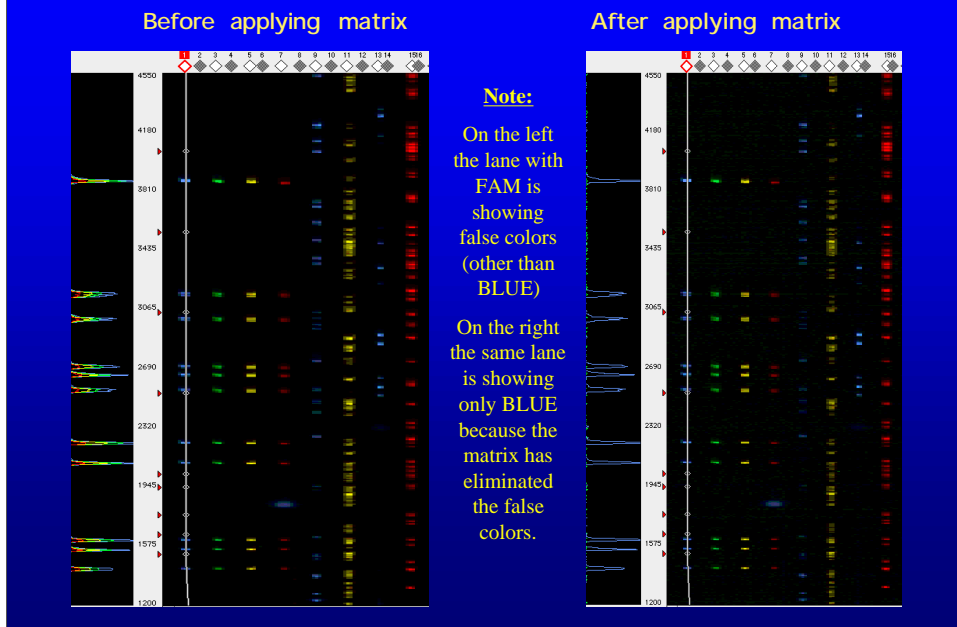
	Fragment Analysis at the CSL Oregon State University
	<p>Researcher</p> <ul style="list-style-type: none"> Marker discovery, e.g. database searches, sequencing, preliminary screening w/[F]dntps Design and purchase labeled primers Perform PCR single- or multi-plex[*]; Post-PCR multiplex if desired Submit sample sheet and samples Downstream analysis 	<p>CSL</p> <ul style="list-style-type: none"> Discuss researcher's goals and provide guidance for obtaining good results Pour slab gel for the ABI 377 Prepare and aliquot master mix Denature samples, load and run gel Post-run preliminary analysis: GeneScan and Genotyper

In the core facility (CSL) at Oregon State University the researchers bring the samples which they have developed, amplified, diluted and multiplexed. We prepare and add the master mix (internal lane standard), run the samples on a slab gel, convert the raw data to numbers and return the data to the researchers for further analysis.

Nature Biotechnology (Feb.2000) Vol 18: pp233-234 details a method to reduce costs for fluorescent labeling of PCR primers. It is a way that one of the researchers labels both strands of the samples run on a non-denaturing (SSCP) gel.

Spectral Overlap - Importance of Matrix



Understanding the effect of spectral overlap, using matrices to overcome or reduce spectral overlap is critical to producing reliable data. Matrix standards are run for each machine and each set of gel running conditions. In the run window no matrix is selected ('none'); steps for creating a matrix are found in the GeneScan User's Manual (Section 5).

In the panels above we can see the usefulness of applying the matrix. Lane 1 is activated, showing the peak pattern on the left of the gel image. In the panel on the left, before a matrix is applied, there is evidence of all colors visible beneath the 'actual' or expected blue signal. The panel on the right shows the identical gel after a matrix is applied. The signals from the other wavelengths (for green, yellow and red) have been removed, allowing the blue signal to appear without any cross-talk.

When a matrix is bad or ineffective, the cross-talk can be so strong as to make genotyping difficult. Close scrutiny can often confirm the correct dye / locus, but the use of a strong matrix can avoid confusion by eliminating overlap of the spectral signal.

Fluorescent Dyes and Filter Sets

	Filter Set A, D	Filter Set C	Strength (rfu)
FAM	Blue	Blue	High
Fluorescein	Blue	Blue	High
TET	Blue	Green	Mid
HEX	Green	Yellow	High
ROX	Red	Red	Mid
TAMRA	Yellow	Red	Low
CY3	Green	Yellow ?	Mid
JOE	Green	?	Low
NED	Yellow	?	Mid
Texas Red	Red	Red	Mid

Virtual filter sets capture the fluorescent emissions at specific (but different) wavelengths. As the laser tracks across the gel, the fluorophores are excited at one wavelength and emit light at another. For example, Tamra is collected as yellow or red depending on which filter set is used for the Run Module. To distinguish between Fam and Tet, you must use filter set C to visualize them as different colors.

For details about the filter set excitation and emission wavelengths refer to the GeneScan Reference Guide, pages 4-10, 4-11 and 4-12.

Post-PCR Multiplexing

- Use knowledge of rfu strength for each dye
- Calculate values using Beer's Law, or
**** do a dilution series of dyes independently ****
- Then multiplex from PCR rxns according to results

Example:

1 locus labeled w/ 6-FAM	1 μ l
1 locus labeled w/ HEX	2 μ l
1 locus labeled w/ TET	3 μ l
Total volume	= 6 μ l

Final dilutions:

6-FAM	1 / 6
HEX	1 / 3
TET	1 / 2

Note: if you multiplex loci with the same dye, it is important to have a distinct distance between the highest fragment of one locus and the lowest fragment of the next.

Sometimes a dilution series is not absolutely accurate - See tutorial by Lynn Petrakova on this subject.

By multiplexing samples throughput can be significantly increased.

It is critical to have a matrix capable of separating the fluorescent signal for the dyes used in the multiplexed sample. Also, fragments labeled with the same dye should be separated sufficiently (by size) as to have no difficulty determining the allele calls.

Getting Started on the ABI 377



- Selecting gel type
- Selecting comb type
- Selecting internal lane standard
- Selecting Run Parameters
 - Size range of fragment = length of run
 - 350bp = 2hr; 500bp = 3hr; 1.1Kb = 5hr

There are numbers of ways to customize slab gels.

- chemistry: denaturing or non-denaturing
- polyacrylamide type / concentration
- number of samples / style of comb
- size standard choices
- choice in run parameters: virtual filter sets, length of time

Electrophoresis Conditions: Slab Gel

Conditions

36 cm plates
6M Urea, 5% Long Ranger
3000V, 60mA, 200W, 51C

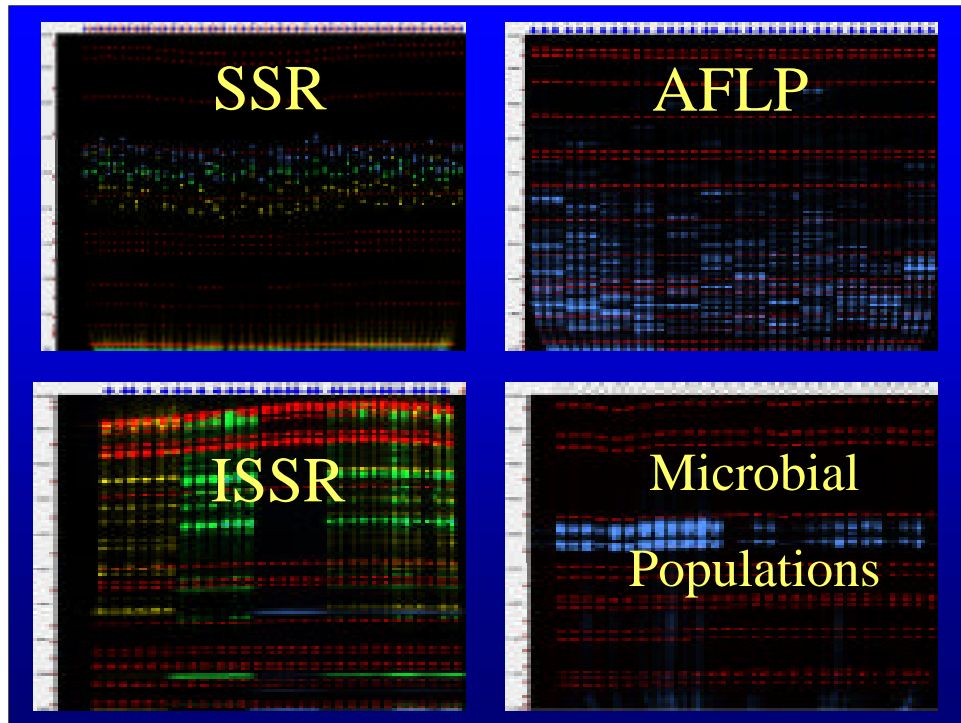
12cm plates
0.4 X MDE + glycerol
2000V, 40mA, 20W, 16C

Samples

Microsatellites (SSR, STR, VNTR)
AFLPs
ISSRs
Microbial Populations

← SSCP

I have tried longer (48cm) plates and higher % Long Ranger for the normal (non SSCP) gels; the results have not been remarkable (significantly different), and I now standardize those samples as described above.



