NSW Police Force

NSW GOVERNMENT Health

PHASE 1 EVALUATION REPORT OF CYBERGENETICS TRUEALLELE® EXPERT SYSTEM

NSW REVIEW TEAM
JULY 2011

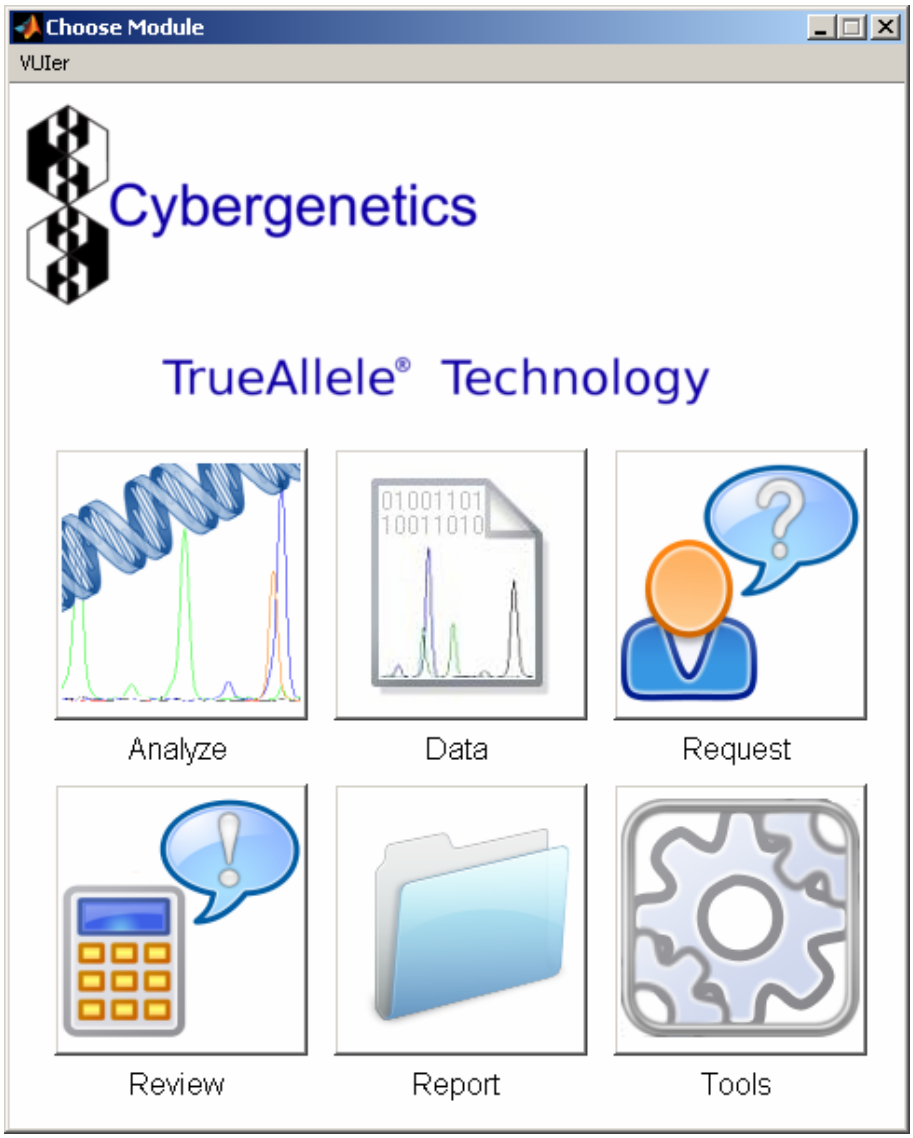


TABLE OF CONTENTS

1. KEY FINDINGS SUMMARY

1.1 INFORMATION GAIN

TA generally provides LRs that are significantly higher than those calculated under current methods. There is ample evidence that TA computer processing preserves all the identification information in the DNA data which translates into computation of LRs for samples which cannot be interpreted under current methods, and LRs generally a number of orders of magnitude higher than for samples currently interpreted.

1.2 SENSITIVITY

TA provides LRs for single source samples with extremely low template DNA and also a substantial number of mixtures that are too complex for any statistical evaluation under current methods. In addition, TA provides LRs for minor components of mixtures where current methods are limited to reporting an LR for the major component.

1.3 SPECIFICITY

15 known contributors were used in the construction of the mixtures. There was no instance where an LR>1 was calculated for any person as a contributor to any mixture other than the ones which they were known to contribute to. In addition, reference samples from relatives of the known contributors were also compared to the inferred genotypes. A small number of positive LRs were observed where there was significant allele sharing with a relative in a mixture, however the log LR values were generally negative.

This study tests the ability of TA to accurately infer genotypes from sets of laboratory-generated data with contributors of known genotypes. Plots of match scores for major and minor contributors in two person mixtures are generated. In most of these mixture types the inferred genotypes reflect the known genotypes, or display uncertainty that is consistent with the nature of the data. (Exceptions to this in certain mixture types were identified during the course of this evaluation and will be discussed under limitations).

1.4 REPRODUCIBILITY

All samples were uploaded to TA, the uploaded data analysed in duplicate and reproducibility was calculated. The process is inherently stochastic and it was expected that replicates would usually differ by small amounts and that outlier results would occur. It was demonstrated that results with higher match scores generally have greater reproducibility.

1.5 EASE OF USE/END USER SUPPORT

TA is very user friendly and provides good opportunity for the analyst to explore the data analysis and TA's interpretation using the several different modules/views. Cybergenetics has been very supportive in all areas throughout phase 1 of the evaluation. Technical advice, prompt response to queries from the review team and their assistance with data analysis has been helpful during this process. It has been

demonstrated that Cybergenetics has the capability to provide significant support to any group implementing TA within their laboratory.

1.6 TIME EFFICIENCES

All samples were analysed for 50,000 cycles although this could have been reduced for the more straightforward samples. TA typically required 10 hours to carry out a 2 person mixture analysis or 15 hours for a 3 person mixture and might require longer depending on the complexity of the data. A single processor channel is therefore at best limited to solving 3 mixed samples running over a 24-hour period. To protect against outlier results, samples may require duplicate or even triplicate processing depending on laboratory policy. A high through put laboratory will need to invest in the appropriate number of parallel processor channels to meet demand. Time required for data upload and results review will also require consideration.

1.7 POTENTIAL DIFFICULTIES

One of the purposes of this study is to assess the strengths and weaknesses of TA. It has been recognised that some data will require more careful interpretation than others and this study has helped identify such data. These areas include:

- Mixtures with equal or nearly equal contributions of DNA from 2 contributors. TA does not consistently perform well in this situation. Several examples were encountered where it was expected that there would be uncertainty spread across the possible genotypes at a locus, however the genotype probability distribution heavily weighted one or two possible genotypes. Depending on the genotype of the suspect, this could either reduce or inflate the LR at this locus.
- In cases with similar levels of contribution from 2 or more persons a lot of 'crossing over' was observed in the MCMC history data generated by TA. This decreases the ability to resolve the genotypes but we have not yet established whether this always has an adverse affect on the overall LR or not.
- In samples where the minor contributor(s) are at low levels, modelling of stutter is inconsistent and often not supported by the data. Cybergenetics have indicated that more informative priors, based on laboratory test results, will be put back into the system by the end of 2011 and that this will improve the capability of the system to deal with stutter behaviour. This review will not be limited to stutter but will include other end user laboratory dependant settings such as peak variance.
- Artefacts, such as spikes or pull-up that are not removed from the data prior to the analysis stage were found to interfere with the subsequent interpretation.
- Currently TA gives no probability as to the number of contributors and analysis requests must specify the number of unknown contributors and identify all known contributors. It will run any request and try to determine the most likely genotypes for the specified number of contributors regardless of how badly the request fits the data. Cybergenetics have indicated that they will incorporate the capability to give a probability to the number of contributors by the end of 2011.

- Reproducibility between duplicate runs is not always achieved and instances with significant differences were identified.

These issues are discussed further in this report. Laboratory policy in relation to the implementation of TA may need to address these issues.

1.8 STANDARDISATION

There is a move towards standardisation of DNA interpretation across Australian jurisdictions and the implementation of an expert system such as TA could assist in this endeavour. TA provides a significant advancement in the direction of standardisation by eliminating the need for guidelines and thresholds currently used in the different laboratories. However, there may be some variation in the results due to different analyst requests, reproducibility of runs, and variation at the initial data analysis stage.

1.9 OJECTIVITY

The computer processing of the data is inherently objective because the computer has no knowledge of any reference profile when the genotype probability distributions are generated. This complete objectivity is a considerable advantage in the courtroom environment provided the algorithms adequately account for the variation associated with all the parameters that have a significant effect on the PCR process.

1.10 ADDITIONAL FEATURES

Persons known to be contributors, for example the complainant, to a mixture can be included in requests selected and an analysis carried out with fewer unknowns which strengthens the interpretation of the inferred unknown profile(s). TA also incorporates a number of useful additional features which enhance the capability of the system. The most notable feature is the capability to carry out interpretation on multiple amplifications and/or multiple items. The system also has a 'degradation' feature which can be activated and while this increases the analysis time it may enhance the interpretation.

1.11 GENERAL ACCEPTANCE

Recent SWGDAM guidelines allow for a probabilistic approach to DNA interpretation. During the course of this evaluation, attention has been given to the presentation of TA match statistics in various court matters (outside Australia). The recent review and recommendation by the New York State DNA subcommittee to allow /endorse TA use in the New York crime laboratory has been noted. We have also noted other laboratories have shown interest in TA and held discussion with scientists testing the TA system at NIST and other users. Reference has been made to the many peer reviewed publications in relation to TrueAllele.

1.12 MATHEMATICAL MODEL

The mathematical model for TrueAllele is presented in attached extract (document 4) from *Validating TrueAllele DNA Mixture Interpretation* amended by John West. The original paper is in press but can be viewed at Cybergenetics website.

2. Phase 1: Evaluation of Cybergenetics TrueAllele Expert System

2.1 BACKGROUND

TrueAllele is an expert computer system for objective automated interpretation of STR data using a continuous probabilistic model, which gives a weight to a genotype based on the fit of the peak heights to the proposed combination. This model has significant advantages over binary methods that apply thresholds and therefore may result in loss of information. The goal in DNA interpretation is to extract as much information as possible from the evidence. Interpretation of complex mixtures and low template DNA is constrained by threshold values. In contrast, TrueAllele mathematically models the quantitative data and has no "threshold" issues. The way forward in DNA interpretation is generally accepted to be the adoption of a probabilistic approach where the uncertainty around the evidence data is accounted for and therefore thresholds may not be needed. In theory, TrueAllele should have a much greater ability to identify correct hypotheses if the modelling parameters adequately reflect variability in peak height as a function of peak height, locus and allele. Stutter and baseline noise will introduce challenges to the modelling parameters. Peak heights have variation which can be assessed in a probabilistic model whereas thresholds have absolute values so can't reflect variation. A probabilistic model that can calculate the uncertainty around every data element instead of using a threshold solution will preserve the information in the data. It has been demonstrated by the NSW review and many other groups that the information gain is significant using TA analysis and also that TA can generate LRs for many samples which under current human review would be inconclusive. This is of significant value to the justice system.

One of the desirable requirements of an expert system is that it facilitates inter-jurisdictional standardisation in the interpretation of STR data and capability in respect of this is evaluated. Standardisation has been targeted as a critical consideration in respect to DNA interpretation.

As robotic platforms are increasingly utilised to facilitate high throughput of DNA samples, the implementation of expert systems may assist in handling the increase in evidence data by reducing interpretation time.

