. Following completion of the EXO/SAP addition program on the Biomek, quick spin the plates at 1500rpm for 30 seconds.
- 3.2. Seal each plate with a dental dam being sure each well is properly covered.
- 3.3. Place each plate in a 384 block of a thermalcycler.
- 3.4. Run the "EXO-SAP" thermalcycler program, which is as follows:
 - 37°C for 30 minutes
 - 80°C for 15 minutes
 - 10°C forever
- 3.5. When the thermalcycler programs has finished, remove the plates and quickspin them at 1500rpm for 30 seconds.
- 3.6. Place trays in the refrigerator at 4°C until they are ready to be sequenced.

Revisions:

6/24/05: Sigma Water was added to the Materials and Equipment Section.

dH2O was removed as an additive to the EXO/SAP brew recipe.

Sigma Water was added to the EXO/SAP brew recipe.

Changed grammar and wording throughout protocol.