These are examples of some types of fragment analysis at OSU

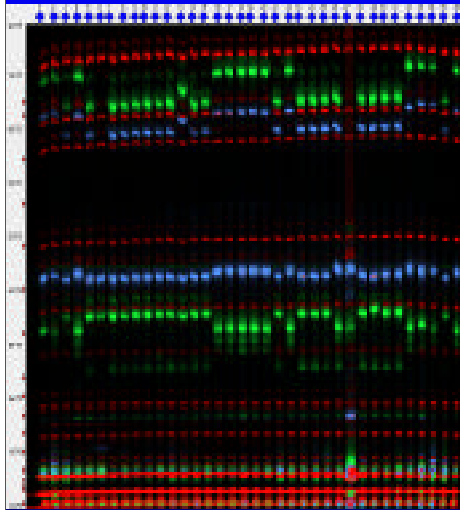
SSR - short simple repeat (microsatellite), used for genotyping

AFLP - amplified fragment length polymorphism, used for fingerprinting

ISSR - inter-ssr, an anchored microsatellite primer is used for fingerprinting

Microbial Population - ribosomal primers used on dna extracted from
soil and water samples

Electrophoresis Conditions: SSCP Slab Gel



SSCP

12 cm plates
0.4X MDE + 2g glycerol
2000V, 40mA, 20W, 15C

This is an example of an SSCP gel processed at OSU.

The researcher has labeled each strand of the PCR product using a different fluorophore for each strand.

Master Mix Cocktails and Volumes per sample

Type of Gel	Amount of Sample	Master Mix Components	Amount of Master Mix Added	Amount Loaded into Gel
Regular ,denaturing In tubes	Dried pellet	Formamide: 34 ul Loading Dye: 15 ul Internal standard: 8 ul	1.5 ul	~ 1.5 ul
Regular, denaturing In tubes	0.5 ul aliquot total	Formamide: 30 ul Loading Dye: 11 ul Internal standard: 8 ul	1.3 ul	~1.5 ul
Regular, denaturing In microtiter plate	0.5 ul aliquot total	Formamide: 130 ul Loading Dye: 25 ul Internal std: 12 ul	3.2 ul	~1.0 ul
SSCP (MDE), non-denaturing In tubes	0.5 ul aliquot total	Formamide: 108 ul 100mM NaOH: 18 ul Loading Dye: 18 ul Internal std: 18 ul	4.5 ul	~1.5 ul

This table shows:

1. How we accept samples from the researcher: volume / type
2. Our 'standardized' master mix components and volumes added
3. Amount of denatured sample we load onto the gel.

Comb Type	Pros	Cons
36-well square tooth	Able to combine several jobs	Potential for leakage between samples during loading
48-well square tooth	<ul style="list-style-type: none"> • Able to combine several jobs • More samples per gel 	<ul style="list-style-type: none"> • Need silane to keep wells separated • Need 10M NaOH to remove silane • More hand work to load; pos. leak
Sharks tooth: 96-well (loading 48)	<ul style="list-style-type: none"> • More samples per gel • Easier to load • Leakage less of a factor 	<ul style="list-style-type: none"> • Initial set-up • Half-microtiter plate
Sharks tooth: 96-well (loading 96)	<ul style="list-style-type: none"> • More samples per gel 	<ul style="list-style-type: none"> • Initial set-up • Large potential for erroneous fragment calls from leakage
Membrane	<ul style="list-style-type: none"> • Load on the bench • Storage of loaded comb • No leakage 	<ul style="list-style-type: none"> • Signal strength decreases with more samples • Inserting comb

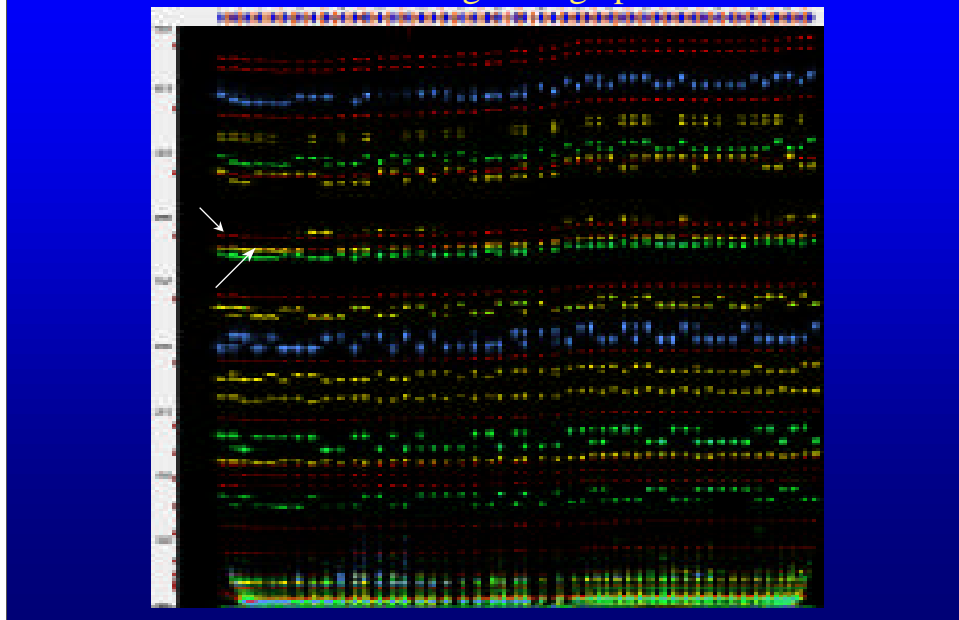
Currently used in our facility:

36-well square tooth combs

96-well sharkstooth comb, loading 48 samples

96-well membrane combs. The technique for inserting these combs between the plates is not difficult to learn, and it is wonderful for increased throughput. The total volume for the denatured aliquot of multiplexed sample plus internal lane standard is 0.8 ul per lane.

48 samples loaded using the 96-well sharkstooth comb showing leakage problem



The arrows indicate leaks which would be problematic in allele calling if all 96-wells were loaded in the sharkstooth comb.

Leakage such as this is unacceptable as the data for the intervening lanes could be confused with the spilled sample. In fragment analysis it is supremely important that each lane is absolutely representative of the sample for that lane. Without this assurance data is unreliable.

Does it matter which standard I use?

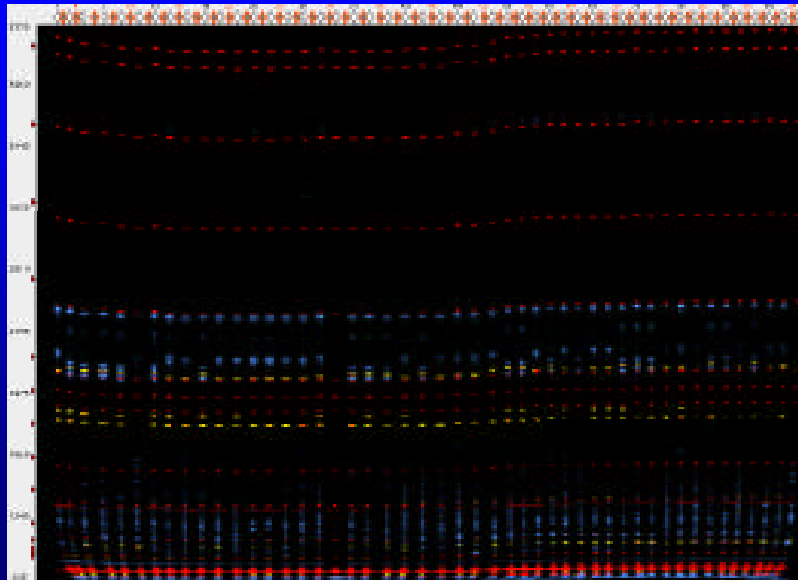
gs400hd	CXR	gs500	gs1000	gs2500
				1181
				1115
			928	
				827
			677	
			674	
			539	536
		500		
		490		490
				470
		450		
			421	
400	400	400		
380	375			
360				361
340	350	350		
320	325	340		
300	300	300	299	
290				286
280	275		275	
				269
260				
	250	250		
240			244	238
				233
220	225			222
200	200	200		
190				186
180	180			
				172
160	160	160		
150		150		
	140	139		
120	120		118	116
100	100	100	108	109
90				94
	80		81	
		75	76	
60	60		64	

Consider that the **Local Southern** algorithm requires two standard peaks above the largest unknown fragment and two standard peaks below the smallest unknown fragment in the sample.