The aim of the validation study is to test the ability of True Allele to deduce genotypes of the persons contributing DNA to a set of laboratory-constructed profiles (Document 1). Mixtures of varying ratios and complexities were constructed to explore the limits of TA's capability in resolving the contributor profiles. In particular the focus of the assessment is on specificity (accuracy), sensitivity, information gain and reproducibility. In addition the study tested the lower limits of detection with single source profiles.

3. SENSITIVITY AND INFORMATION GAIN

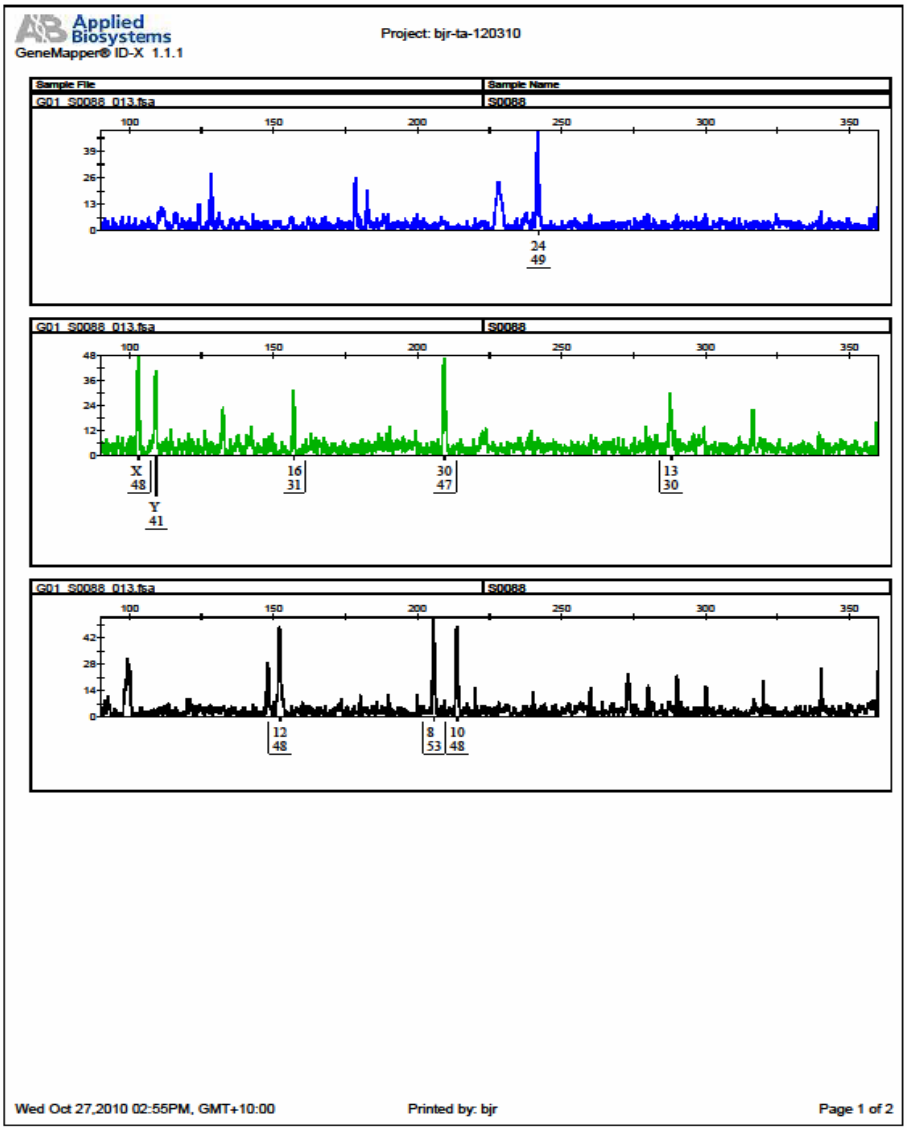
3.1 SINGLE SOURCE SAMPLES

Single source profiles from known sources were amplified at various DNA input amounts ranging from 1ng to 16pg. TrueAllele generated LRs for low level DNA which demonstrated a significant information gain and increase in sensitivity against current capability. Samples S0093, S0088 and S0102 are extreme low level samples (16pg) which demonstrate the capability of TA to use all the information within the data beyond the scope of current laboratory methods which are restricted by a reporting threshold value of 50RFU causing all data below 50RFU to be lost. The removal of a threshold poses some risk that baseline noise and artefacts will be included in the analysis but this should decrease the inferred posterior probability and therefore the likelihood of the suspected source. With TA, unconstrained by a threshold, the criminal justice system will be presented with DNA evidence, which is not currently reportable. Generally the inferred genotypes seem reasonable, or display appropriate uncertainty in genotype distribution. All possible allele pairs are considered right down to baseline albeit with very low posterior probability. These samples appear to have reached the limits of reasonable interpretation by TA in clean samples and may be adversely affected by casework samples where baseline artefacts are more likely to occur. TA is less effective in weak samples when one allele of a heterozygote approaches baseline. In this event TA overweights the probability of a homozygote. An example of this can be seen in S0107 in the table on page 10 showing the information gains for single source samples. In this instance, current dropout based methods assign a higher probability to the possiblility of a heterozygote contributor and therefore perform better.

S0088_ vs A252035

The LR calculation assumes one unknown contributor in the evidence relative to a AU_CAU human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 38.5 million

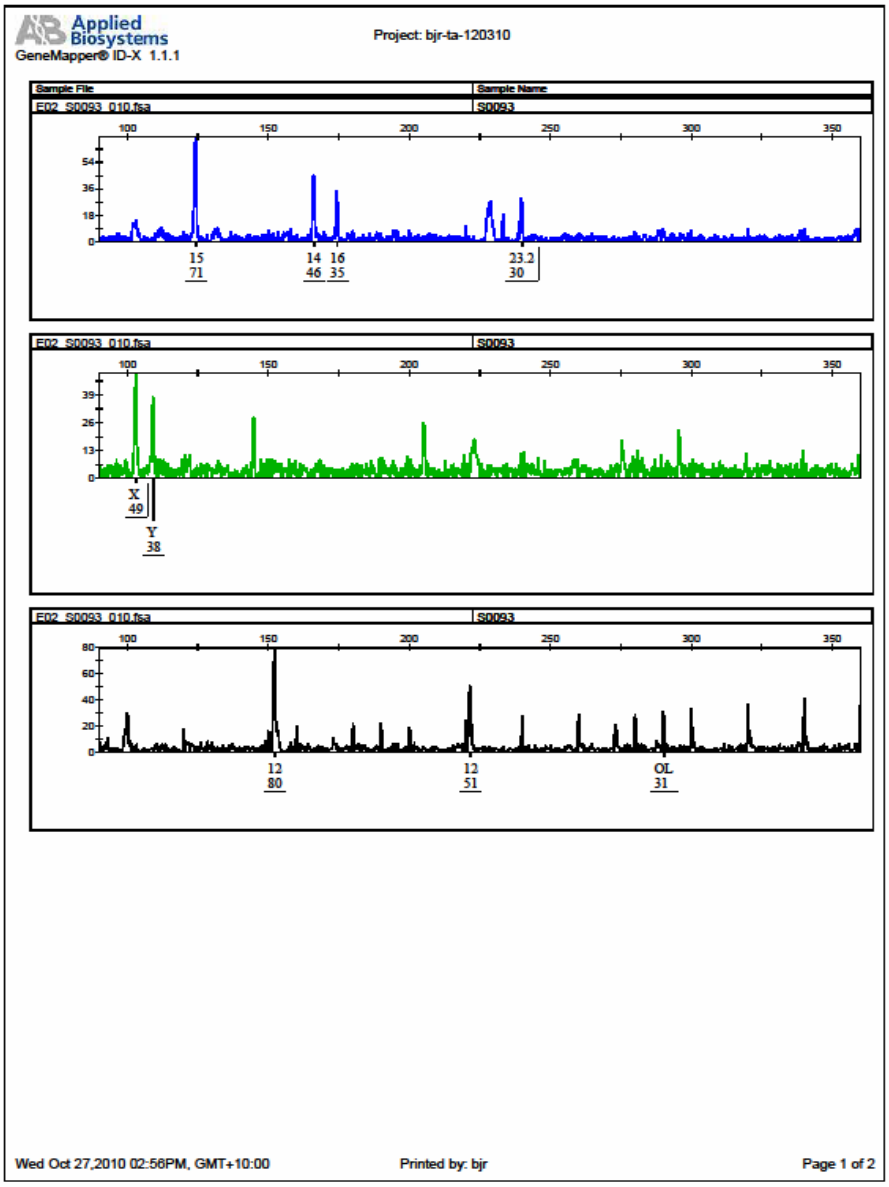
The joint LR is approximately 38.5 million
The log (LR) information is 7.68



S0093_1 vs A251475

The LR calculation assumes one unknown contributor in the evidence relative to an AU_CAU human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 328 thousand

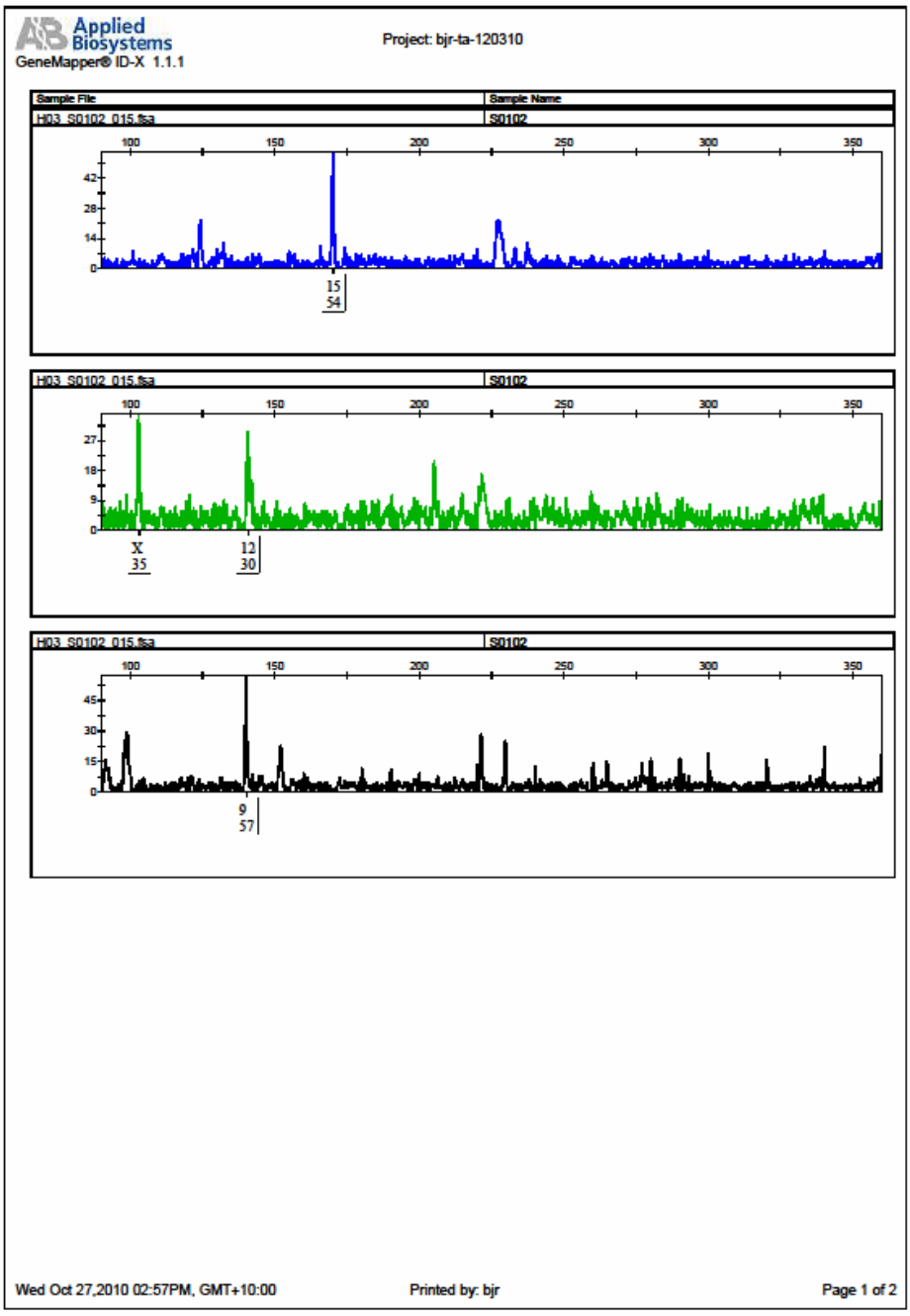
The joint LR is approximately 328 thousand
The log (LR) information is 5.51



S0102-1 vs A251972

The LR calculation assumes one unknown contributor in the evidence relative to an AU_CAU human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 421.

