1. Scanning Polyacrylamide Gels

1.1 Turn on the scanner and computer.

1.2 Allow the laser in the scanner to warm up. (A red light will come on if there is an error, usually due to the scanner lid not being properly closed).

1.3 After completion of electrophoresis:

1.3.1 Remove gel from tank and rinse both sides of the glass plates thoroughly with dH_2O.

1.3.2 Dry completely with a low lint cloth (e.g. KimWip) so that there are as few marks, dust, or streaks on the plates as possible.

1.3.3 If black spacers are not used, cover the white spacers by placing black electrical tape on the long plate.

1.4 Place the gel into the scanner:

1.4.1 By lifting the lid to the unit.

1.4.2 Placing the gel so that the short plate is on top and the bottom part of the gel (where the dye front should be) is resting on the movable bar. Make sure that bar is as close to the end of the plate as possible so as not to interfere with the scanning.

1.4.3 Close the lid properly to the unit.

1.5 Once your computer is turned on:

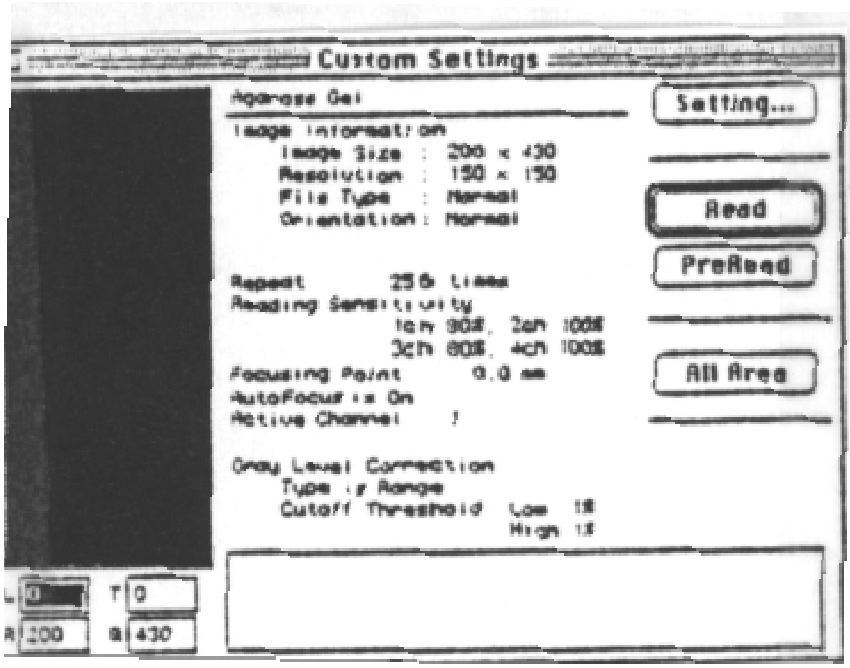
1.5.1 Desktop files as well as the Macintosh hard drive icon (located in the upper right hand portion of the screen) will appear.

1.5.2 Double click on the hard drive icon. This will list all of the files on the hard drive.

1.6 To open Read Image Software:

Click on the PowerPlex Scan icon. Choose the correct icon; that depends on whether black spacers were used or if black tape was used to cover the black spacers.

1.7 The screen will appear that looks similar to this:



1.8 Check to make sure scanning parameters are correct for Powerplex (See table below).

	FM BIO 100*	FM BIO 1
Material Type	Fluorescent	acrylamide gel
Orientation	Fig Horizontal	Normal
Resolution: Horizontal	150dpi	150dpi
Vertical	150 dpi	150dpi
Gray Level Correction Type	range	range
Cutoff Threshold: Low (background)	50%	50%
High Signal	1%	1%
Reading Sensitivity	80%	100% (535nm channel) 80%(585nm channel) 100% (650nm channel)
Focusing Point	n/a	-1.8mm for disposable gels 0.0mm for manual gels

