1 Preparation of Human DNA Standards:

*This only needs to be done as needed

1.1 Label seven autoclaved screw cap tubes A-G.

1.2 Vortex the DNA standard A to mix it thoroughly.

1.3 Transfer 120 µl of DNA standard A into the tube labeled A.

1.4 Aliquot 60 µl of TE buffer into the six remaining tubes labeled B-G.

1.5 Add 60 µl of DNA Standard A (tube A) to the 60 µl of TE buffer in tube B. Vortex to mix well.

1.6 Add 60 µl of DNA Standard B (tube B) to the 60 µl of TE buffer in tube C. Vortex to mix well.

1.7 Add 60 µl of DNA Standard C (tube C) to the 60 µl of TE buffer in tube D. Vortex to mix well.

1.8 Continue the serial dilution through tube G.

1.9 If the dilution steps are performed as described, the seven DNA standard tubes will have the following concentrations of human DNA:

DNA Standard	Concentration (ng/µl)	Quantity DNA per 5 µl (ng)
A	2	10
B	1	5
C	0.5	2.5
D	0.25	1.25
E	0.125	0.625
F	0.0625	0.3215
G	0.03125	0.15625

1.10 Slot Blotting:

***Prior to slot blotting pre-warm the Hybridization and Wash Solutions in a 50°C water bath or incubator until all solids have dissolved into solution.

1.10.1 Determine the number of samples to be analyzed including the seven Human DNA Standards, the DNA Calibrators 1 and 2 (in the kit), and the one blank (spotting solution only). Aliquot 150µl of spotting solution into a new labeled reaction tube for each sample.

1.10.2 Label eight of the tubes as follows: A, B, C, D, E, F, G, H and label two tubes as follows: DNA Calibrator 1, DNA Calibrator 2.

1.10.3 Vortex the seven standards and the two calibrators. Add 5 µl of each solution to the corresponding tube of spotting solution.

1.10.4 Add 5 µl of each test sample to the remaining tubes of spotting solution.

1.10.5 While wearing clean gloves, cut a piece of Biodyne B membrane approximately 11 x 8 cm. If desired, cut the right corner to mark the orientation of the membrane. Be sure to make any labeling markings on the membrane at this time. Place the membrane in the hybridization tray and cover with 50 ml of Pre-Wetting solution for a few minutes.

1.10.6 Begin with both knobs on the apparatus turned to the OFF positions. Place the top plate of apparatus over membrane and turn clamp knob to the ON position. Turn on the vacuum. Turn vacuum knob to the ON position to allow the vacuum to pull the samples slowly through the membrane. Test apparatus to be sure vacuum is functioning by gently pulling the top plate up. If plate separates from apparatus, turn vacuum off and repeat by gently pressing top plate down as the vacuum is turned on. When the plate is suctioned to the apparatus, continue.

1.10.7 Using a new pipette tip for each sample, load the entire volume of each sample into separate wells. Leave the vacuum on until all of the samples have been drawn through the membrane (~30 seconds). Inspect that each sample slot has a uniform blue band.

1.10.8 Turn off the clamp. Turn off the vacuum source. Disassemble the slot blot apparatus and remove the membrane. PROCEED DIRECTLY TO HYBRIDIZATION. DO NOT LET THE MEMBRANE DRY OUT.

1.11 Hybridization: (in 50°C rotating water bath)

1.11.1 Pour off pre-wetting solution and rinse the hybriboat with dH_2O.

1.11.2 Pre-hybridization: Transfer the membrane to a hybriboat with 100 ml pre-warmed hybridization solution. Add 5 ml of 30% hydrogen peroxide. Place the lid on the tray. Use a lead weight to keep the tray from floating in the water bath. Rotate in a 50° C water bath for 15 minutes. Pour off solution.

1.11.3 Hybridization: Add 30 ml hybridization solution to the hybriboat containing the membrane. Tilt the tray to one side and add 20 µl of Quantiblot D17Z1 probe to the solution. Place the lid on the tray. Rotate in 50°C water bath for 20 minutes. Pour off the solution.

1.11.4 Rinse the membrane briefly in about 50 ml pre-warmed wash solution by rocking the tray for several seconds. Pour off the solution.

1.11.5 Stringent Wash/Conjugation: Add 30 ml of the pre-warmed wash solution to the hybriboat. Tilt the tray to one side and add the Enzyme Conjugate:HRP-SA to the wash as follows:

For Chemiluminescent detection: Add 90 µl of the conjugate

Place the lid on the tray. Rotate in 50° C water bath for 10 minutes. Pour off solution.

1.11.6 Rinse the membrane thoroughly for approximately 30 seconds in about 50 ml pre-warmed wash solution by rocking the tray at room temperature. Pour off the solution. Rinse again for approximately 1 minute. Pour off solution.

1.11.7 Wash the membrane by adding 50 ml of pre-warmed wash solution to the tray. Place lid on the tray. Rotate at room temperature on an orbital shaker for 10 minutes. Pour off solution.

1.11.8 Rinse the membrane briefly in 50 ml of citrate buffer by rocking the tray. Pour off the solution.

1.12 Chemiluminescent Detection:

1.12.1 To 5 ml ECL Reagent 2 add 5 ml ECL Reagent 1. Do not prepare this mixture more than 5 minutes before use. Add the 10 ml ECL Reagent to the membrane in the tray and shake for approximately 1 minute at room temperature. Pour off solution.

1.12.2 Place membrane in a development folder, squeeze out the excess reagent, and heat seal the edges. Take to darkroom, place in a lumisette and put next to the Reflection file (the gray film). Tape in place (optional). Expose to film for at least 10 minutes at room temperature.

1.12.3 In darkroom under the red lights, remove tape (if used) and membrane from film and feed film into machine when the machine indicator ready light is lit. As the film feeds the red light will go out (it may not if the film is small) when the red light comes back on, make sure that no film is out and the white lights can be turned on.

1.12.4 When film is developed the signals can be compared and quantitated.

Revision History		
Effective Date	Revision	Reason

NCSBI Forensic Biology Section	DNA SOP	Effective Date: July 19, 2004
Title: **Quantiblot DNA Quantitation**		Revision 04

	Number	
Unknown	00	Original Document for DNA Tech Procedures
January 4, 2002	01	Addition of Speed-Vac Procedure (Change in DNA Technical Procedures)
November 26, 2002	02	Procedure Updates; Collation of Procedures and use of WP Outline; change in page numbering (Change in DNA Technical Procedures)
August 7, 2003	03	Addition of Table of Contents; no substantive changes in any procedure. (Change in DNA Technical Procedures)
July 19, 2004	04	Made "DNA Quantitation" SOP a separate document and changed name to "Quantiblot DNA Quantitation". No technical change in SOP.

APPROVAL SIGNATURES	Date
Author/Title (Print)	
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