While I dislike the gs2500 ladder because both strands are labeled, and it is very time consuming to verify the standard in each lane, it is useful for those researchers who need data for large fragments. The largest standard fragment I call is the 1181, which gives researchers data to 1.1 Kb.

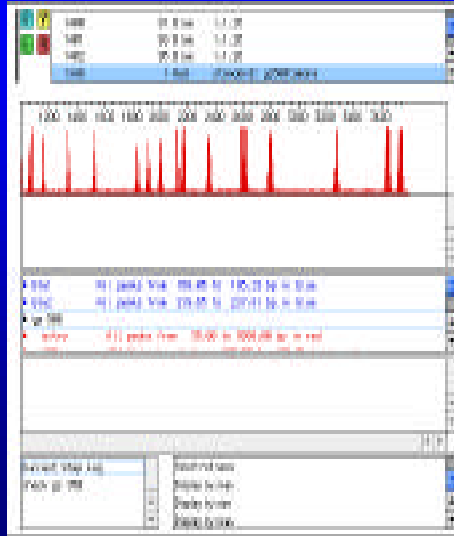
The most frequently used standards are gs500 either in ROX(filter set A) or TAMRA(filter set C), and the 400hdROX.

Do I need to add standard to each lane?

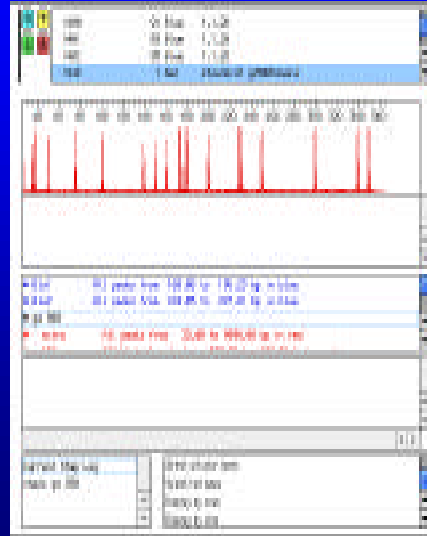


When a gel is not uniformly polymerized, there is inconsistency in the way the sample moves through the gel. With an internal lane standard in every lane, the software can compensate for any anomalies (well, most) due to this condition.

Align: By Scan



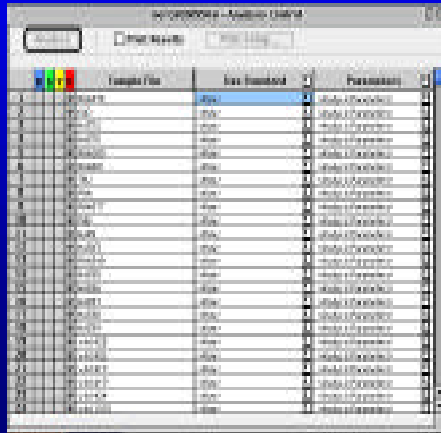
Align: By Size



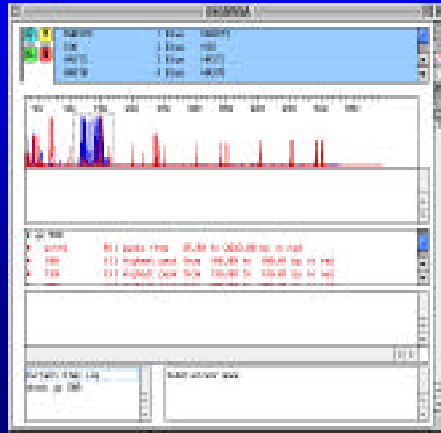
The align by size tightens up the standard bands to a level of uniformity not seen in the align by scan. The align by scan indicates the variation between lanes due to non uniformity in the way the sample ran through the gel.

Analysis: Main Windows

GeneScan



Genotyper



These are the two analysis windows which represent the two sides to the ABI analysis software package. GeneScan turns the raw signal into sizes by assigning numbers to the unknowns as calculated from the standard curve developed for the internal lane standard used for each lane.

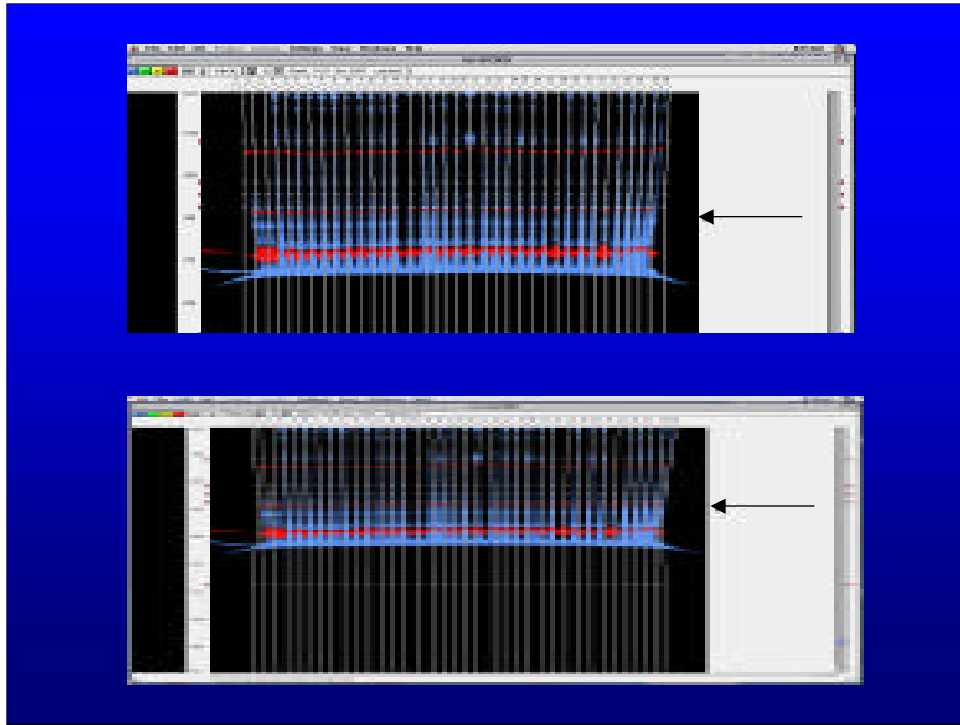
Genotyper takes the output from GeneScan and allows an electropherogram to be plotted for downstream analysis of fragment data.

GeneScan Analysis

Getting Started

1. Look at the Gel Image
2. Track lanes and verify traces
3. Set Analysis parameters
4. Extract Lanes
5. Choose Standard
6. Analyze

The beginning of Analysis should begin with looking at the Gel Image for any obvious problems. Tracking algorithms help greatly to get the sample lanes aligned. However, verification by eye is critical. Once a lane has been extracted one can return to the image for re-extraction if the tracking was faulty, but it saves time if do this evaluation up front.