1.9 To adjust parameters:

1.9.1 Click on the PowerPlex Scan icon on the desktop and then “Setting”.

1.9.2 To get to the Powerplex mode, click on MATERIAL TYPE, select POWERPLEX.

1.9.3 Click on all Channels that you will be scanning on:

Channel 1-- 605 nm
Channel 2 -- 505 nm -- DDDD (Gamma Str)
Channel 3 -- 585nm -- CTTV
Channel 4 -- 650nm -- CXR

**Note: 1) The FMBIO II Instruments have an Autofocus feature. Autofocusing occurs during the first scan after the instrument has been turned on. The 605 nm filter is provided for Autofocusing, however, the 585nm filter is also suitable. To ensure proper Autofocusing and operation of the instrument, always be sure that either of these filters (585 or 605) are in Channel 1 (Autofocus channel) whenever the instrument is turned on or for the first scan of the day.*

2) CXR -- When using CXR, you must first scan with Channel 1 at 585nm and Channel 2 at 505nm THEN remove the 585 nm filter in Channel 1 and replace it with the 650 nm filter. Select Channel 1 only on the ReadImage window. Keep all scanning and area parameters the same as for the previous scan with the 585 and 505 filters.

1.10 Define a scan area by:

1.10.1 Selecting an area to PreRead by clicking on ALL AREA (entire scannable area) or by using the mouse to drag out a rectangular area in the image scan window.

1.10.2 Click PREREAD and the instrument will perform a brief scan of the defined area.

1.10.3 Based on the PreRead results, select area to be scanned by dragging inside the scan window with the mouse (Be sure your defined area includes all lanes as well as high and low bands).

1.10.4 When all parameters are set, click READ (A new screen will appear which asks you to name the image) and which file you need to save to.

1.10.5 Name the image and click on the SAVE.

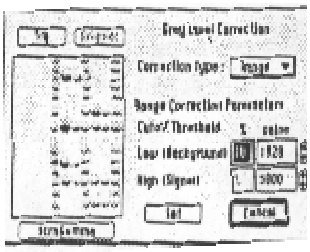
1.11 At this point the counter for the FMBIO II -- will count down in minutes, until it reaches "0" at which time the scanning is complete.

***Note Do not stop or pause the instrument until scanning is complete. Also do not change the scan area parameters between scans of the same*

gel. This will create different size image files and 3-color analysis will not be possible.

1.12 At the completion of scanning, when 2 or 3 channels are selected, a suffix (.1 CH, .2CH, CXR) is automatically added to the end of the file name.

1.13 To view scanned images, click on the designated file on the FMBIO II.



2. COLOR SEPARATION

2.1 Move file from server to a designated folder (optional).

2.2 Open the file and select CH.1 or CH.2 image.

2.3 For Database Images: Click on "File" and select "New". "Select Multi Analyze Image Box" appears. Open of the channels and save new project with file name.

2.4 Adjust Background and Signal Strength (Note: Some analysts may elect to adjust the background AFTER Color Separation. This is acceptable)

2.4.1 Select MULTI (top menu bar)
2.4.2 Select DISPLAY MODE: MONO

2.4.3 Click on Gray Level icon or choose Gray Level Correction in the Image menu. The Gray Level Correction dialog box appears. You can click on this dialog box and move it around over the scanned image. The left side of the dialog box acts like a transparent opening letting you frame regions of interest.

2.4.4 Adjusting the background (if necessary) by one or both of these methods:

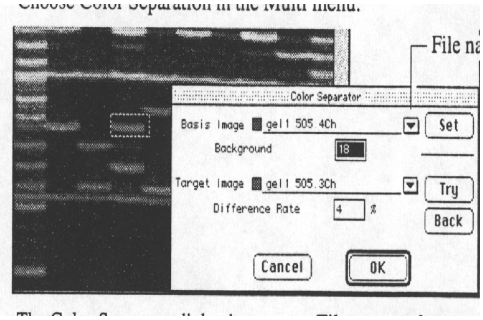
2.4.4.1 Double click on the "%" beside of in the "Low (Background)" box to highlight the number (if not highlighted already). Manually adjust by typing in a number accordingly (NOTE: A general guideline is that the Background Signal be set at 70-85%. Optimal numbers may vary depending on signal of bands and background noise in the gel).

2.4.4.2 Double click on the "%" beside of in the "Low (Background)" box to highlight the number (if not highlighted already). Draw a box around area of background and the machine will automatically adjust the setting.