The joint LR is approximately 421
The log (LR) information is 2.62



Results obtained with TrueAllele (TA) calculation and DAL's spreadsheet that includes dropout as p(2-p) for single source samples. For comparison purposes the results are expressed using log(LR) to measure the amount of information obtained. These results indicate the information gain obtained with most of the weak samples.

Sample	Plot ID	TA	DAL
S0086_1	1	12.178	9.525
S0088_1	2	7.494	0.602
S0092_1	3	12.185	8.575
S0093_1	4	5.417	0.845
S0096_1	5	13.508	11.979
S0097_1	6	5.517	3.498
S0101_1	7	11.53	11.67
S0102_1	8	2.763	1.613
S0106_1	9	11.716	11.946
S0107_1	10	5.04	7.216

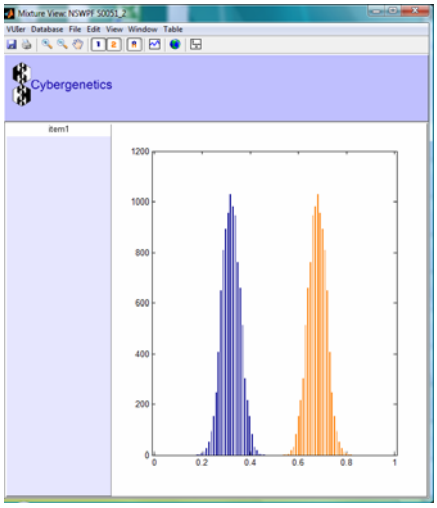
3.2 TWO PERSON MIXTURES

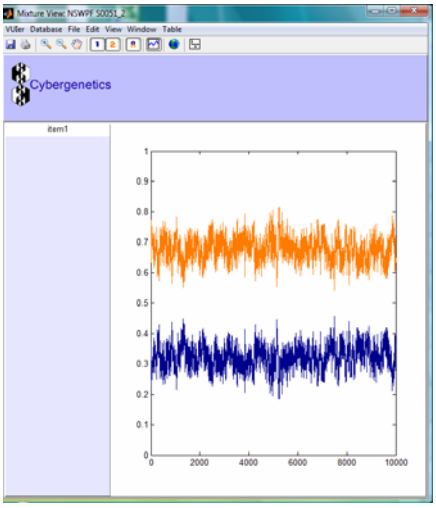
Under DAL's current methods, 10% of the 2 person mixtures could not be interpreted in any way and therefore failed to provide any weight of evidence to the justice system. A major contributor or an LR for 2 contributors ((S+ 1U)/2U) could be calculated for the remaining mixtures. The mixtures with no calculations were all low template mixtures (total DNA <300pg).

Results obtained with TrueAllele Calculation and DAL's spreadsheet using Hp 1 known and 1 unknown and Hd 2 unknowns or where possible, RMP for a resolved contributor, are presented in attached document 2A *Information gain -2 person mixtures*

While generally less complex than 3 person mixtures, deconvolution of 2 person mixtures into individual profiles can be challenging depending on the ratio of the two contributors. TrueAllele demonstrates a significant information gain in the interpretation of many 2-person mixtures.

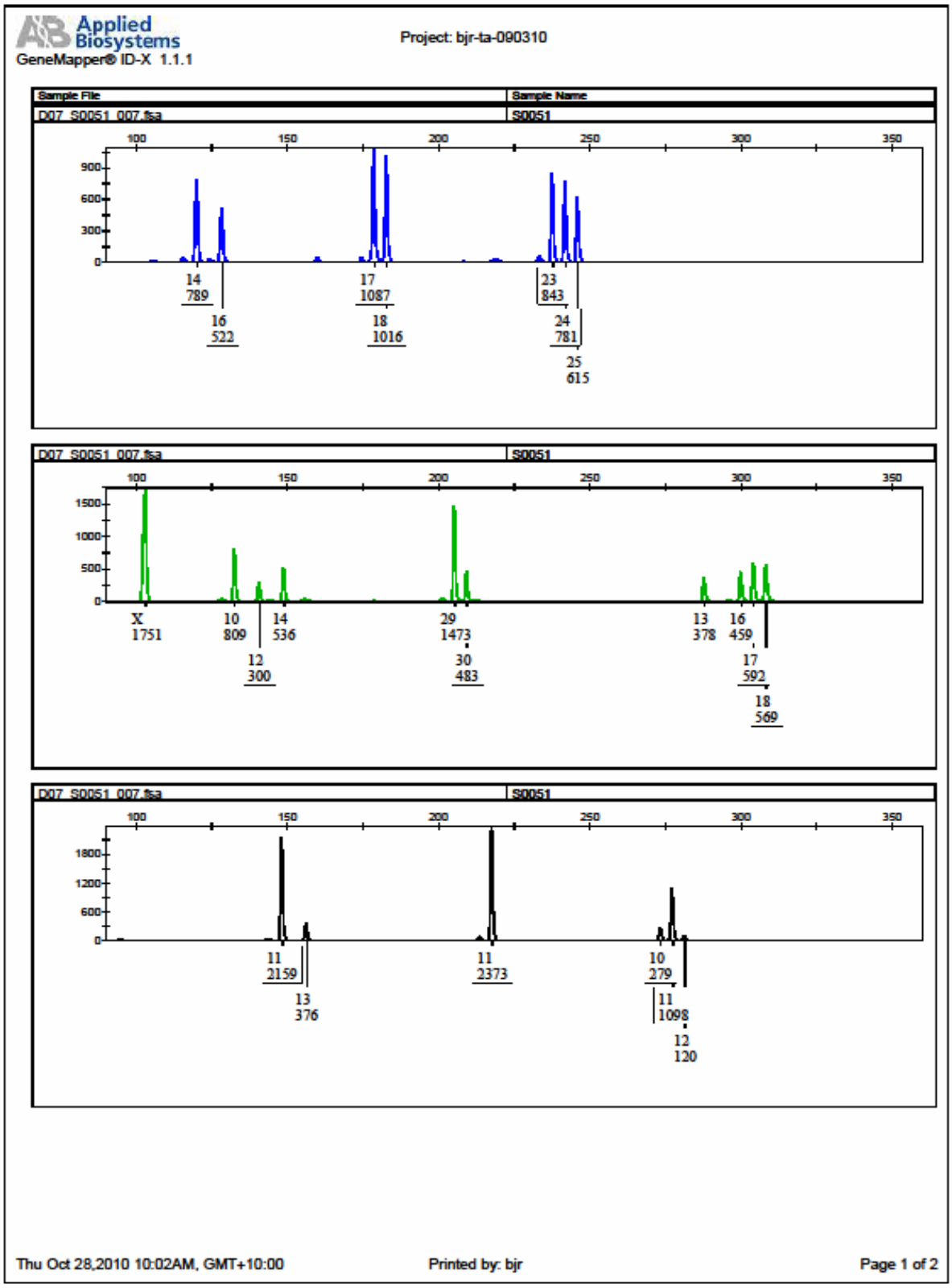
The following example (S0051) shows a mixture (total DNA 150pg), which could not be deconvoluted using current manual review, however TA has resolved the mixture with inferred genotype probability distributions that are consistent with a manual review of the evidence data. This mixture is particularly challenging to resolve because there is a high level of allele sharing with only a single locus with 4 alleles. When the mixture weight separates clearly (as shown in the following views) only a few genotypes are possible and these are more certain as indicated in the match reports (see page 13 and 14).





Mixture Variance: NSWPF S0051_2

Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.322	0.038	[0.248, 0.396]
item1	2	0.678	0.038	[0.604, 0.752]



Profiles of the known contributors to S0051:

	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
A252033 C1 (.322)	16,16	17,18	25,25	XX	10,12	30,30	13,16	11,13	11,11	10,12
S0082 C2 (.678)	14,14	17,18	23,24	XX	10,14	29,29	17,18	11,11	11,11	11,11

TA match report
S0051_2 vs. A252033

The LR calculation assumes two unknown contributors in the evidence relative to a AU_CAU human population.
The match rarity between the evidence and suspect is 8.22 billion.

The joint LR is approximately 8.22 billion.
The log(LR) information is 9.91.

		Likelihood Ratio	Genotype Probability Distribution				Weighted Likelihood		
	allele pair		Likelihood		Questioned		Reference	LR	log(LR)
locus	x	l(x)	q(x)	r(x)	s(x)	l(x)*s(x)	l(x)*r(x)		
D13S317	11, 11	1	1	0.0913	1	1	0.09129	10.953	1.040
D18S51	13, 16	1	1	0.0331	1	1	0.03314	30.173	1.480
D21S11	30, 30	0.446	0.493	0.0616	1	0.44579	0.02745		
	29, 29	0.523	0.446	0.0433			0.02266		
	29, 30	0.031	0.060	0.1033			0.00321		
						0.44579	0.05332	8.360	0.922
D3S1358	14, 14	0.797	0.389	0.0148			0.01180		
	14, 16	0.126	0.334	0.0624			0.00788		
	16, 16	0.077	0.277	0.0658	1	0.07702	0.00507		
						0.07702	0.02475	3.111	0.493
D5S818	11, 13	1.000	1.000	0.1167	1	0.99971	0.11665	8.570	0.933
D7S820	10, 12	0.708	0.735	0.0801	1	0.70833	0.05675		
	11, 12	0.227	0.168	0.0631			0.01432		
	10, 11	0.053	0.090	0.1085			0.00579		
						0.70833	0.07736	9.155	0.962
D8S1179	10, 12	0.992	0.989	0.0265	1	0.99199	0.02633		
	12, 12	0.007	0.008	0.0205			0.00015		
						0.99199	0.02653	37.392	1.573
FGA	24, 24	0.260	0.393	0.0173			0.00450		
	23, 23	0.233	0.337	0.0224			0.00522		
	25, 25	0.507	0.269	0.0063	1	0.50660	0.00320		
						0.50660	0.01293	39.191	1.593
vWA	17, 18	0.838	0.922	0.1107	1	0.83842	0.09278		
	18, 18	0.121	0.047	0.0423			0.00513		
	17, 17	0.040	0.032	0.0724			0.00293		
						0.83842	0.10084	8.314	0.920

TA match report
S051_2 contributor 2 vs. S0082

The LR calculation assumes two unknown contributors in the evidence relative to an AU_CAU human population.
The match rarity between the evidence and suspect is 50.8 billion.

