DNA Analyst Training
Laboratory Training Manual

Protocol 3.07
Slot Blot (Chemiluminescence)

This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S DNA INITIATIVE

INTRODUCTION

STR profiles are largely dependent on the quantity of human DNA present introduced into the reaction. Slot blot quantitation can help determine whether the DNA is human or non-human.

DNA quantitation must be accomplished on all samples by yield gel and/or slot blot before amplification. Slot blot is required for all unknown samples. It is not required for standard samples. The decision to use slot blot on standard samples is left to the discretion of the analyst.

SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices

Warning: Treat all reagents/samples as potential biohazards

Warning: The following are considered hazardous reagents:

Sodium Dodecyl Sulfate (SDS) is harmful if inhaled or ingested. It potentially causes skin and eye burns. Special protection: Wear a dust mask or respirator; use of a fume hood is required.

Sodium Hydroxide is a corrosive chemical which may be fatal if swallowed or absorbed through the skin. It causes severe eye skin, digestive and respiratory tract burns.

PREPARATIONS

DNA Quantitation Reagents: Equivalent preparations may be purchased commercially, if available.

Slot Blot Hybridization Solution

20% SDS	25 ml
SSPE 20X	250 ml
ddi H2O or equivalent	725 ml

Citrate Buffer

Sodium Citrate Na3C6H5O7.2H2O 92 g
adjust pH to 5.0 with Citric acid monohydrate C6H8O7.H2O≈30 g
ddi H2O or equivalent to 5 liter.

500 mM EDTA pH 8.0

EDTA-2H2O@Na2	930.5 g
ddi H2O or equivalent	4.0 liter
NaOH pellets	5-100 g

When fully dissolved, add more NaOH to bring the pH to 8.0 (EDTA does not go into solution until the pH nears 8.0.) Adjust the volume to 5.0 liter. Autoclave.

4M NaOH

NaOH	800 g
ddi H2O or equivalent	4200 ml

Adjust volume to 5.0 liter.

SSPE 20X

500 mM EDTA pH 8.0	200 ml
NaCl	1050 g
NaH2PO4.H2O (monohydrate)	138 g
pH to 7.4	
ddi H2O or equivalent to	5 liter

Prewetting Solution

ddi H2O or equivalent	85 ml
4M NaOH	10 ml or 400 mM NaOH
500 mM EDTA pH 8.0	5 ml

20% SDS

PCR- SDS	1000 g

ddi H2O or equivalent to 5 liter

Heat gently to about 65°C and stir to dissolve. Use a nuisance mask. Prepare the reagent in the fume hood.

Slot Blot Standards from Quantiblot kit - see kit insert.

Slot Blot Standards from Promega Human Genomic DNA (240 ng/µl).

Dilute 5 :l Human Genomic DNA (240 ng/:l) with 595 :l TE-4 to make Standard A (10 ng/5 :l final concentration).

Carry out five serial doubling dilutions of this solution (mixing 100 :l aliquots of sample and 100 :l aliquots of TE-4 for each step) to obtain solutions containing: 5 ng/5 :l (Standard B), 2.5 ng/5 :l (Standard C), 1.25 ng/5 :l (Standard D), 0.6 ng/5 :l (Standard E), 0.3 ng/5 :l (Standard F), 0.15 ng/5 :l (Standard G) and 0.075 ng/5 :l (Standard H).

TE^{-4}

1.0 M Tris pH 8.0	10 ml
500 mM EDTA pH 8.0	0.2 ml

ddi H2O or equivalent to 1.0 liter. Autoclave.

West Dura Chemiluminescent reagents

Pierce Chemical Company
Prepare as instructed below

Slot Blot Wash Solution

20% SDS	25 ml
SSPE 20X	75 ml
ddi H2O or equivalent	900 ml

Spotting Solution

ddi H2O or equivalent	85 ml
4M NaOH	10 ml
500 mM EDTA pH 8.0	5 ml
Bromthymol blue	200 :l

INSTRUMENTATION

Standard Laboratory Instrumentation
Syngene Bio Imaging System (CCDBio)
Slot Blot Apparatus
Vacuum Pump

SOFTWARE

Syngene GeneSnap software, version 6.0 or higher
Hitachi SlotQuantTM software, version 2.1.5.5 or higher

MINIMUM STANDARDS & CONTROLS

A human genomic DNA standard is used to prepare eight DNA standards. The eight DNA standards represent the following quantities: 10, 5.0, 2.5, 1.25, 0.6, 0.3, 0.15, and 0.075 ng. Results are interpreted by comparing the signal intensity of the DNA test sample to the signal intensity obtained from the DNA standards.