2.4.5 Adjusting the signal manually (if necessary) double clicking on the "%" number and typing in a number. Adjusting the high value field down, will increase the intensity of your image (NOTE: A general guideline is the signal be set at 10,000 for Channel 1 and 3000 for Channel 2. Optimal numbers may vary depending on signal of bands and background noise in the gel).

2.4.6 Test the area of interest by clicking on TRY. Repeat, until you are satisfied with your image. Then click SET to apply changes.

NOTE: Some analysts may elect not to adjust background until after color separation



2.5 Perform Initial Color Separation.

2.5.1 Go to Menu bar.
2.5.2 Click on MULTI.
2.5.3 Click on COLOR SEPARATION.

2.6 The COLOR SEPARATION dialog box opens. File names of components of the multicolor image appear on 2 lines labeled Basis Image and Target Image.

2.7 Use the file name pop-up to (downward facing arrow next to the SET button) choose a Basis Image file.6.

2.8 Enlarge the image as necessary. On the Image, use the Marquee tool to draw a rectangle inside an isolated band that MATCHES the color assigned to the Basis Image file for CH1.

2.9 Click the SET button.

2.10 Use the file pop-up to choose the Basis Image file for CH.2.

2.11 Draw a rectangle inside the chosen band that MATCHES the color assigned to this Basis Image file.

2.12 Click the SET button.

2.13 Click OK.

2.14 Click OK when asked to “Create Separate Images”.

2.15 Go up to the top menu bar.

2.15.1 Click on MULTI
2.15.2 Go down to DISPLAY MODE

2.15.3 Click on MONO (This brings up the black and white image of the file that is highlighted in the project window).
2.15.4 View each channel to insure no "bleed through" is occurring. If there is still "bleed through", repeat COLOR SEPARATION.
2.15.5 If your images are correct, go to 1-D GEL ANALYSIS.

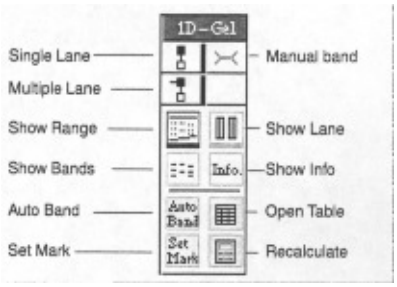
3. STR-FMBIO Analysis (1-D Gel Analysis)

3.1 Once scanning is complete and image is saved to desktop or HD (hard drive), double click on your personal file (This opens up your file and allows you to choose what image you want to analyze).

3.2 Double click on file containing image of interest, i.e, popdbase 561-575. The main file opens containing sub-files generated from Channel 1 (CTTV loci) and Channel 2 (DDDD loci)

3.3 Double click on file of interest. FMBIO Analysis will momentarily appear on the screen. The image will appear next. Make sure that FMBIO 8.0 (or newest revision) is used by going up to the small computer screen icon located at top right corner of screen. Click and hold down mouse and scroll down to FMBIO 8.0 (or newest revision). A check mark appear when program is on.

3.4 From the menu bar at the top of the screen, click on and hold down TOOLS. Scroll down to 1-D Gel and release the mouse button. The 1-D gel menu appears in the menu bar and the corresponding tools appear on the left side of the image window:



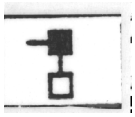
3.5 MIGRATION AREA BAR A migration area bar also appears at the top and bottom of your image when 1-D Gel is selected. Adjust to just above

the top band and just below the bottom band by holding arrow on left square of bar and moving it up or down.

3.6 MULTILANE SELECTOR TOOL. The Multilane Selector Tool will define the area of the scan that will be analyzed.

3.6.1 To select the multilane selector tool, click on the following icon from the 1-D Gel menu bar:

3.6.2 Click and drag the multilane selector tool from the center of lane #1 to the center of the last lane (above migration bar area). Release the mouse button.



3.6.3 Using the mouse, pull down below bottom migration area bar.

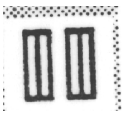
3.6.4 Click mouse (Multi Lane Window appears).