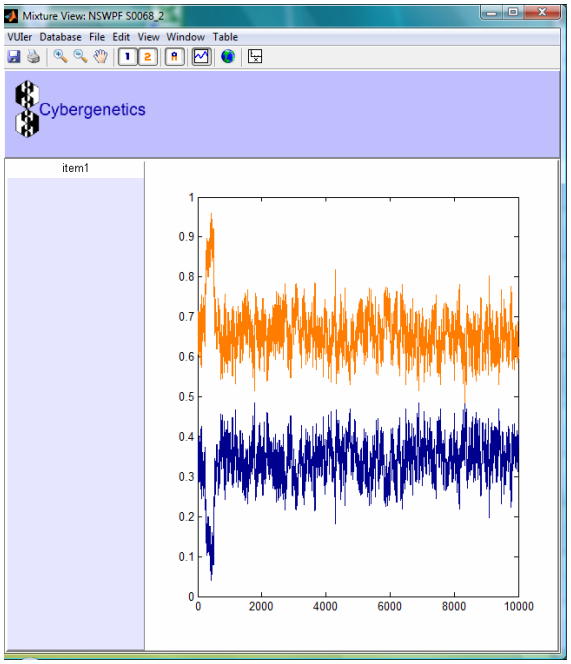
The joint LR is approximately 50.8 billion.
The log(LR) information is 10.70.

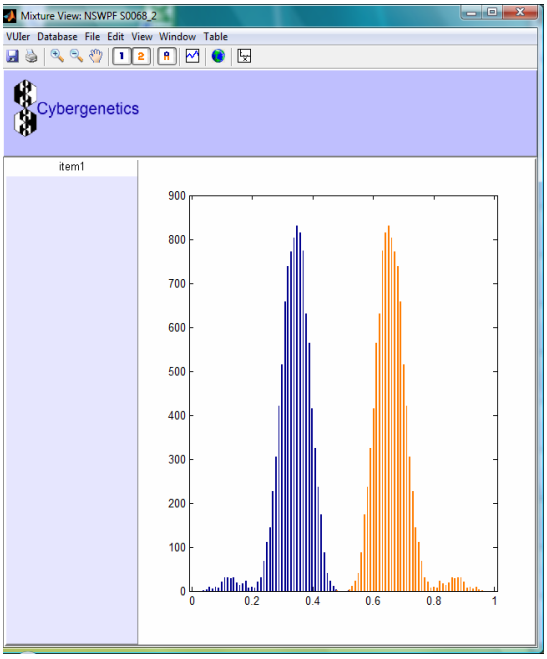
			Genotype Probability Distribution						Weighted Likelihood	
	Likelihood Ratio allele pair		Likelihood		Questioned		Reference		Suspect	Numerator
	Denominator		LR	log(LR)						
locus	x	l(x)	q(x)	r(x)	s(x)	l(x)*s(x)	l(x)*r(x)			
D13S317	11, 11	1	1	0.0913	1	1	0.09129	10.953	1.040	
D18S51	17, 18	1	1	0.0175	1	1	0.01754	57.026	1.756	
D21S11	29, 29	0.738	0.553	0.0433	1	0.73835	0.03198			
	29, 30	0.262	0.447	0.1033			0.02703			
						0.73835	0.05901	12.512	1.097	
D3S1358	14, 16	0.325	0.723	0.0624			0.02030			
	14, 14	0.675	0.277	0.0148	1	0.67489	0.01000			
						0.67489	0.03030	22.274	1.348	
D5S818	11, 11	1	1	0.1374	1	1	0.13741	7.277	0.862	
D7S820	11, 11	1.000	1.000	0.0427	1	0.99992	0.04270	23.414	1.369	
D8S1179	10, 14	0.990	0.999	0.0361	1	0.99015	0.03570	27.661	1.442	
FGA	23, 25	0.445	0.393	0.0238			0.01058			
	24, 25	0.374	0.337	0.0209			0.00782			
	23, 24	0.181	0.270	0.0394	1	0.18106	0.00713			
						0.18106	0.02552	7.094	0.851	
vWA	17, 18	0.917	0.969	0.1107	1	0.91666	0.10144			
	18, 18	0.083	0.031	0.0423			0.00350			
						0.91666	0.10499	8.731	0.941	

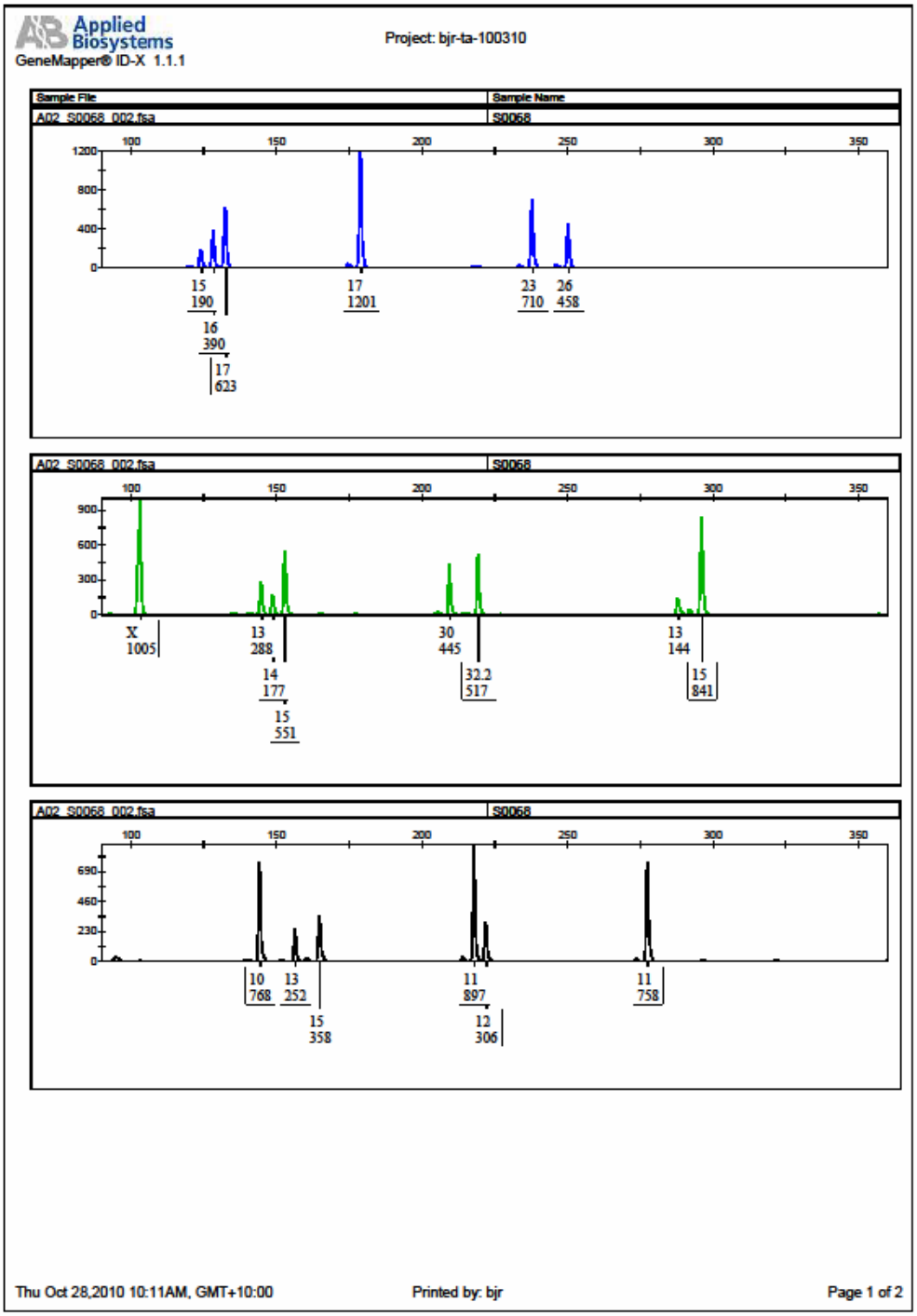
S0068 is a 2-person mixture constructed from related individuals therefore has a high degree of allele sharing. Under human review, this mixture cannot be resolved into individual profiles, however TA accurately infers genotypes with high probabilities generating significant match statistics. The mixture weight separates well and infers genotypes with high certainty. As seen in the MCMC history, there was an initial period where the chain had not converged however it quickly settled down to consistent mixture weights and therefore provided a reliable genotype distribution.

Mixture Variance: NSWPF S0068_2

Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.339	0.056	[0.229, 0.449]
item1	2	0.661	0.056	[0.551, 0.771]







Profiles of the known contributors to S0068:

	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
A251973 C1 (.339)	15,17	17,17	23,26	XX	14,15	30,32.2	13,15	10,13	11,11	11,11
A251474 C2 (.661)	16,17	17,17	23,26	XX	13,15	30,32.2	15,15	10,15	11,12	11,11

TA match reports for S0068:
S0068_2 contributor 2 vs. A251474

The LR calculation assumes two unknown contributors in the evidence relative to a AU_CAU human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 2.28 trillion.

The joint LR is approximately 2.28 trillion.
The log(LR) information is 12.35.

			Genotype Probability Distribution			Weighted Likelihood		Likelihood Ratio	
locus	allele pair x	Likelihood l(x)	Questioned q(x)	Reference r(x)	Suspect s(x)	Numerator l(x)*s(x)	Denominator l(x)*r(x)	LR	log(LR)
D13S317	11, 12	0.501	0.706	0.1746	1	0.50059	0.08742		
	11, 11	0.499	0.294	0.102			0.05092		
						0.50059	0.13834	3.618	0.559
D18S51	15, 15	1	1	0.0283	1	1	0.02828	35.355	1.548
D21S11	30 , 32.2	0.778	0.943	0.0481	1	0.77751	0.03738		
	32.2, 32.2	0.22	0.053	0.0121			0.00266		
						0.77751	0.04023	19.324	1.286
D3S1358	16, 17	0.823	0.934	0.105	1	0.82286	0.08643		
	17, 17	0.177	0.066	0.0469			0.00832		
						0.82286	0.09475	8.684	0.939
D5S818	10, 15	1	0.996	0.0016	1	0.99981	0.00164	607.319	2.783
D7S820	11, 11	1	1	0.0511	1	1	0.05114	19.554	1.291
D8S1179	13, 15	0.732	0.908	0.0717	1	0.73223	0.05253		
	15, 15	0.267	0.091	0.0162			0.00432		
						0.73223	0.05688	12.874	1.11
FGA	23, 26	0.853	0.786	0.0125	1	0.85345	0.01066		
	23, 23	0.144	0.213	0.029			0.0042		
						0.85345	0.01486	57.426	1.759
vWA	17, 17	1	1	0.0823	1	1	0.08235	12.143	1.084