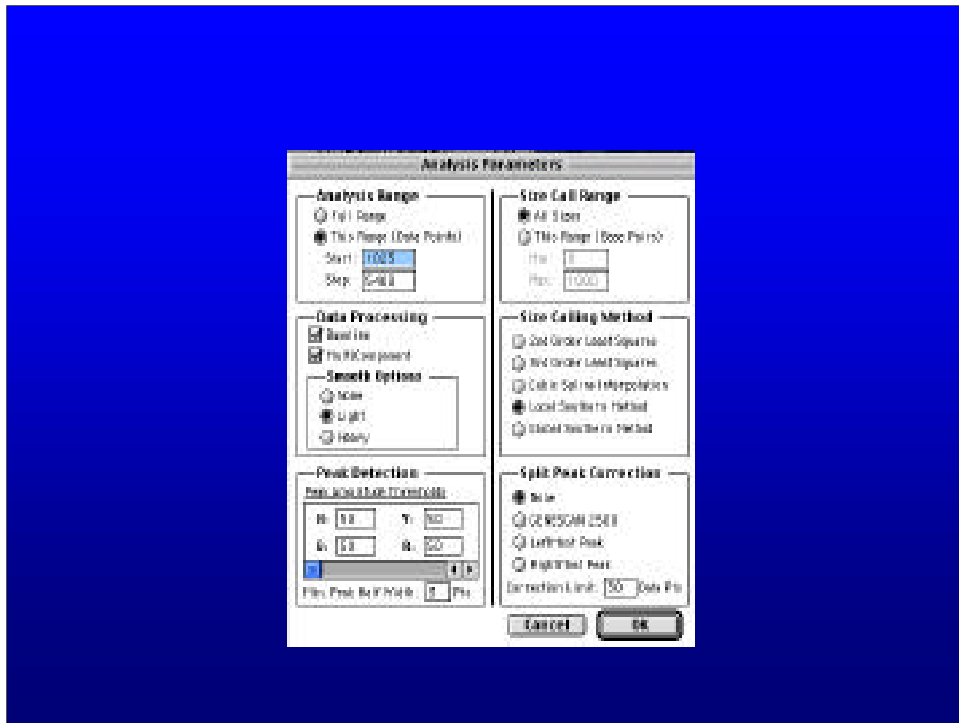
After autotracking, look closely at the beginning of the gel (dye front) to verify that the traces intercept all of the standard bands.

GeneScan Analysis

Getting Started

1. Look at the Gel Image
2. Track lanes and verify traces
3. Set Analysis parameters
4. Extract Lanes
5. Choose Standard
6. Analyze

This set of steps is outlined here and detailed in the following slides.



This dialog box for Analysis Parameters is located under the Settings Menu.

I set up the range to start above the dye front and below the lowest distinguishable internal lane standard band.

I like light smoothing for the majority of fragments. This feature will smooth over peaks caused by gel inconsistency but also allows for single-base differences to be distinguished.

I vary the Peak Detection thresholds according to the individual gel requirements. I prefer using 50 (rfus) as the default, if possible.

I always use Local Southern Method for Size Calling.

Sizing Questions and Choices

• **Global methods** are less affected by anomalies in run times of single size standard fragment; **will not** give normalized data lane-to-lane, gel-to-gel

→ • **Local methods** are less affected by changes in electrophoresis conditions or in analysis range; **will** normalize data across lanes and gels

• **Accuracy** sizes that are close to actual size as determined by sequencing

→ • **Precision** (=reproducibility) generates the same size consistently for a given fragment, independent of whether it is close to the actual sequenced size

It is important to understand the different choices for the analysis software.

This slide gives some explanation regarding different approaches to the data.

The arrows indicate the considerations which I feel are most useful for the work in our core facility.

Forensic Sci.Int.(1998) 2:81-90 highlights variation in sizing due simply to the actual sequence rather than the sequence length, that is a sequence where the AC / GT ratio is greater than 1 will migrate through a slab gel faster than the inverse (GT / AC > 1).

Which Size Calling Method Should I Use?

Local Sizing Methods

- **Local Southern Method**
 - Generally recommended by ABI
 - Most useful for precise data
- **Cubic Spline Interpolation**
- **GeneScan** calculates the best fit least squares curve for all samples, regardless of size calling method you use.

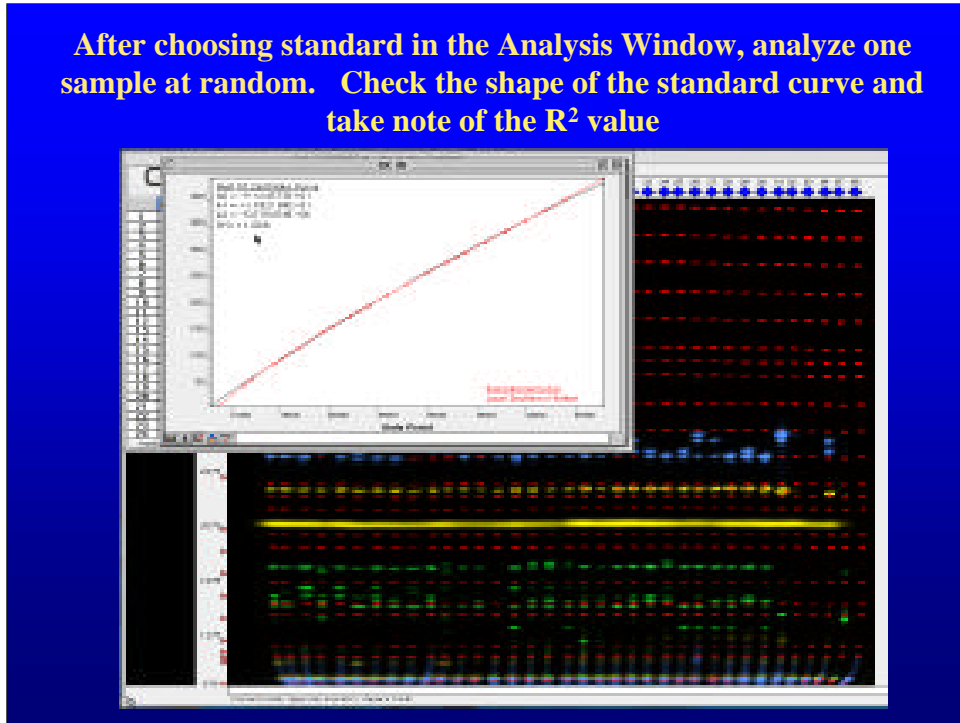
Global Sizing Methods

- **Least Squares (2nd, 3rd Order) and Global Southern Method**
 - Uses all standard peaks for standard curve; does not allow assignment of size to the individual standard peaks



Local Southern Method is the sizing algorithm of choice in the CSL.

After choosing standard in the Analysis Window, analyze one sample at random. Check the shape of the standard curve and take note of the R² value

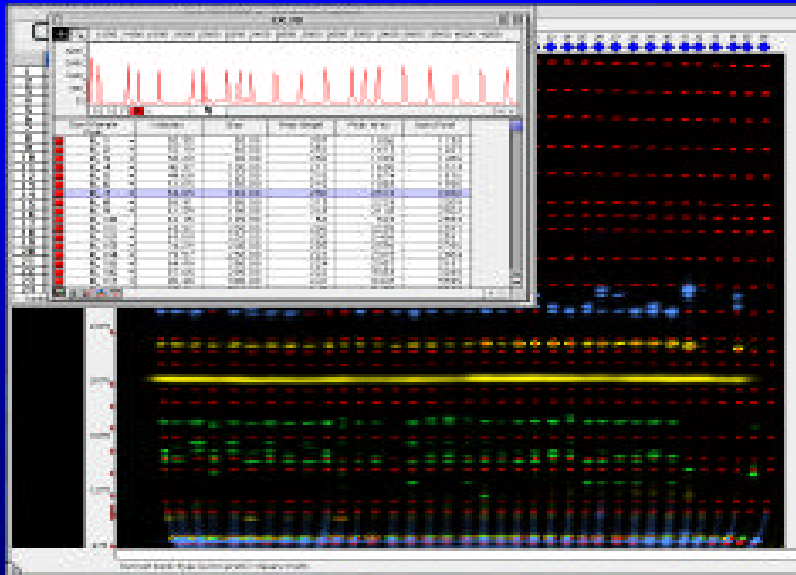


The way I verify that a standard I have chosen will work for the gel being analyzed is to analyze the standard in any random sample. If the Analysis Log pops forward there will be a message detailing any problem that the software had for labeling the standard fragments. If the Analysis Log window does pop forward, it is likely that you will need to define an internal lane standard specifically for this gel.

If the Analysis Log does not pop forward, double click (in the Analysis Control window) on the sample that was analyzed. At the bottom left of the window are several icons, one of which is for the standard curve. Click on it to visualize the curve for this sample. If it appears straight, then it is probably a good match.