3.6.5 Enter # of lanes on gel.

3.6.6 Click on OK. (Lanes are now identified by the computer with blue vertical lines).

3.7 CENTERING OF LANES. Once the computer recognizes the correct number of lanes by placing blue vertical lines on each lane, each lane must be centered.

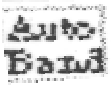
3.7.1 Click on the following icon until you get a single blue center line for each lane (Clicking on this icon several times changes the display from 3-line to center line to no lines).



3.7.2 Click on arrow icon found in the menu bar. Go up to top of blue center line on each lane. Click on the line until a small box appears. Using the mouse, place the arrow on the box, click and move the line until centered over the lane (This moves line on lane left or right). Go to bottom of lane and click on the box. With the FMBIO II, you must click at the bottom of the lane for the small box to appear. (This moves line on lane diagonally). Center all lanes by using the mouse as described above.

3.7.3 Once all lanes are centered , press on keyboard "apple" + A to get "flashing lines".

3.8 AUTOBAND. This function instructs the computer to identify bands.

3.8.1 Click on 

3.8.2 Computer will identify bands in all lanes that were centered. If you look at bottom of gel scan you will see displayed (Autoband: lane #) as it goes through autobanding.

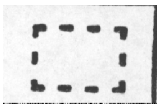
3.8.3 Once autobanding is complete, red and yellow horizontal bands will appear on bands (vertical blue lines will still be visible).

3.8.4 Remove vertical blue lines by clicking on the following icon until no blue lines are visible.

3.8.5 Click on the icon until only red bands are visible (Clicking on this icon will allow you to go from yellow & red bands, to red bands only, to no visible bands.

3.9 EDITING BANDS. At this point you can add or delete bands that the computer misidentified.

3.10 To add bands, click on peak finder and dogbone. Place dogbone on top of band of interest and click. A red band will appear denoting addition (recognition) of band.

3.11 To delete bands you can use: 

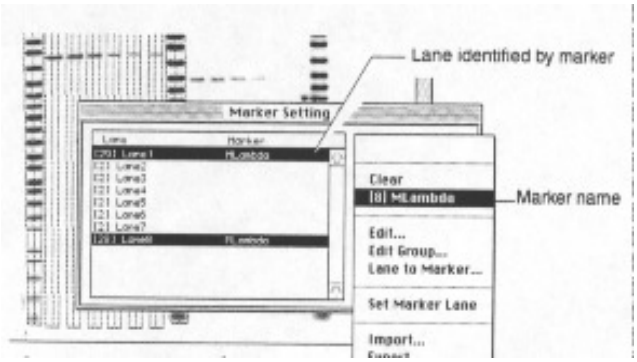
3.11.1 The dogbone. Place on top of band to delete and click. Band will turn from red to blue. Hit the delete button. The computer no longer recognizes this band.

3.11.2 The marquee .The marquee allows you to draw a box around the band or bands you want to delete. Once you have the box around the desired band(s), they will also turn blue. Hit the delete button. The computer no longer recognizes the band(s).

3.12 SET MARK . Once all band editing is complete you are ready to tell the computer what standards to use to calculate unknown band sizes.

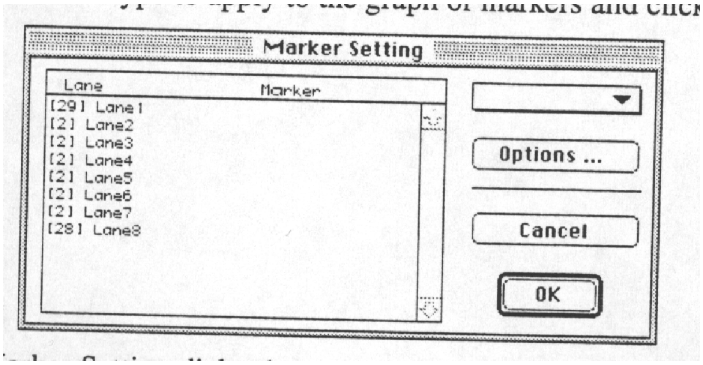
3.12.1 Click on the "set mark" icon.