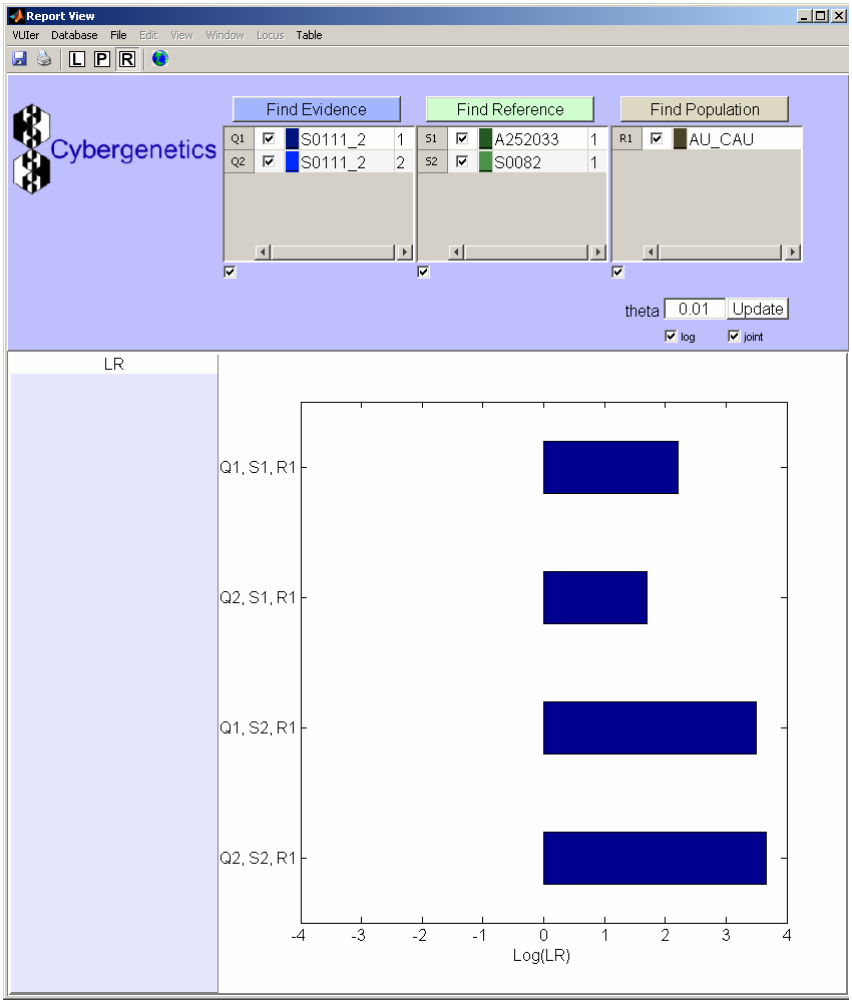
S0068_2 vs. A251973

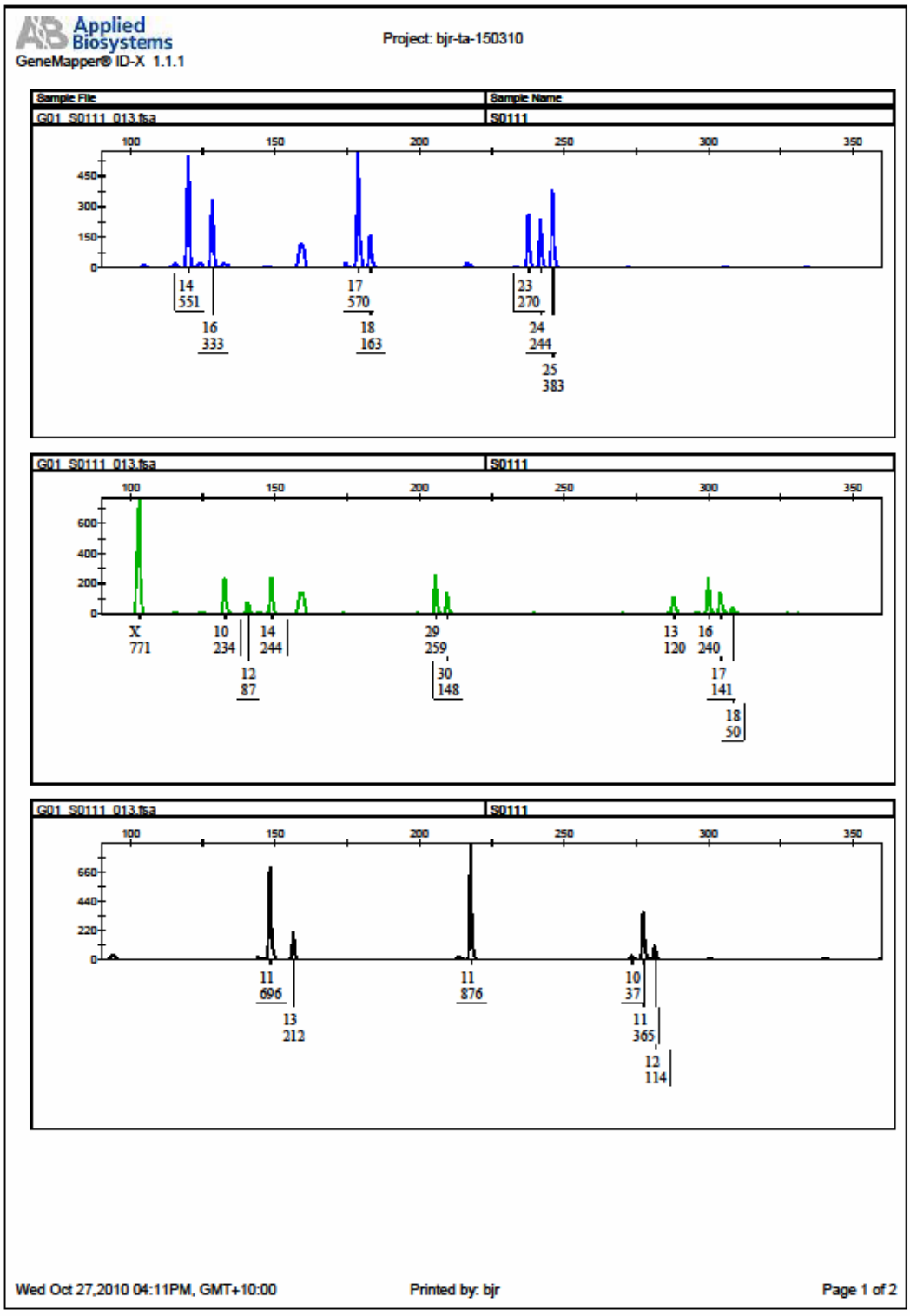
The LR calculation assumes two unknown contributors in the evidence relative to a AU_CAU human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 23.1 billion.

The log(LR) information is 10.36.
The joint LR is approximately 23.1 billion.

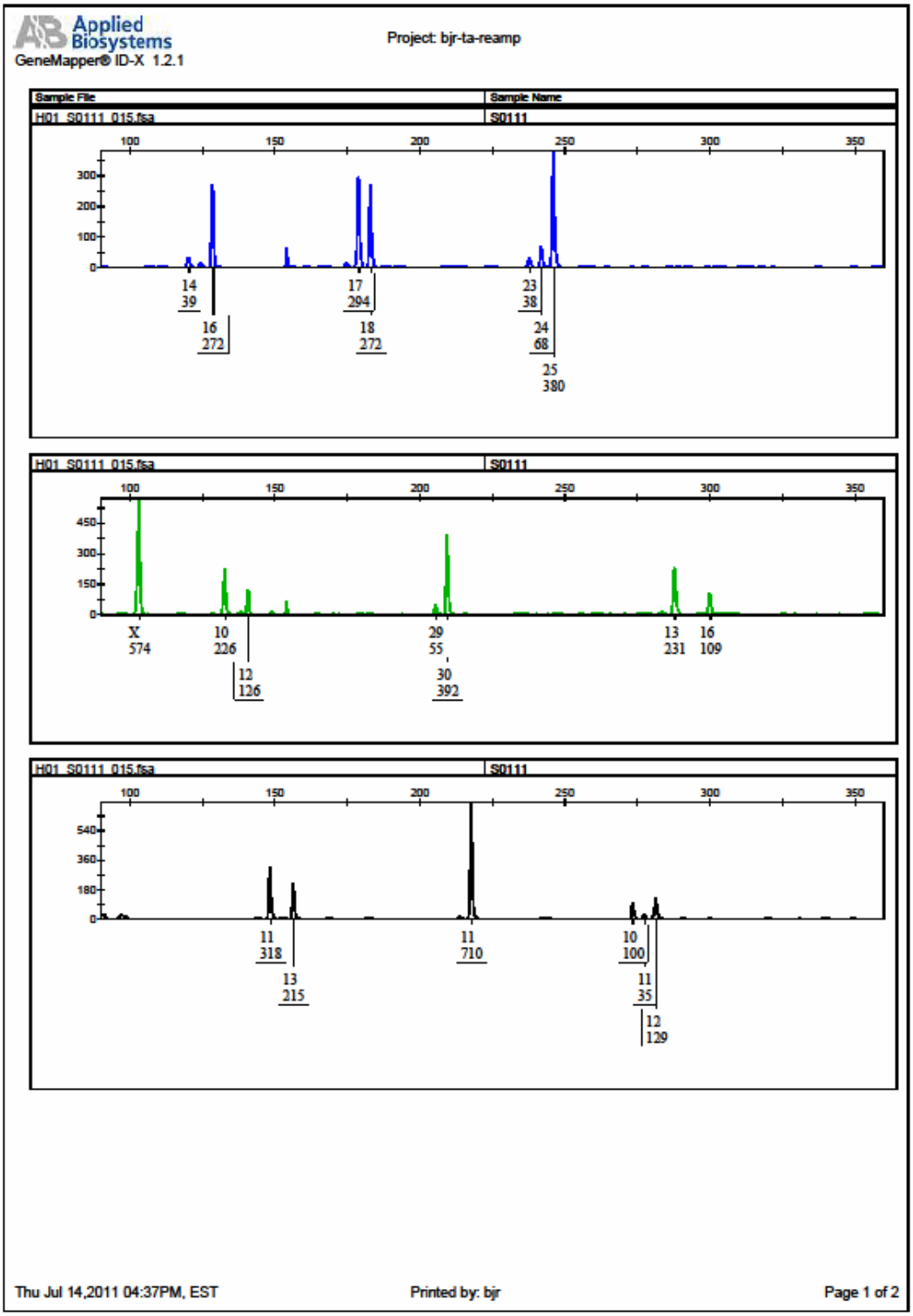
			Genotype Probability Distribution			Weighted Likelihood		Likelihood Ratio	
	allele pair	Likelihood	Questioned	Reference	Suspect	Numerator	Denominator	LR	log(LR)
locus	x	l(x)	q(x)	r(x)	s(x)	l(x)*s(x)	l(x)*r(x)		
D13S317	11, 11	0.771	0.702	0.102	1	0.77124	0.07863		
	12, 12	0.215	0.281	0.0909			0.01957		
	11, 12	0.007	0.015	0.1746			0.0012		
						0.77124	0.09971	7.735	0.888
D18S51	13, 15	0.932	0.978	0.0397	1	0.93209	0.03701		
	13, 13	0.067	0.021	0.0208			0.00139		
						0.93209	0.03844	24.246	1.385
D21S11	30 , 32.2	0.829	0.906	0.0481	1	0.8293	0.03987		
	30 , 30	0.041	0.057	0.0711			0.00294		
	32.2, 32.2	0.082	0.018	0.0121			0.00099		
	31 , 32.2	0.034	0.012	0.0155			0.00053		
						0.8293	0.04469	18.558	1.269
D3S1358	15, 17	0.933	0.917	0.1096	1	0.93335	0.1023		
	15, 16	0.045	0.067	0.1412			0.0063		
	15, 15	0.02	0.015	0.0819			0.00165		
						0.93335	0.11035	8.458	0.927
D5S818	10, 13	0.817	0.971	0.0226	1	0.81715	0.01849		
	13, 13	0.012	0.024	0.0317			0.00037		
						0.81715	0.01947	41.974	1.623
D7S820	11, 11	0.999	0.998	0.0511	1	0.99943	0.05111	19.529	1.291
D8S1179	14, 15	0.753	0.845	0.0449	1	0.75346	0.03384		
	15, 15	0.175	0.055	0.0162			0.00283		
	13, 14	0.026	0.053	0.1274			0.00332		
	13, 13	0.037	0.039	0.1113			0.00414		
						0.75346	0.04454	16.915	1.228
FGA	23 , 23	0.143	0.672	0.029			0.00416		
	26 , 26	0.463	0.211	0.003			0.00138		
	23 , 26	0.031	0.09	0.0125	1	0.03075	0.00038		
	22 , 23	0.001	0.013	0.0569			0.00007		
	18.2, 23	0.016	0.006	0.0031			0.00005		
						0.03075	0.00657	4.682	0.67
vWA	17, 17	0.999	0.997	0.0823	1	0.99854	0.08223	12.118	1.083