Double check this by clicking on the icon for the peak data (furthest left). The peak calls below the graph which have dots beside them represent those peaks which the software assigned as one of the 'official' standard peaks. The next slide illustrates this way of double checking the standard by highlighting one peak call and verifying which peak is chosen in the plot.

Return to the plot view, click a random size standard in the list and check that it is called correctly



This indicates that the colored peak is represented by the shaded row in the table. Verify that they are correctly assigned before proceeding to analysis of the entire set of samples for this gel.

Typical Analysis Problems, or The Analysis Log is your friend

Examples:

- 1 -Missed standard peak
- 2-Exceeds detection limit of 250 peaks of a given dye
- 3-Off-scale peaks

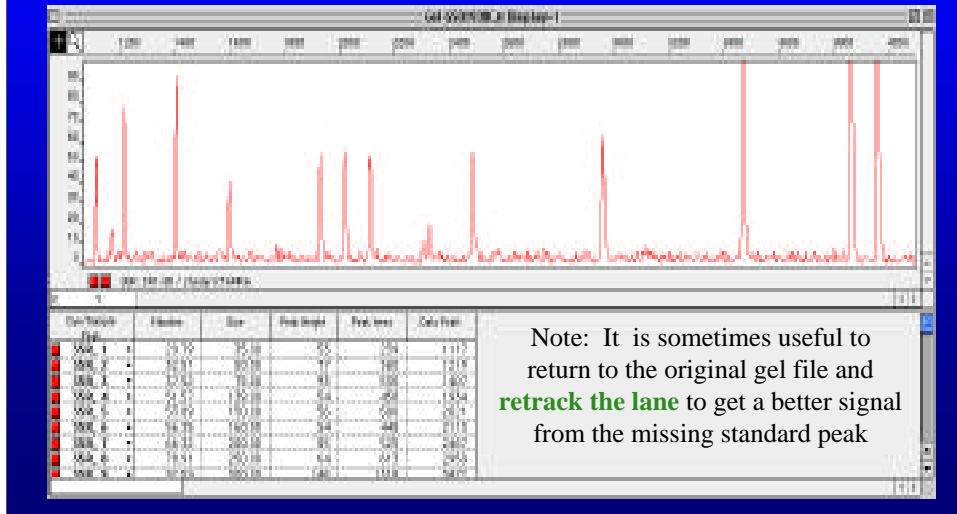
Use the Analysis Log as a tool to get each sample to be correctly sized. If the Analysis Log does not pop forward after analysis, you can proceed to Genotyper. However, in the following slides there are three examples of problems which I see on a regular basis with hints how I overcome each.

Analysis log: Missed Standard Peak

```
>> CR1-29 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
>> CR1-30 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
>> CR1-31 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
!!! Size standard matching skipped the peak defined as 75 bps.  
>> CR1-32 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
>> CR1-33 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
!!! Size standard matching skipped the peak defined as 139 bps.  
>> CR1-34 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
!!! Size standard matching skipped the peak defined as 139 bps.  
>> CR1-35 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
!!! Size standard matching skipped the peak defined as 100 bps.  
>> CR1-36 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
!!! Size standard matching skipped the peak defined as 100 bps.  
>> CR1-37 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
!!! Size standard matching skipped the peak defined as 200 bps.  
>> CR1-38 (B, R): Analysis completed.  
>> Size Calling completed. Matched Size Range: 35 - 350 bps  
>> CR1-39 (B, R): Analysis completed.
```

When one standard band has not been determined by the analysis software, the analysis log will tell you which sample and which peak are being called into question.

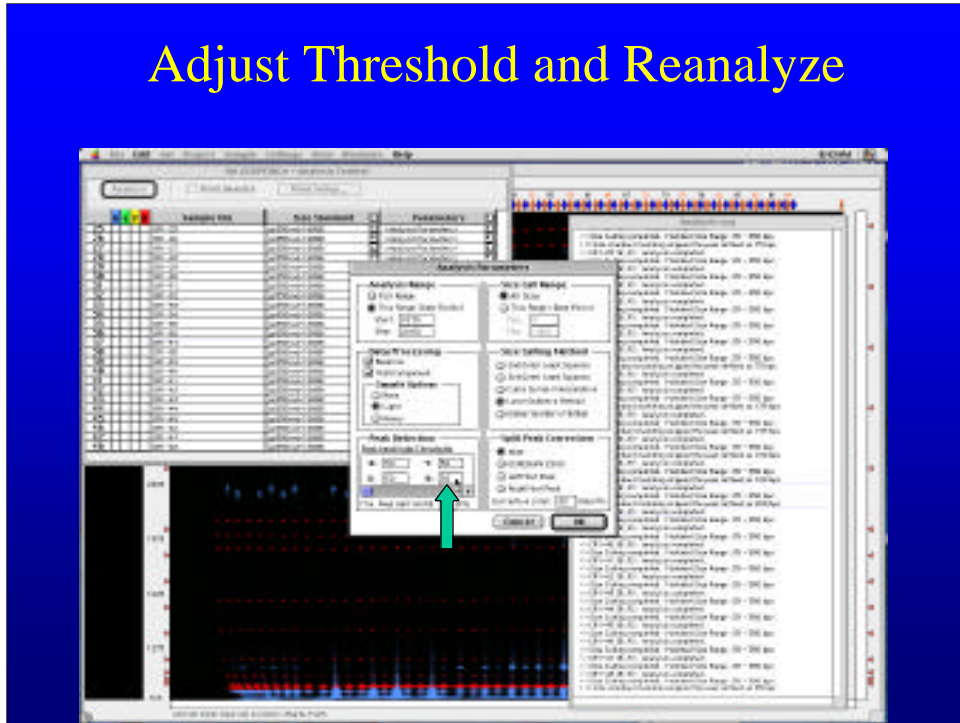
Look at the Results Control Window:
note height of standard peak which was
missed, reduce the threshold, reanalyze



Using the Results Control Window I look at the graph of the standard in the sample indicated in the Analysis Log. In this example the 100bp peak is shorter than the 50rfu threshold set in the Analysis Parameters.

Reopen the Analysis Parameters dialog box and change the threshold to accommodate the peak height for the short standard band; then reanalyze that sample. Sometimes I reanalyze the entire sample set in the Analysis Control window.

Adjust Threshold and Reanalyze



This is the Analysis Parameters dialog box indicating where to make the threshold adjustment for reanalyzing the samples.

Analysis Log: Maximum number of peaks exceeded

```
>> PAR2-14 (G, R): Analysis completed.
>> Size Calling completed. Matched Size Range: 37 - 1181 bps
>> PAR2-15 (G, R): Analysis completed.
!!! Exceeded the detection limit of 250 peaks for dye R.
>> Size Calling completed. Matched Size Range: 37 - 361 bps
>> PAR2-16 (G, R): Analysis completed.
!!! Exceeded the detection limit of 250 peaks for dye R.
>> Size Calling completed. Matched Size Range: 37 - 361 bps
>> PAR2-17 (G, R): Analysis completed.
>> Size Calling completed. Matched Size Range: 37 - 1181 bps
>> PAR2-18 (G, R): Analysis completed.
!!! Exceeded the detection limit of 250 peaks for dye R.
>> Size Calling completed. Matched Size Range: 37 - 361 bps
>> PAR2-19 (G, R): Analysis completed.
>> Size Calling completed. Matched Size Range: 37 - 1181 bps
>> PAR2-20 (G, R): Analysis completed.
!!! Exceeded the detection limit of 250 peaks for dye G.
>> Size Calling completed. Matched Size Range: 37 - 1181 bps
>> PAR2-21 (G, R): Analysis completed.
!!! Exceeded the detection limit of 250 peaks for dye R.
>> Size Calling completed. Matched Size Range: 37 - 361 bps
>> PAR2-22 (G, R): Analysis completed.
!!! Exceeded the detection limit of 250 peaks for dye R.
>> Size Calling completed. Matched Size Range: 37 - 361 bps
>> HA89-01 (G, R): Analysis completed.
>> Size Calling completed. Matched Size Range: 37 - 1181 bps
!!! Size standard matching skipped the peak defined as 109 bps.
>> P21-01 (G, R): Analysis completed.
!!! Exceeded the detection limit of 250 peaks for dye R.
>> Size Calling completed. Matched Size Range: 37 - 361 bps
```

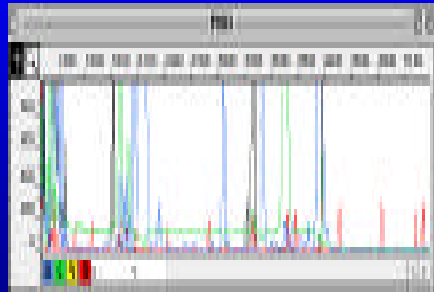
The maximum number of peaks collected for each color is 250. If the software detects peaks beyond this number it will alert you, in the analysis log, by describing which color has exceeded this limit, and in which sample this has occurred.