Critical Reagent: Nylon Membrane.
(Refer to pdi_lab_pro_2.01, Quality Assurance).

PROCEDURE OR ANALYSIS

Slot Blot Membrane Development

1. Place at least 150 :l spotting solution in all tubes to be used. Add 5 :l of each standard and known volumes of the test samples into the appropriate tubes.

2. Wet the membrane in prewetting solution and assemble the slot blot apparatus with the membrane in place. Apply a vacuum to the apparatus.

3. Load the samples into the center of the wells of the slot blot apparatus being careful not to puncture the membrane or introduce bubbles. The four corners of the blot area used should be loaded with samples, to assist in positioning the grid for computer calculations.

4. Turn on the vacuum to the wells. When all of the wells go dry turn off the vacuum.

5. Place the membrane in prewarmed slot blot hybridization solution. At this point, the membrane may be stored in hybridization solution in the refrigerator.

6. Process up to four membranes together in one container. Prehybridize the membranes in a box containing 100 ml prewarmed hybridization solution and 5 ml 30% H2O2 at 50°C for 15 minutes with rotation. Pour off the hybridization solution.

7. Add 30 ml prewarmed slot blot hybridization solution. Add 20 :l D17Z1 probe. Incubate at 50°C for 20 minutes with rotation. Pour off the solution.

8. Briefly rinse the membrane in slot blot wash solution.

9. Add 30 ml prewarmed slot blot wash solution. Add 90 :l HRP-SA enzyme conjugate. Incubate at 50°C for ten minutes with rotation. Pour off the solution.

10. Rinse the membrane for one minute with 100 ml prewarmed (50°C) slot blot wash solution at room temperature with rotation. Repeat.

11. Rinse the membrane for ten minutes with 100 ml prewarmed (50°C) slot blot wash solution at room temperature with rotation.

12. Rinse briefly in 100 ml citrate buffer. Drain off as much solution as possible.

13. Process membranes individually. Mix 5 ml of each West-Dura substrate reagent and pour this mixture over the membrane. Rotate for at least five minutes so that the entire membrane is saturated.

14. Place the membrane in plastic and wipe off excess solution.

Image Capturing with GeneSnap

1. Place the membrane on the transilluminator in the darkroom of the CCDBio.

2. Position the membrane and focus. Close the darkroom door.

3. Open the iris completely. Select no filters and no light in the software.

4. Using the “series capture” icon select a series of cumulative exposures. This range of exposures will reflect increasing saturation. Saturation of the 10 ng standard should be attained for the series to be complete.

5. Select an image which shows no or minimal saturation at the 5 ng standard and incomplete saturation for the 10 ng standard.

6. Optimize the selected image and save the results as a .sgd file. The selected image should also be printed for the case file.

7. Export the image and save the results as a .tif file.

SlotQuantTM Analysis

1. Using the SlotQuantTM program, open the image for quantitation. Start a new slot blot analysis.

2. Maximize and magnify the image to 200%. Optimize the image grey scale and contrast so that the standards and positioning samples are easily seen.

3. Create a grid for the slot blot image.

4. Under “Slot Blot - Preferences,” make the following adjustments when necessary: Under “Part Quantity”: Adjust the area size for partial quantification for the horizontal (H) and vertical (V) setting in order to avoid artifacts that may be affecting readings. Under “Quantification”: Establish the minimum quantification limit (MQL) as 99% of the lowest standard.
5. Set the grid markers by designating standard series in the appropriate positions. A previously saved grid marker can be used at this step.

6. Calculate the concentration of DNA in each non-standard position.

7. Print and review the standard curve analysis for quantitation. R2 values should be between 0.99 and 1.0 for optimal quantitation data. A copy should be included in each applicable case file.

8. Review and print spreadsheet data (to include volume and ng reading) which documents the calculated results for each sample. This data may be saved and printed in the form of an Excel spreadsheet, if desired. The data generated will be compared with a visual interpretation of the same results prior to accepting the calculated DNA concentration. A copy should be included in each applicable case file.

9. If low or high scale plateaus exist for the standard series, a second analysis of the CCDBio image may be done. Another set of grid markers (standards), another calibration curve, and another concentration calculation may be done for the image. A minimum of three standards must be included in any standard curve. Standards included should be of the high, middle and low ranges, when possible. Data outside the range of the standards used will be interpreted with caution.

10. Save the project before closing the SlotQuantTM software.

11. Electronic data, including the optimized image (.sgd and .tif files), the spreadsheet, and the calibration curve, will be archived on CD-R.

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