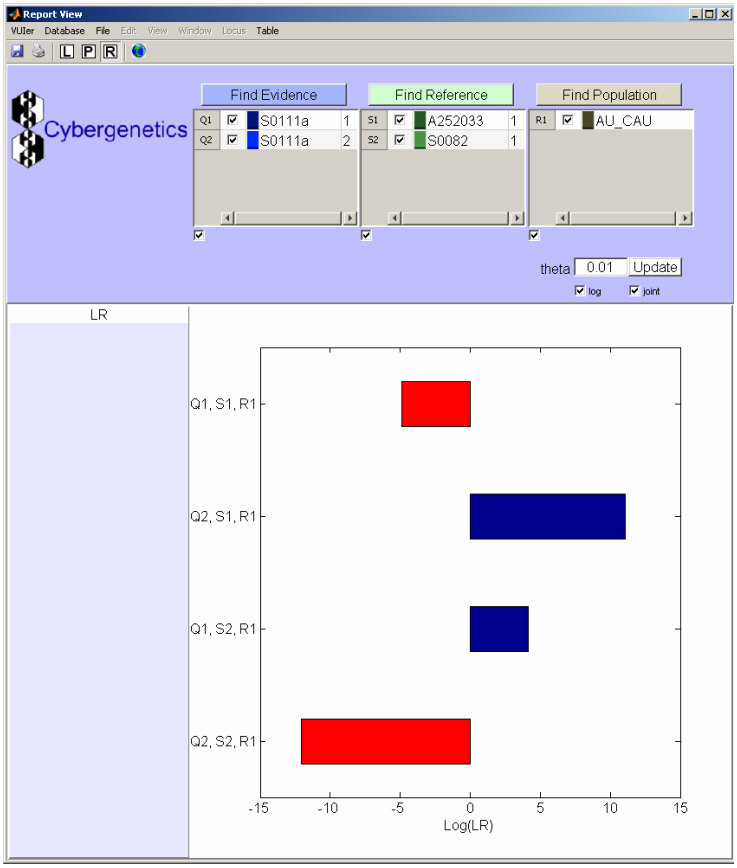
2 person mixtures at very low levels (total input DNA of 75pg) were analysed by TA testing its sensitivity. S0111 is a challenging low-level mixture that provides no useful information under current methods. TA provides a gain in information in this instance.




Applied Biosystems
GeneMapper® ID-X 1.1.1
Project: bjr-ta-150310
Sample File
G01_S0111_013.fsa
Sample Name
S0111
14 551
16 333
17 570
18 163
23 270
24 244
25 383
X 771
10 234
14 244
12 87
29 259
30 148
13 120
16 240
17 141
18 50
11 696
13 212
11 876
10 37
11 365
12 114
Wed Oct 27,2010 04:11PM, GMT+10:00
Printed by: bjr
Page 1 of 2

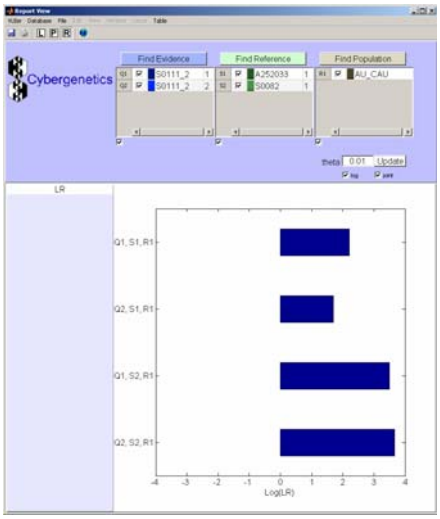
Resampling was carried out in approximately the same proportions however the proportions in the EPG varied considerably. TA analysis of this data again provided useful information in terms of the LRs. The match statistics are high for both contributors.



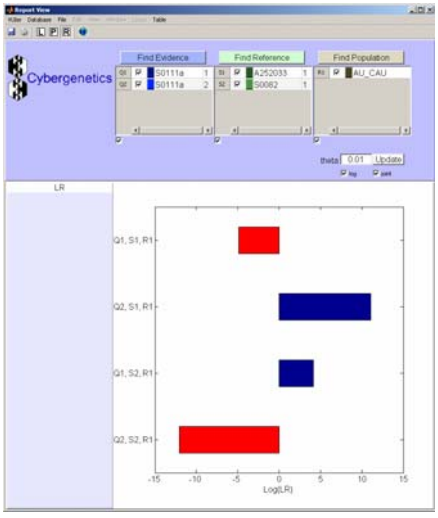


Using the feature that allows the system to use the information from 2 separate amplifications to generate a single LR provided **an additional** increase in information for suspect 2 (third bar down in graph) to a log LR of 5.81 for the joint amplification analysis.

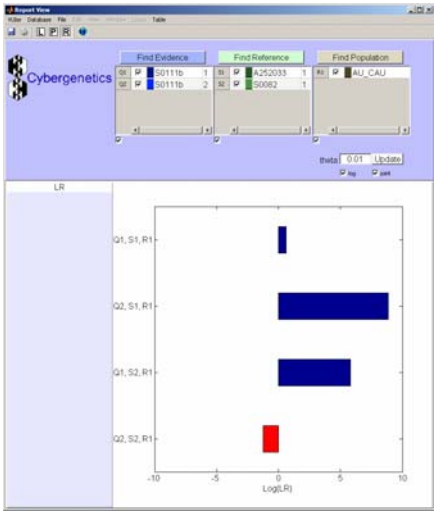
Log LR Sample 1



Log LR Sample 2



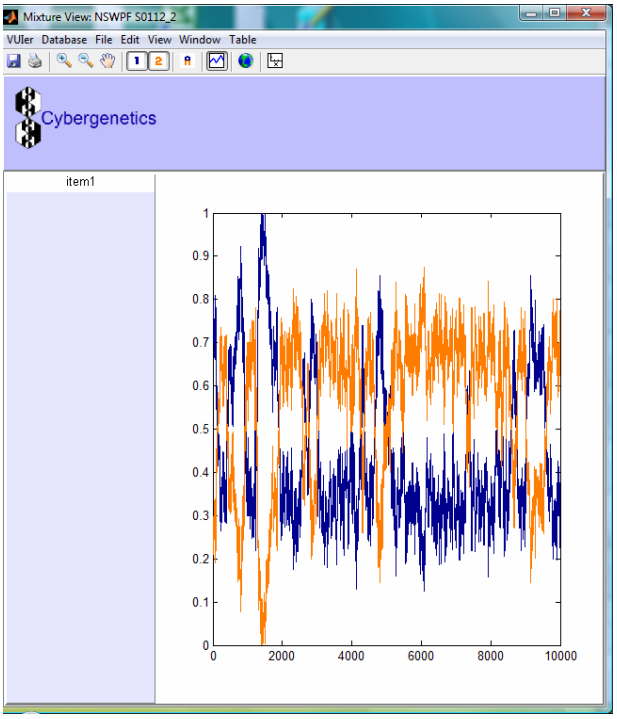
Log LR Joint Amp



Note: Log LR scale varies on X axis

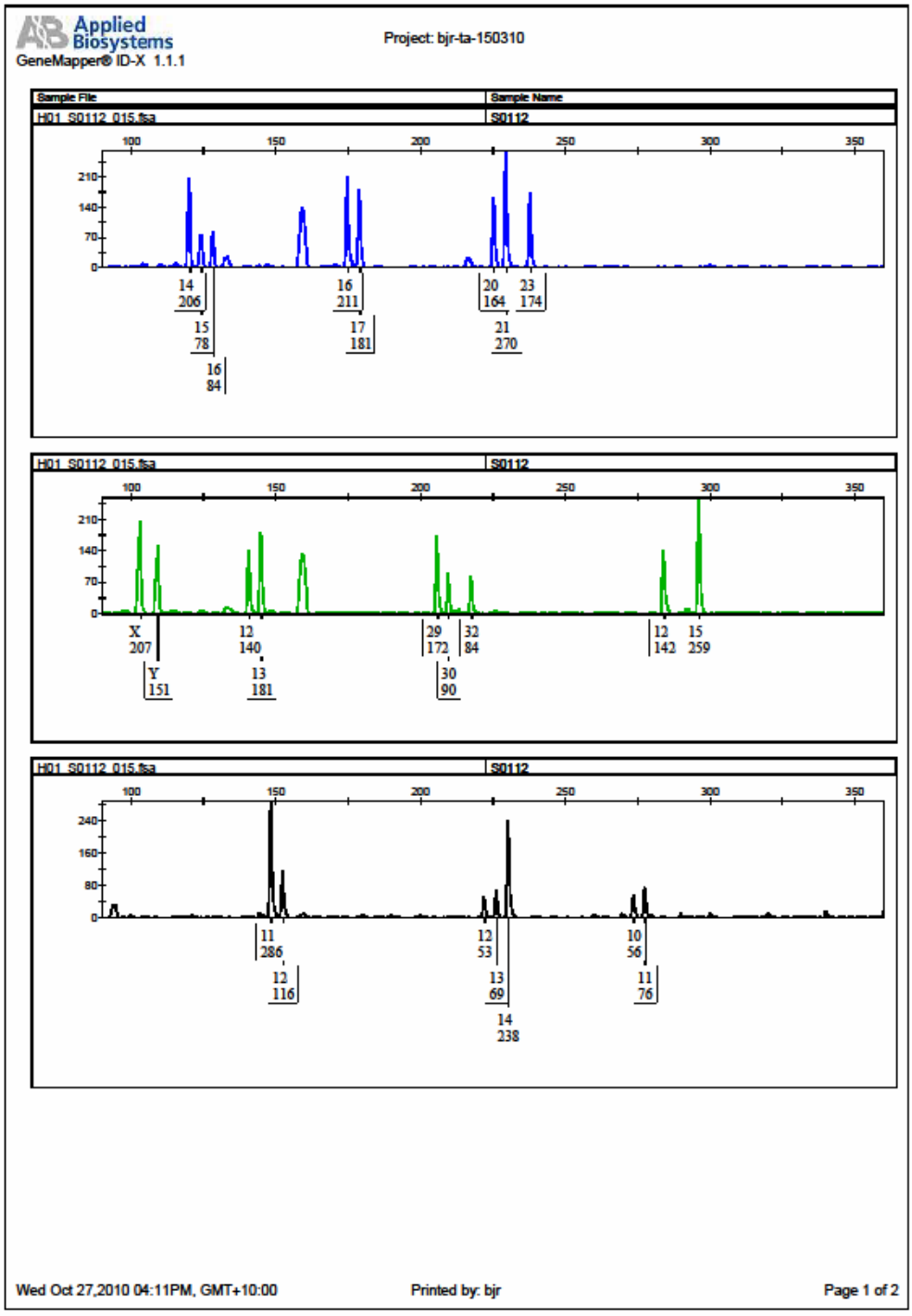
TA has the capability to analyse multiple amplifications or to use multiple samples in the analysis which results in an information gain. The application of multiple sampling from different areas of an item has not been explored and is not current practice however the feature is available if required.