Use the Results Control window (illustrated in next few slides) to determine the best way to get data from samples with this problem.

Exceeding detection limit of 250 peaks

Usual cause = High baseline

1. Check matrix, install new matrix and reanalyze.
2. Raise threshold of specific dye above background
3. Restrict analysis range to two standard peaks above and below unknown peaks



This plot window shows all colors in a single sample.

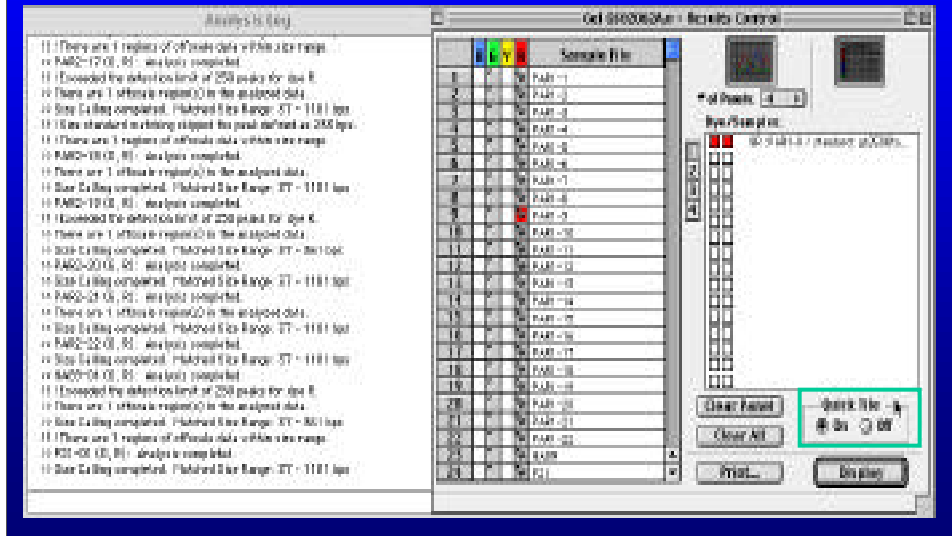
The signal from the green dye shows a high baseline which explains how the maximum number of peaks detected was exceeded.

There are three ways I have found (listed above) to get around this problem: Check the matrix: double click on any sample in the GeneScan project file. When that sample data opens, click on the icon at the lower left with the **i** symbol. The matrix used for analysis is listed in this information window. Check to see that this was for the correct filter set (used for the run). Sometimes the most recent matrix is not as good as another, so if I'm having matrix problems I will try various matrices and reanalyze.

If installing a new matrix makes no difference, I look at the height of the elevated baseline in the Results Control window, go back to the Analysis Parameters and change the threshold for the sample with the high baseline to eliminate the baseline. I adjust these parameters only for the sample(s) which had the problem.

I would rather not restrict the analysis range as stated in #3 above, but I will if I cannot get sufficient data any other way.

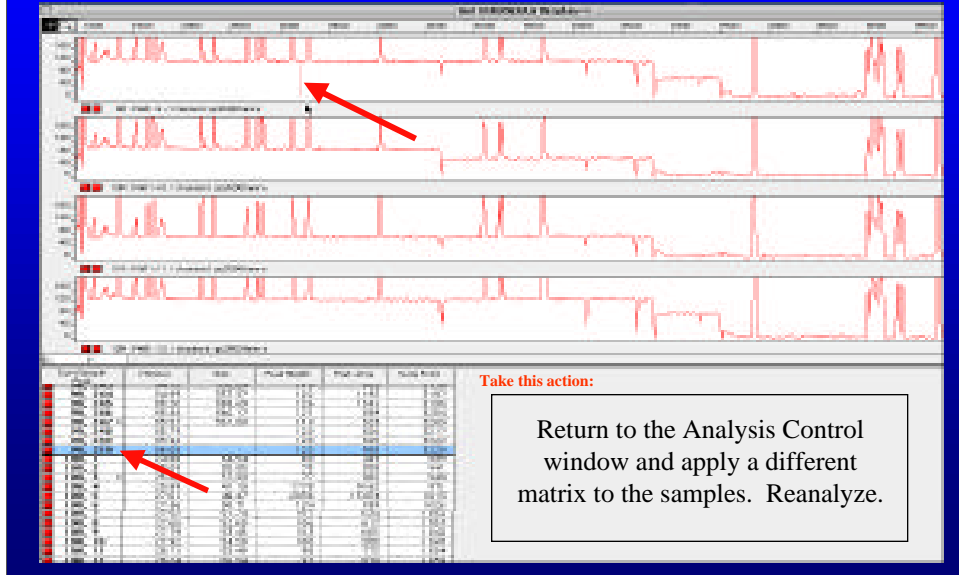
Check several lanes of the offending dye in a quick tile display



This shows the Results Control window with the Quick Tile display ON.

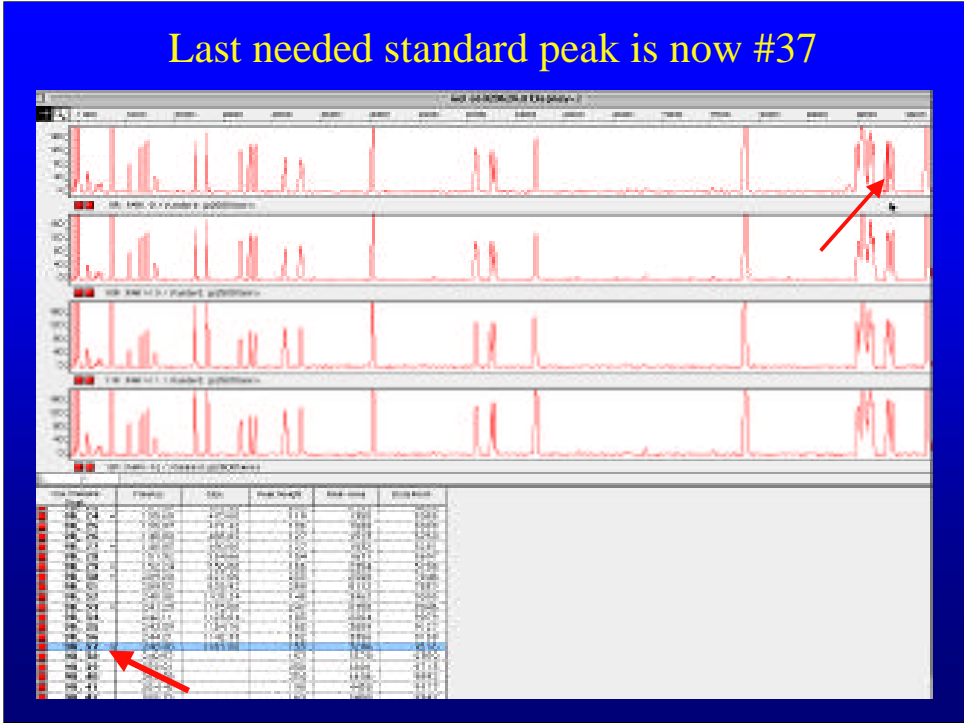
The samples indicated in the Analysis Log are highlighted in the Results Control window and then displayed.

The maximum number of peaks (#250) was reached in the first third of the gel run:



These plots are in the Quick Tile display so that we can see each lane independently. The arrow on the top panel shows where the 250th red peak is between 269 and 286bp.