3.12.2 The following screen will appear:



3.12.3 Make sure Natural Logarithms is checked. Click OK.

3.12.4 The Marker Setting window will appear: The Marker Setting dialog box displays the defined lanes with parentheses indicating the number of defined bands in each lane.



3.12.5 For Powerplex , you should have the following number of ladder bands.

[35] ppCTTV ladder [52] pp2.1 TMR
[37] ppCTTVa ladder [86] pp2.1 Fluroscein
[36] DDDD ladder
[14] CXR ladder

3.12.6 Scroll up and down the Marker Setting window to check that the correct number of ladder bands has been identified.

3.12.7 If all ladders do not contain the correct # of bands. Cancel out of this screen and go back to Editing Bands.

3.12.8 If all ladders are correct press shift and click on each ladder lane in the Marker Setting window. They will turn black once highlighted as seen below.

3.12.9 Click on down arrow in the Marker Setting window menu. Go to Group and click on desired ladder , i.e., ppCTTV.+ppCTTV will now appear on the lane next to the highlighted ladders.

3.12.10 Click on OK (window will close).

3.13 RECALCULATE. Now that bands have been marked and the computer knows what ladder to use, the unknown sample band sizes can be calculated.

3.13.1 Go up to 1-D Gel on the top menu bar.
3.13.2 Go down to Setting.
3.13.3 Go down to Table Info
3.13.4 Click on what items you want calculated. Always click on bp. If determining % stutter click on OD. Deselect the other options.
3.13.5 Click on Recalculate icon.

3.14 TABLE INFO. The above calculated data is placed in a table format.

3.14.1 Click on Table icon.
3.14.2 A table will appear with calculated data. You must SAVE the data before exiting the program.
3.14.3 Go up to the top menu bar. Click on FILE.
3.14.4 Go down to SAVE ANALYSIS.
3.14.5 Go over to desktop or HD. Click on it (This brings up files found either on the desktop or HD).
3.14.6 Scroll down until you find your personal file. Click on it (Now you file is opened and the table data will be saved in it).
3.14.7 Click on SAVE. Display at bottom of this screen will already read, i.e.:
RD251-266.1CH.DAT --- for data file.
3.14.8 Repeat for Channel 2 image.

3.14.9 Close out of program by clicking on little box in the upper left hand corner of your image. If program asks if you want to save changes to this image. Click SAVE.

YOU ARE NOW READY TO DO STAR CALL ON THE ABOVE DATA. IN ORDER TO ACCESS STAR CALL YOU MUST CLOSE OUT OF THIS PROGRAM AND GO INTO MICROSOFT EXCEL.

4. STaR CALL

4.1 Turn on computer by holding down far right button with arrow pointing left on it.

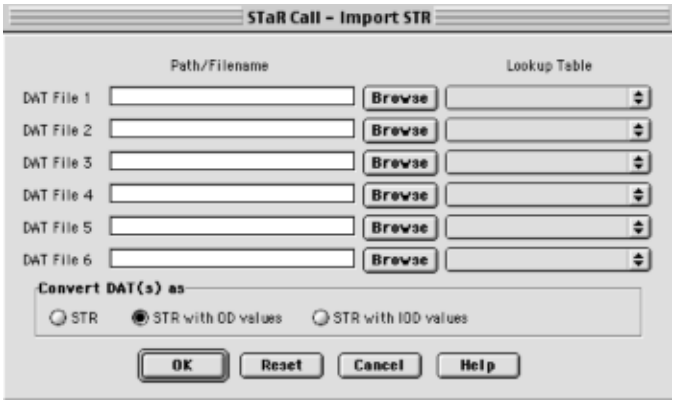
4.2 Open Microsoft Excel by clicking on the Microsoft Excel icon on the desktop or go up to top left corner of screen, click on the "Apple". Scroll down to Microsoft Excel alias or use the Microsoft Excel icon on the desktop.

4.3 New screen appears with a menu bar across the top and a "codisdbf.xls" dialog box will appear briefly. (DO NOT CLOSE OUT of this dialog box. It must be opened for STaR CALL to operate. It is the lookup table containing known bp sizes of the standards). A table titled "Workbook 1" will then appear.