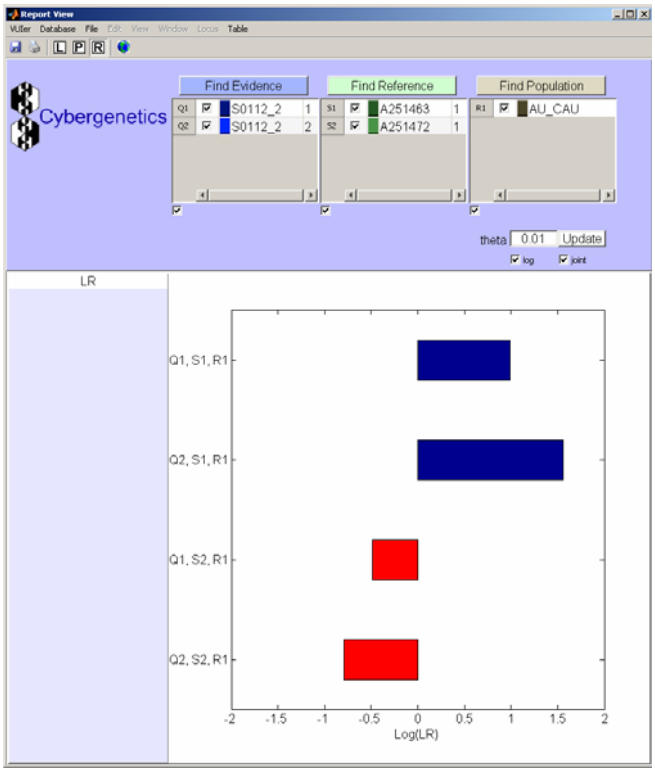
A different 2-person mixture (S0112) at the same low template of 75pg did not perform well. While we have just discussed a good result at 75pg, TA may have entered the limit of its capability/sensitivity in this range. TA has been unable to settle on definite mixture weights and oscillates widely over the range of possible mixture weights.



Mixture Variance: NSWPF S0112_2

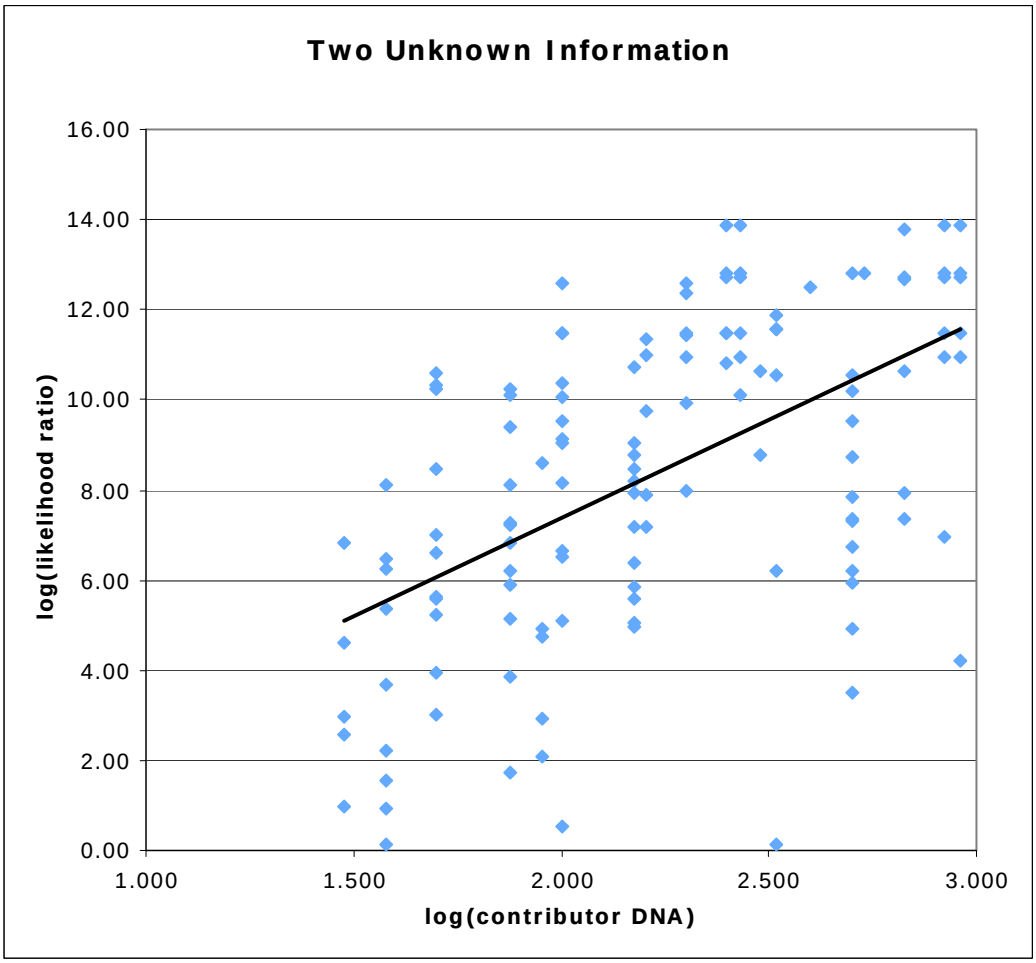
Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.440	0.179	[0.089, 0.791]
item1	2	0.560	0.179	[0.209, 0.911]


Applied Biosystems
GeneMapper® ID-X 1.1.1
Project: bjr-ta-150310
Sample File
Sample Name
H01_S0112_015.fsa
S0112
100
150
200
250
300
350
210
140
70
0
14
206
15
78
16
84
16
211
17
181
20
164
23
174
21
270
H01_S0112_015.fsa
S0112
X
207
Y
151
12
140
13
181
29
172
32
84
30
90
12
142
15
259
H01_S0112_015.fsa
S0112
240
160
80
0
11
286
12
116
12
53
13
69
14
238
10
56
11
76
Wed Oct 27,2010 04:11PM, GMT+10:00
Printed by: bjr
Page 1 of 2



This TA analysis provided an LR of 36 (log LR 1.55) for contributor 2 and an LR of .3 (log LR –0.49) for contributor 1.

The following graph using the NSW 2-person mixture data shows that as the contributor DNA amount increases, the match statistic increases (reproduced from Australia TrueAllele Validation study: Exploring each axis in more depth . Cybergenetics April 2011)



The amount of contributor DNA is the total template amount multiplied by the mixture weight.

3.3 THREE PERSON MIXTURES

Under DAL's current methods, 26 % of the 3 person mixtures could not be interpreted in any way and therefore failed to provide any weight of evidence to the justice system. The major profile of a single contributor could be inferred from 29% of the mixtures, but no further interpretation or weight could be placed on the minor contributors. An LR for 3 contributors ((S+ 2U)/3U) could be calculated in the other 44% of the mixtures. For the 8 low template mixtures (<0.3ng total), there was only a single mixture which could be interpreted under current guidelines and this was limited to 5 reportable loci.

Under current methods:

Number of 3 person mixtures	Number with no calculation	Number with calculation of major component only	Number with calculation for each of the 3 contributors
75	20	22	33

In contrast, TA resolved all the mixtures into 3 contributors with inferred genotype probabilities for each contributor and these probabilities are used to calculate a match statistic for any source with a known profile. It is this ability to assign a probability to all possible genotypes that translates into match statistics that are significantly higher than those calculated under current methods. Current guidelines require distinct and clearly defined differences in signal intensities between contributors, and this requirement often makes it extremely challenging to resolve mixtures, especially 3 person mixtures, into individual contributor profiles. It is TA's ability to deconvolute mixtures that strengthens the interpretation of the evidence.

The information gains achieved by TA analysis are shown in the attached document 2B (spreadsheets 1-5) *3-person mixtures Information Gain.*

In addition, a series of 3-person mixtures with one contributor degraded were analysed. These mixtures were constructed with varying total DNA template (300pg to 1ng) and contributor ratios. (see document 1 for sample matrix). Under current methods, a major contributor could be identified in 3 of these mixtures and a second contributor profile was resolved for one mixture.

Under DAL's current methods:

Number of 3 person mixtures with 1 contributor degraded	Number with no Calculation	Number with calculation of major component	Number with calculation for an additional contributor
12	9	3	1

TA interpretations were a **significant improvement** on current methods. The attached document 2B (spreadsheet 5 *3-person mixtures Information Gain)* records the log LR values generated for each of the contributors to these degraded mixtures and illustrates the significant information gain achieved with TA analysis. Almost all did not provide an LR >1 for all 3 known contributors. In most instances, the degraded contributor has an LR<1.

TA analysis was also carried out with the 'degraded feature' enabled. The log LR values are presented in the following table. The gain in information over all contributors was seen to be, on average, 1/3 of a log (LR) unit.

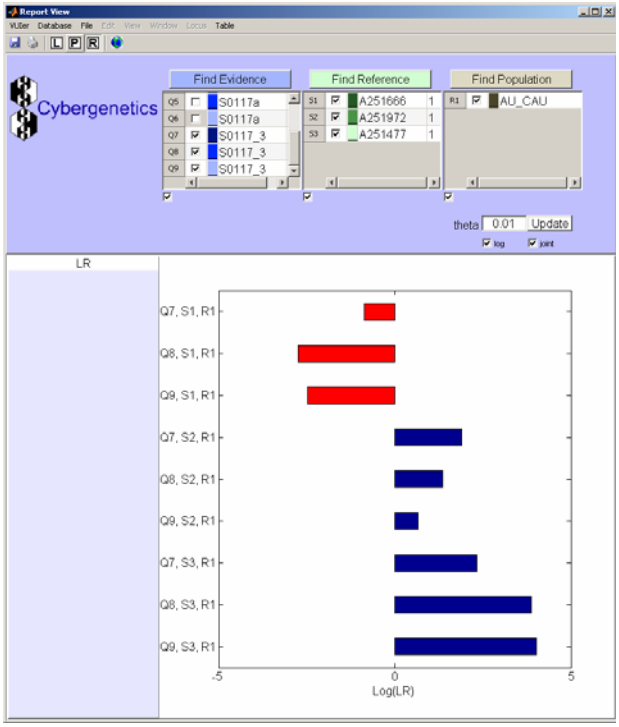
TA Degraded feature enabled-3 person mixtures with one contributor degraded.