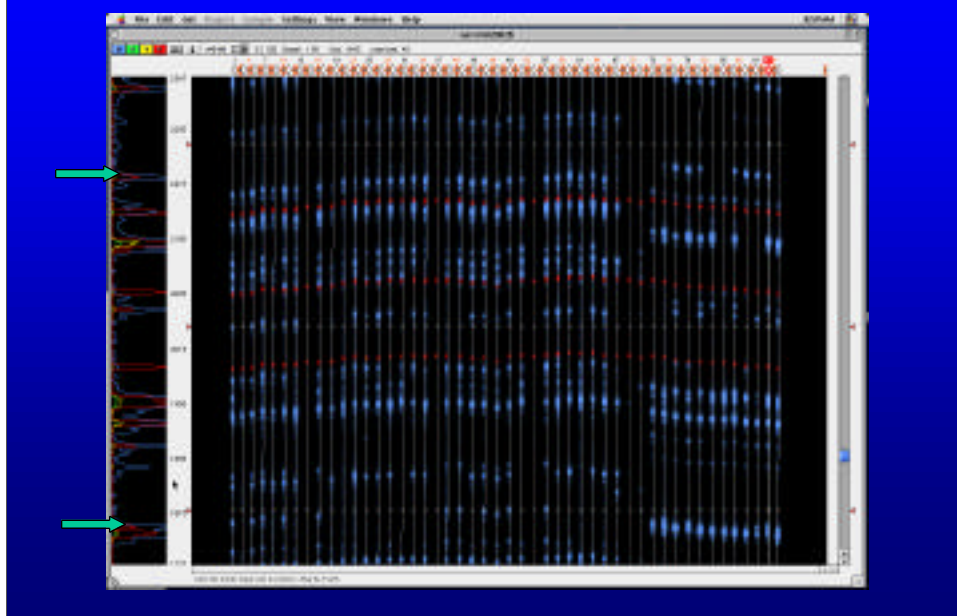
The arrow on the bottom panel confirms which peak is being shown in the upper panel.



After the sample was reanalyzed using a different matrix, the high baseline was eliminated. Now the last peak in the standard (1181bp) is peak # 37.

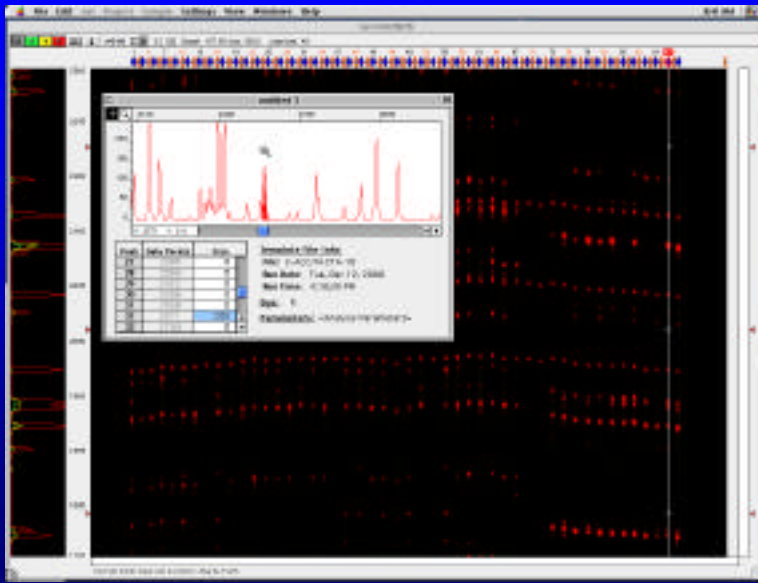
Strong Spectral Overlap - Likely a Matrix Problem

If samples are overloaded, often matrix manipulations are ineffective

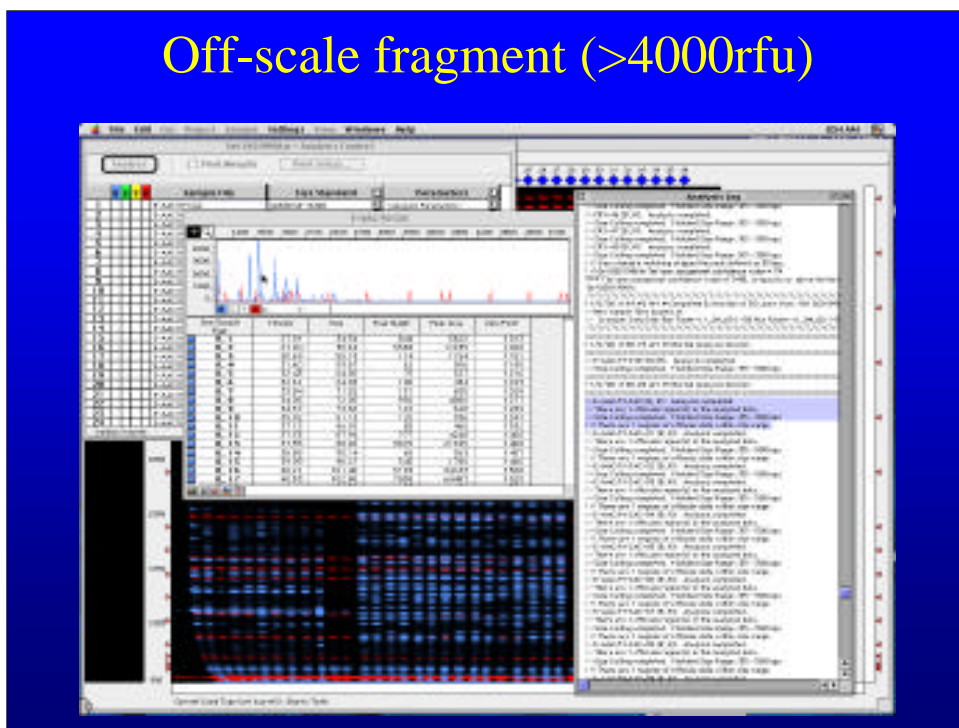


The arrows on the left are indicating red peaks caused by spectral overlap. These peaks are not part of the standard ladder.

Problems in calling standard peaks caused by strong spectral overlap



Off-scale fragment (>4000rfu)

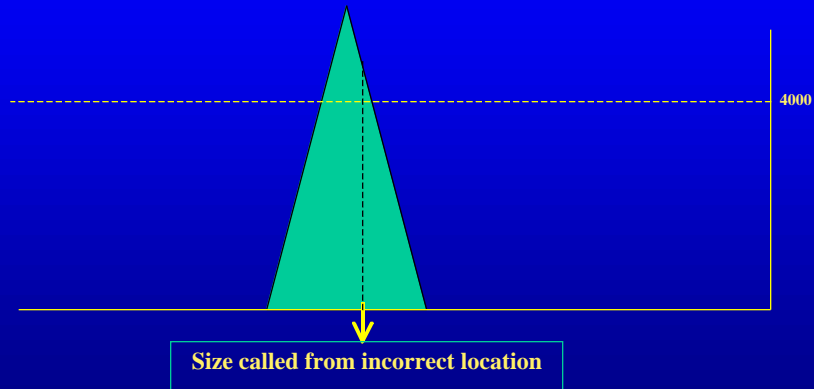


Sometimes samples are overloaded where the software is unable to accurately make a size determination. The message that off-scale signals were detected will indicate which samples need to be scrutinized.

Any peak above 4000rfus is suspect regarding sizing. When the analysis log indicates a sample has off-scale peaks I check to be sure that this does not refer only to the dye front. Then I copy the Analysis Log information onto a text document and put it into the researcher's electronic folder.

I warn them initially about apparent polymorphisms, if the samples are off-scale. However, once I've given them the copy of the analysis log I leave it up to them to decide whether to believe the data or not.

False data from overloaded sample



GeneScan cannot distinguish the top of a fragment whose fluorescence is too strong. Peak height for most 'reliable' data is between 150 - 4000 rfus. 3000 rfus is optimal.

This cartoon is to show that sometimes the software will give a size for a peak, even when the exact location (size) of that peak cannot be determined.

Genotyper

1. Using a macro to verify the standard peaks
2. Filtering the unknown peaks

We use the Genotyper software package for two main purposes:

Verifying the standard peaks are correctly called is critical to being confident in the calls for the unknown fragments.

Sizing the unknown peaks, and filtering these peaks is useful, especially with microsatellite data.

