Quantiation of DNA

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SUMMARY

This protocol outlines the procedure of cleaning up sequencing reactions.

MATERIALS & REAGENTS

Materials/Reagents/Equipment	*Vendor*	*Catalog Number*
DISPOSABLES		
96 well PCR plates	ISC BioExpress	T-3082-1
Stack Rack 1uL-10 uL Natural Tip	USA Scientific	1111-0200
TermalSeal Tape	ISC BioExpress	T-2417-5
REUSABLES		
Foil Stripper	Orochem Technologies	OT-0592
REAGENTS/CHEMICALS		
Water, HPLC Grade	Aldrich	7732-18-5
0.5X TBE Buffer	Homemade	
GenePure LE Agarose	ISC Bioexpress	
Ethidium Bromide		
10X Loading Dye	Homemade	
EQUIPMENT		
Plate Vortexer	VWR Scientific Products	
Flour-S MultiImager	Bio Rad	

PROCEDURE

Inactivation of Enzyme

1. After the plate has sat over night in the incubator, place it in the MJ Thermal Cycler at the program name 65 (65°C for 10 minutes).
2. Place the plate on ice **IMMEDIATELY**. Then place the plate in the 4°C refrigerator until it is ready for sequencing reaction setup. The plate can be kept in the 4°C refrigerator for up to a week. When the sequencing reaction is finished the RCA amplified product can be stored in the -20°C freezer B for long-term storage.

3. Add 25μL of water, HPLC grade, to dilute the amplified DNA and vortex for 7 minutes on the plate vortexer.

Quantitation: Gel Image using Agarose Gel

1. Prepare a 1% agarose gel to check the quantity of the DNA. For a full gel rig use 300 uL of 0.5X TBE buffer and 3.0 g of GenePure LE Agarose plus 5.0 uL ethidium bromide. For a half gel rig use 200 uL of 0.5X TBE buffer and 2.0 g of GenePure LE Agarose plus 4.0 uL ethidium bromide. For a small gel rig use 100 uL of 0.5X TBE buffer and 1.0 g of GenePure LE Agarose plus 3.0 uL ethidium bromide.
2. Place mixture in the microwave for about a minute but make sure to watch so that it does not bubble over.
3. Swirl it under cold water for a few minutes and let it cool for 3 minutes.
4. Add the required amount of ethidium bromide and swirl for about a minute.
5. Pour the mixture in the gel rigs and let it solidify for at least 30 minutes.
6. In a full skirted PCR plate add 3 uL of 10X loading dye and 5 uL of the RCA product. Mix with the pipet up and down about 10 times.
7. After the gel has been solidified, load the gel and let it run at 100 V for about 20-40 minutes. Watch the gel so that it does not over run.
8. Take an image of the gel using the BioRad Fluor-S Multiimager.
9. First place the gel in the imager.
10. On the desktop press Quantity One icon.
11. Go to File Fluor-S. Press the SELECT button and select nucleic acid gels then select ethidium bromide.
12. Scan Dimension can be set to 350 mm. Press the POSITION button and position the gel in the right place. Press the FOCUS button and make sure the gel is focused.
13. Select exposure time, which usually is between 20 to 40. Close the imager door and press PREVIEW button. If it looks good press the Live Acquire button.
14. Invert image by selecting IMAGE on the menu bar and select INVERT DATA. Select LIGHTER, then print image. Please refer to the pictures below for good and bad gels.

Recipe for 10X Loading Buffer

30 mL 100% Glycerol
20 mL HPLC grade water
Little Bit of Bromophenol Blue
Little Bit of Xylene Cyanol F

Recipe for 5 X TBE Buffer for 1 L

54 g of Tris Base
27.5 g of Boric Acid
20 mL of 0.5 EDTA ph. 8

Add about 800 mL of water and mix the ingredients. Stir well and then add water up to 1 Liter.
Note: Dilute TBE from 5X to 0.5 X