4.4 From toolbar select "Star Call" > "Options". At this point choose the "descending" button. Later it will be changed back to "ascending".

4.5 From toolbar click on StaR Call, then Import STR.

4.6 A grey box will then appear which will allow the analyst to choose a file or files to open (xx.dat).



Choose "Browse" to locate the desired file (xx.dat). Up to 6 files can be selected. Note: Both PP 1.1 and PP 2.1 files can be imported simultaneously, as long as the samples are the same and the gel lanes are identical.

4.7 Choose Look up Table by using the drop down arrows.{Eg. PP 1.1 choose either CTTV, CTTV + AM, Gamma STR DDDD (New D 7). PP 2.1 choose either (FL) or (TMR).

4.8 Click on:

4.8.1 STR – to calculate bp and genotype
4.8.2 STR with 1OD values -- to calculate bp, genotype, and % stutter.

4.9 New dialog box opens "STaR CALL. Evaluate Select Allelic Ladders".

4.10 Check to ensure your allelic ladders are highlighted and the number of alleles are correct.

4.11 Click OK

4.12 STaR CALL will now perform Genotype Analysis.

4.13 Once STaR CALL has completed its analysis an initial "Summary" screen will appear.

4.14 In the bottom left corner several grey tabs will be visible which represent different tables within the complete workbook such as an individual Excel table for each (xx.dat) file, a Look Up Table for each (xx.dat) file, a merged Excel table which combines all (xx.dat) files and a merged landscape table.

4.15 At this point the analyst should review the "Merged Landscape" table to make sure STaR CALL has functioned properly.

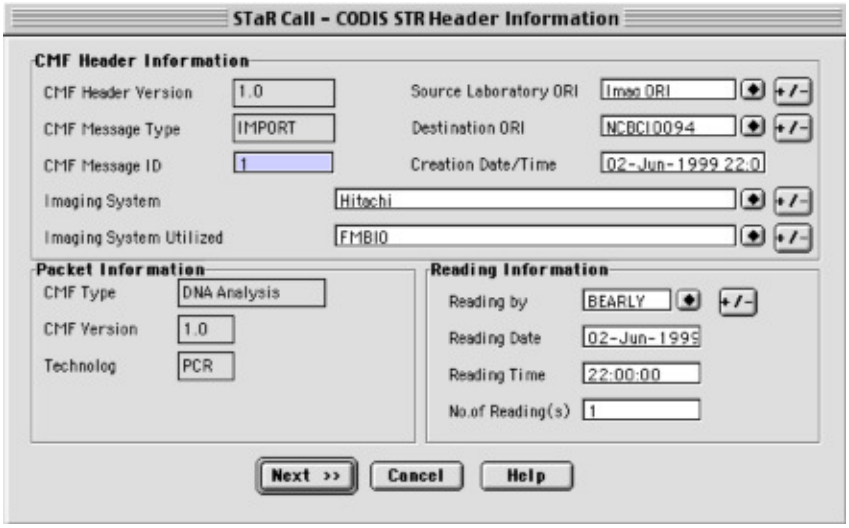
4.16 Evaluate the merged Excel Table to confirm all alleles have been properly identified and the correct # of repeats have been assigned. If alleles have been identified as "Out of Range" the analyst can correct the table by comparing the base pairs of the sample to the base pairs of the allelic ladder. Or the analyst can use the FMBIO Image to manually identify the number of repeats.

4.17 While using the toolbar "STaR CALL" select "OPTIONS". Box will appear allowing the analyst to change the presentation of data in the "Merged Landscape Table". It is suggested that data be viewed as Genotype in Ascending order.

Note: It may be necessary to change the table from ascending to descending during analysis and then back to ascending aiding the analyst to better visualize the data.

4.18 Upon competing corrections return to toolbar "STaR CALL" then to "Create Merged Landscape". This will apply the corrections to the "Merged Landscape Table".

4.19 Return to toolbar "Star Call" then to " CODIS STR Export". A " Star Call Header Information" box will appear. It is <u>important</u> that this box contain all of the correct selected information. If not the file will be unsuitable for importing into CODIS.



The following should be selected.