Using a macro in Genotyper

Purpose: To verify standard peaks called correctly

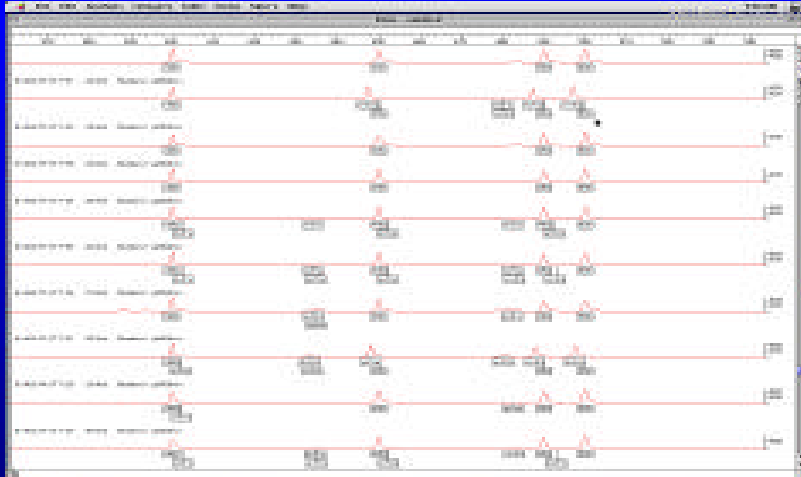
The screenshot displays two windows from the Genotyper software. The left window, titled 'Steps - G402055A', contains a macro script with the following steps: 'Hiams: check:gt:500', 'Select next lanes:', 'Clear Labels:', 'Select category: gt:500', 'Mark selected categories:', 'Label category peaks with the category's name', and 'Show the plot window:'. The right window, titled 'Categories - entitled', shows a list of peaks for the category 'gt:500'. The list includes columns for 'extra', 'IC', 'Height', 'peak', 'from', and 'to'. The 'extra' column contains 'X' for most peaks, indicating they are assigned to the 'extra' category. The 'IC' column contains 'IC1' for all peaks. The 'Height' column contains values like '100.00', '100.05', etc. The 'peak' column contains values like '100.00', '100.05', etc. The 'from' and 'to' columns contain values like '100.00 bp' and '100.01 bp'.

On the left is the macro which I use whenever I begin Genotyper. I have a macro set up for each of the standards we use.

On the right is the category window showing the members of the category set up for the standard. Note: the member 'extra' assigns every peak. Then the exclusive (X) members remove the word extra and assign their name to the peak which sizes within 0.01 bp of their size.

Note: the macro assigns the category name to the peaks rather than the size.

Wrong Peak Chosen



Action to take: Return to the GeneScan project file. Make new size standard for problematic lanes and Reanalyze

This is an example of the software calling the wrong peak as one of the standard fragments.

When the macro is initially run, the plot window will pop forward. Look closely at this window, scrolling through all samples. Look for the word 'extra' indicating a peak which was found but not considered as one of the standard fragments. If an 'extra' peak is located near one of the real standard fragments, look closer to verify that the correct peak was called as the standard peak.

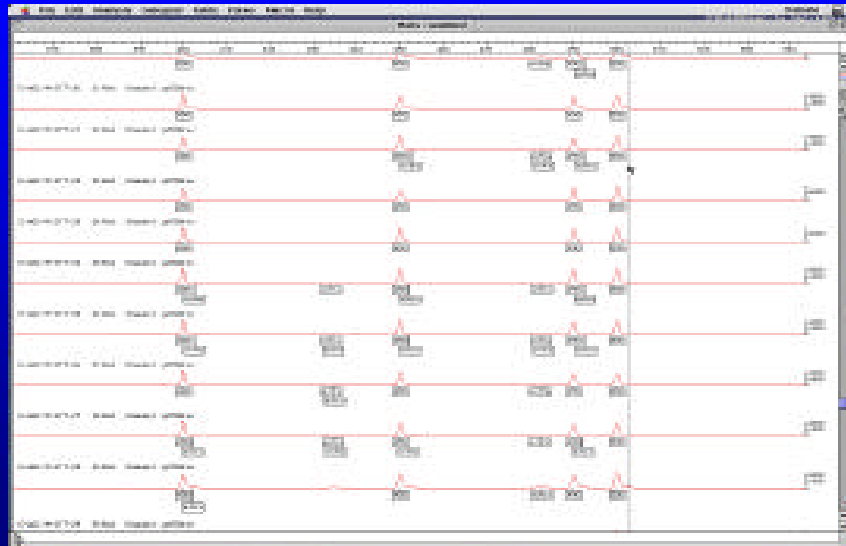
If you find, as in this example, that the wrong peak was called, make a note of which sample is incorrect, return to GeneScan project file and make a new size standard for this sample. Reanalyze.

Return to your Genotyper file, clear the dye lane list (under Analysis), reimport the new analysis files, and run the macro again.

Repeat as often as necessary to get all standard peaks corrected.

Then, continue with analyzing the unknown fragments.

Peak Corrected



This is the corrected version.

Filtering Peaks

Filter Labels

Remove labels from peaks in the size range 0.00 to 100.00

Remove labels from peaks whose height is less than 32 % of the highest peak in a category's range

Remove labels from peaks preceded by higher, labeled peak within 0.00 to 1.60 bp (Higher by at least 5 %)

Remove labels from peaks followed by higher, labeled peak within 0.00 to 3.00 bp (Higher by at least 5 %)

Cancel OK

The checked boxes above causes the removal labels from:

1. Peaks lower than 1/3 height of largest peak in the category
2. Peaks caused by the +A peak
3. Peaks caused by stutter from slippage of Taq over microsatellite amplicons

I use the default filters.

The first filter removes shoulders from PCR products as well as background peaks which may be above the threshold defined during GeneScan analysis.

The second filter is intended to remove a label from a peak which represents the plus-A peak. See the FARG 2001 Study for more information on this phenomenon.

The third filter is intended to remove stutter peaks caused by slippage of Taq during the extension step of PCR.

The values in the boxes can be customized to work within project parameters.

Other Analysis Packages

Using the **PC** Environment to analyze GeneScan output files:

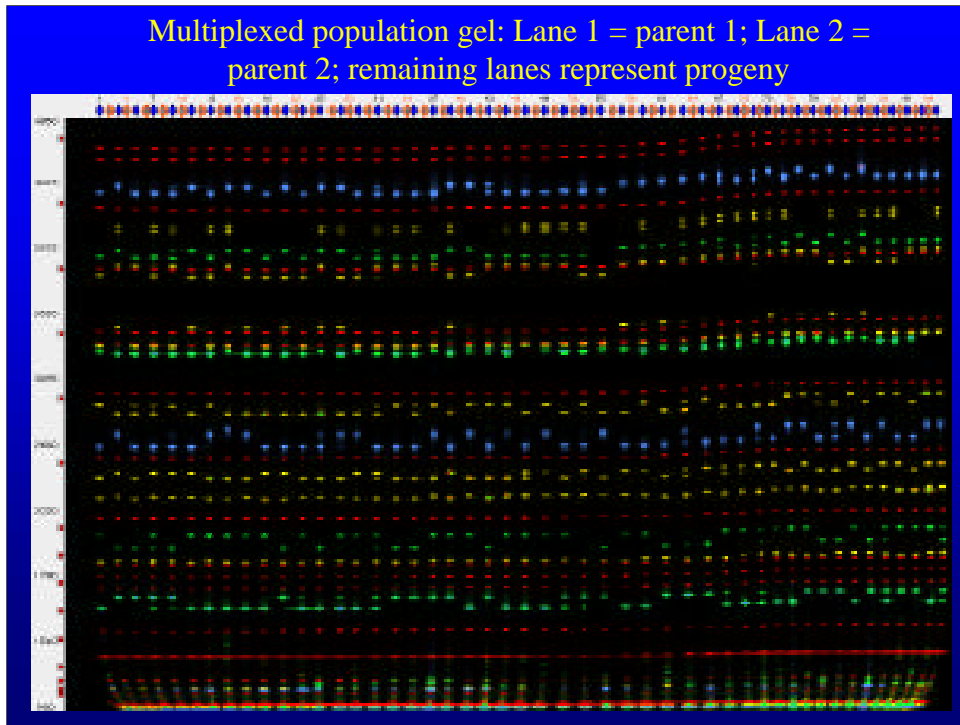
Genographer: Used for **AFLP** analysis:

<http://hordeum.oscs.montana.edu/>

STRand: Used for **Microsatellite** analysis:

<http://www.vgl.ucdavis.edu/strand/>

In the Windows environment there are software packages to do downstream (post GeneScan) analysis. The two websites listed here are available as freeware.



Here is a nice example of a multiplexed gel screening a segregating population with the parents loaded into lanes one and two and the progeny loaded into the remaining 46 lanes. This gel was loaded into every other lane using a multi-channel pipette and a 96-well sharkstooth comb.