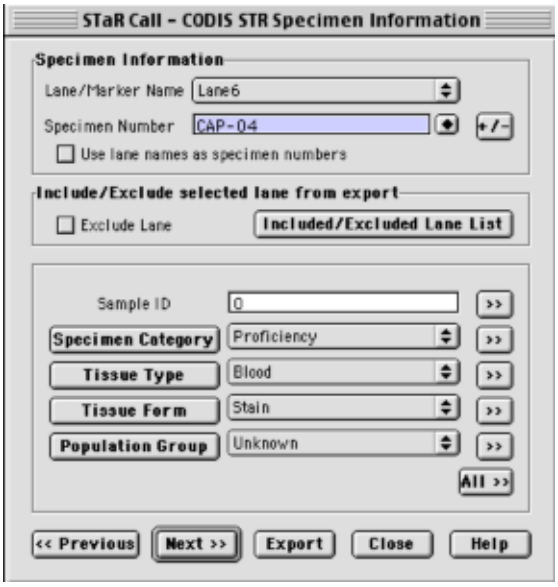
Source Laboratory ORI = Image ORI
Destination ORI = NCBCI0094
Creation Date / Time = N/A
Imaging System= Hitachi
Imaging System Utilized = FMBIO

Reading Information

Reading By = Analyst's CODIS Login name.
Reading Date = Day analysis complete (Eg. 02-Jun-2000). Note: CODIS will not except any dates that are prior to analysts CODIS Start Date.
Reading Time = Optional
Number of Readings = 1

Once complete press "Next".

4.20 "StaR Call-CODIS STR Specimen Information" box will appear.



4.21 Analyst will identify each lane except ones that contain allelic ladders. Type in specimen number in white field at the top of the box. Next select the proper categories from each of the following, Specimen Category, Tissue Type, Tissue Form, and Population Group. This will need to be completed for each specimen and controls. Note: If all or multiple specimens share information the double arrows to the far right or the "All" button followed by double arrows can be pressed after the specimen information has been entered to apply information to all specimens.

4.22 The Include/Exclude box should only be marked if a sample is to be excluded from the Merged Table and/or the CMF file (Common Message Format file used to import data into CODIS). Press "Close". Note: This should not be done until all data has been second read.

4.23 The table should be made to fit the page by using the "Format" function on the toolbar.

4.24 Next go to "File" > "Page Setup" > "Page" select "landscape". Next go to "Header/footer" tab and select the "Custom Header" button. Three boxes will appear. In the left box type the date (eg. 01/24/2000), Center box type Workbook name (eg. DB99-100), Right box type Analyst Name. Press "OK". Press "Custom Footer" button. Center box type " NCSBI", Right box type "Verified _____". Press "OK".

4.25 Return to toolbar "File" > "Save as". Name the file such as (xxxx.wkbk).

4.26 Print the "Merged Landscape Table".

4.27 Once table has been printed and SAVED, delete all of the allelic information from the table and print a second blank table to use for second reads. DO NOT SAVE THE CHANGES.

4.28 Once the second read is complete the analyst will return to computer to create the CMF file. To create CMF file open the desired previously generated Workbook (xxxx.wkbk) file.

4.29 From the bottom left corner select the "merged" tab.

4.30 Next from the tool bar select " Star Call" then "CODIS STR Export".

4.31 Review "Header Information" press "Next".

4.32 Review "Specimen Information". Be sure all specimens that should be excluded are excluded. All controls should also be excluded.

4.33 Press "Export".

4.34 Select where the file should be stored.

4.35 Name the file (eg. 01DB00c.dat) 01=set #, DB =DataBase, 00=year, C=codis/cmf

4.36 Press save.

4.37 The file is ready to import to CODIS.

NOTE: Analysts are not required to make allele calls from STaR Call for case work analysis, but may manually read the gel scans if they prefer.

Revision History		
Effective Date	Revision Number	Reason
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	Combined "FMBIO Analysis (Color Separation)", FMBIO Analysis (1-D Gel Analysis)" and "FMBIO Analysis (StaR Call)" Procedures into a separate SOP entited "FMBIO Analysis". No technical change in SOP.

Approval Signatures	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	