System2	FES_TA2		Log LR		
Evidence	**Contrib**	**Weight**	**A251475**	**S0082**	**A252033**
S0190_3_dgrd	1	0.10	6.077		
S0190_3_dgrd	2	0.76			10.67
S0190_3_dgrd	3	0.14	7.467		
S0192_3_dgrd	1	0.81			11.133
S0192_3_dgrd	2	0.12		7.843	
S0192_3_dgrd	3	0.07		1.552	
S0193_3_dgrd	1	0.53	8.855		
S0193_3_dgrd	2	0.26	5.423	2.977	
S0193_3_dgrd	3	0.22		4.932	
S0194_3_dgrd	1	0.28		8.47	
S0194_3_dgrd	2	0.06			
S0194_3_dgrd	3	0.66	9.043		
S0195_3_dgrd	1	0.20		2.046	0.866
S0195_3_dgrd	2	0.33		4.5	1.893
S0195_3_dgrd	3	0.47		5.734	1.007
S0196_3_dgrd	1	0.32	4.681		
S0196_3_dgrd	2	0.26	3.03		
S0196_3_dgrd	3	0.42	5.26		
S0197_3_dgrd	1	0.32	4.963	3.064	
S0197_3_dgrd	2	0.40	5.613	3.607	
S0197_3_dgrd	3	0.29	5.041	3.432	
S0198_3_dgrd	1	0.34	6.518		
S0198_3_dgrd	2	0.29	4.954		1.359
S0198_3_dgrd	3	0.37	6.562		
S0199_3_dgrd	1	0.77			11.094
S0199_3_dgrd	2	0.13	7.077		
S0199_3_dgrd	3	0.10	5.987		
S0200_3_dgrd	1	0.69			8.32
S0200_3_dgrd	2	0.16	1.835	4.459	
S0200_3_dgrd	3	0.15	1.739	3.782	
S0202_3_dgrd	1	0.69		10.232	
S0202_3_dgrd	2	0.15	5.771		
S0202_3_dgrd	3	0.16	6.095		
S0203_3_dgrd	1	0.44	5.733	3.572	
S0203_3_dgrd	2	0.46	5.382	3.305	
S0203_3_dgrd	3	0.10			0.263

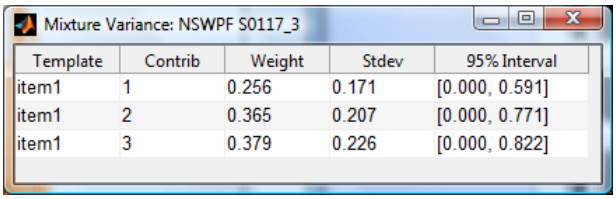
The TA analysis of the degraded mixtures with or without the degraded feature enabled, is a significant improvement on current methods providing evidence for

some of the contributors, however the LR values can be insignificant for one or more contributors providing little or no support for inclusion.

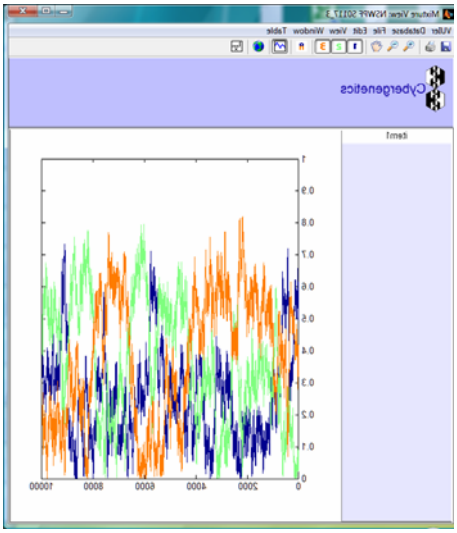
This was also noted to occur in non degraded low template mixtures with relatively equal levels of DNA from the contributors. One known contributor in this mixture example (S0117 see epg) had an LR <1 for each of the 3 inferred contributors, while another contributor had a positive LR against each of the 3 inferred contributors with the highest LR of 10,000 for contributor 3. The highest LR for the third known contributor was limited to 77. So even with this type of extremely challenging data, TA produces a significant LR for at least one of the contributors, which can provide information that is useful in court but provides little information in relation to the additional 2 contributors.

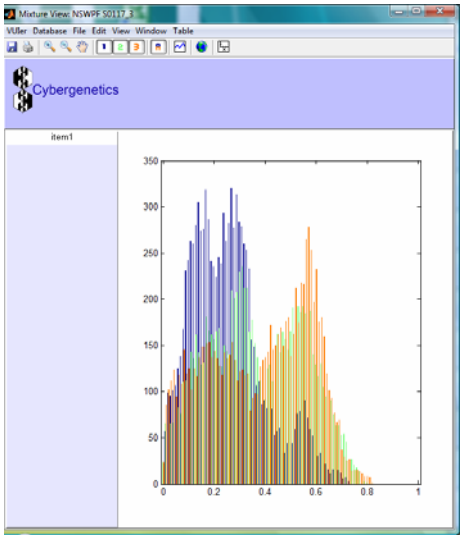


The nature of the mixture with similar levels of contributions results in a challenging mixture interpretation. While it was unable to separate the contributors TA was still able to infer a relatively high probability for inclusion in relation to at least one of the known contributors.


Mixture Variance: NSWPF S0117_3

Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.256	0.171	[0.000, 0.591]
item1	2	0.365	0.207	[0.000, 0.771]
item1	3	0.379	0.226	[0.000, 0.822]





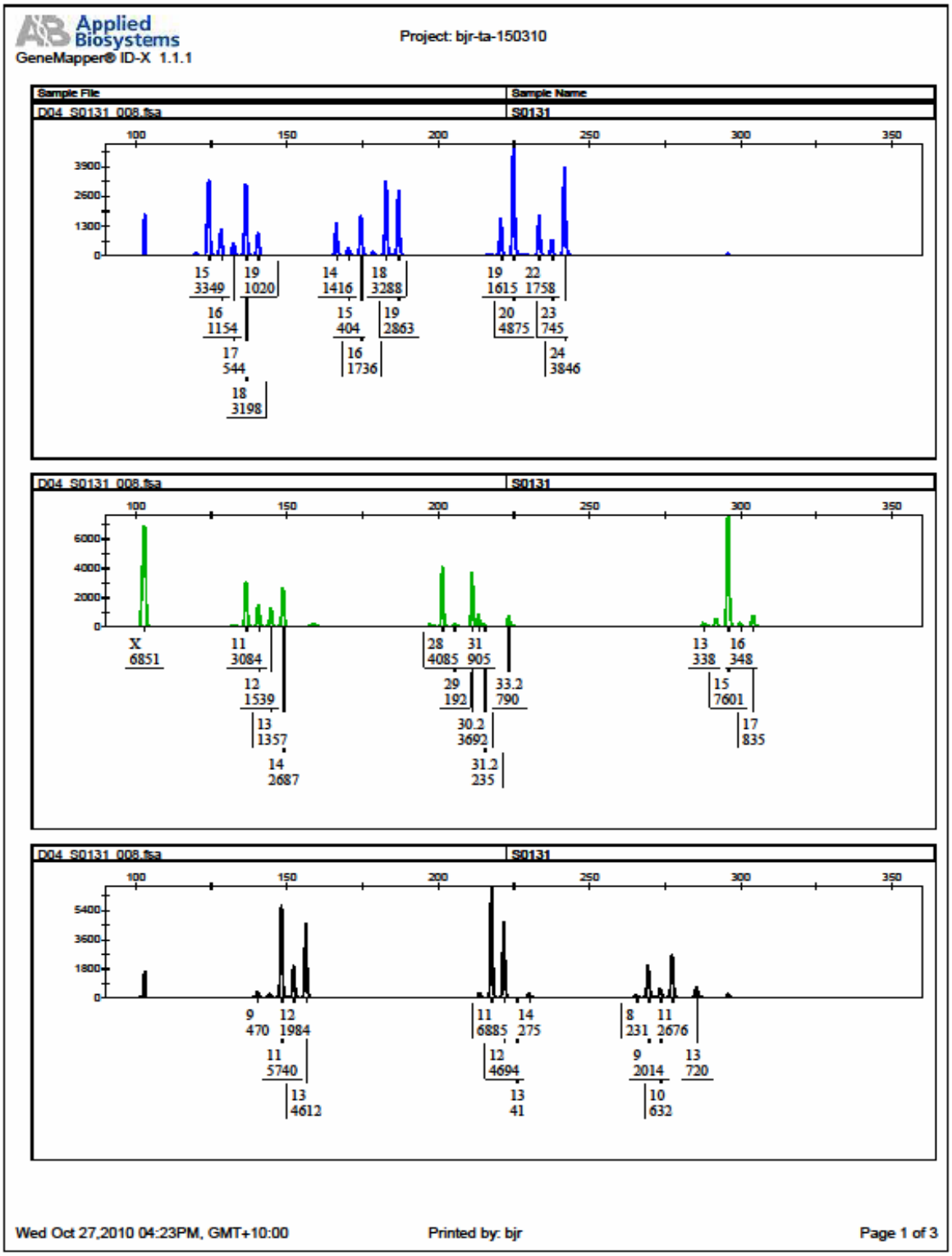
The known profile for the contributor (A251666) which generated an LR<1 is 16,19, 14,16, 19,22, 12,13, 31,33.2, 15,17, 11,12, 11,11, 10,13.

All these alleles are present in the data at very low peak heights. Looking at the MCMC history, neither the blue or green chain spent sufficient time at the lowest mixture weight to infer an accurate weight or genotype.

S0131 provides an example of a 3-person mixture where TA processing results in a significant information gain. The mixture weights are contributor 1 at .729, contributor 2 at .059 and contributor 3 at .212. Current guidelines within each jurisdiction do not support the separation of the contributor profiles in this mixture.

Profiles of the contributors to S0131

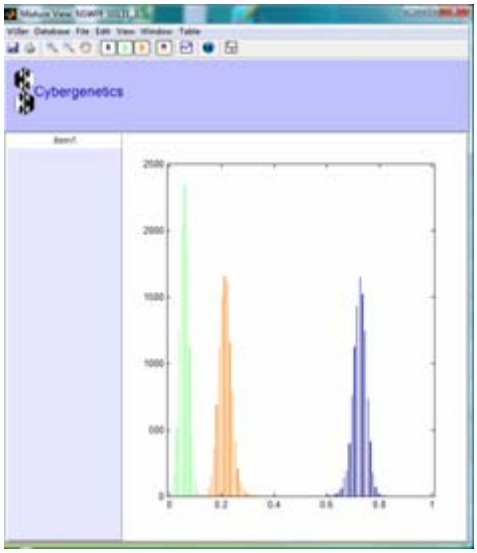
	D3	**vWA**	**FGA**	**Amel**	**D8**	**D21**	**D18**	**D5**	**D13**	**D7**
A251477 C1 (.729)	15,18	18,19	20,24	XY	11,14	28,30.2	15,15	11,13	11,12	9,11
A251972 C2 (.059)	15,17	15,16	22,23	XY	12,14	29,31.2	13.16	9,12	12,14	8,11
A251666 C3 (.212)	16,19	14,16	19,22	XY	12,13	31,33.2	15,17	11,12	11,11	10,13

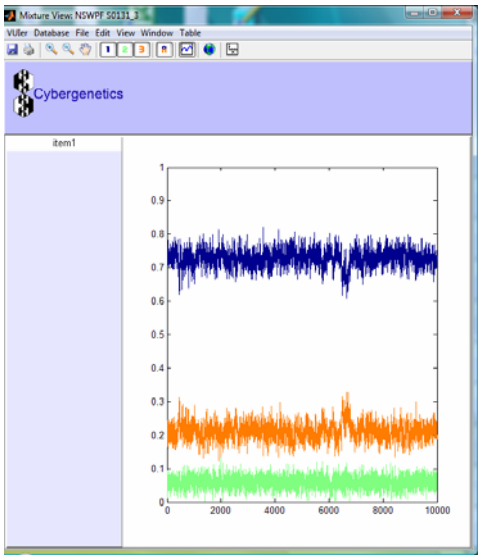

Applied Biosystems
GeneMapper® ID-X 1.1.1
Project: bjr-ta-150310
Sample File
Sample Name
D04_S0131_008.fsa
S0131
15 3349
19 1020
16 1154
17 544
18 3198
14 1416
18 3288
15 404
19 2863
16 1736
19 1615
22 1758
20 4875
23 745
24 3846
X 6851
11 3084
12 1539
13 1357
14 2687
28 4085
31 905
29 192
33.2 790
30.2 3692
31.2 235
13 338
16 348
15 7601
17 835
9 470
12 1984
11 5740
13 4612
11 6885
14 275
12 4694
13 41
8 231
11 2676
9 2014
13 720
10 632
Wed Oct 27,2010 04:23PM, GMT+10:00
Printed by: bjr
Page 1 of 3

TA provides significant gain in information for this mixture:

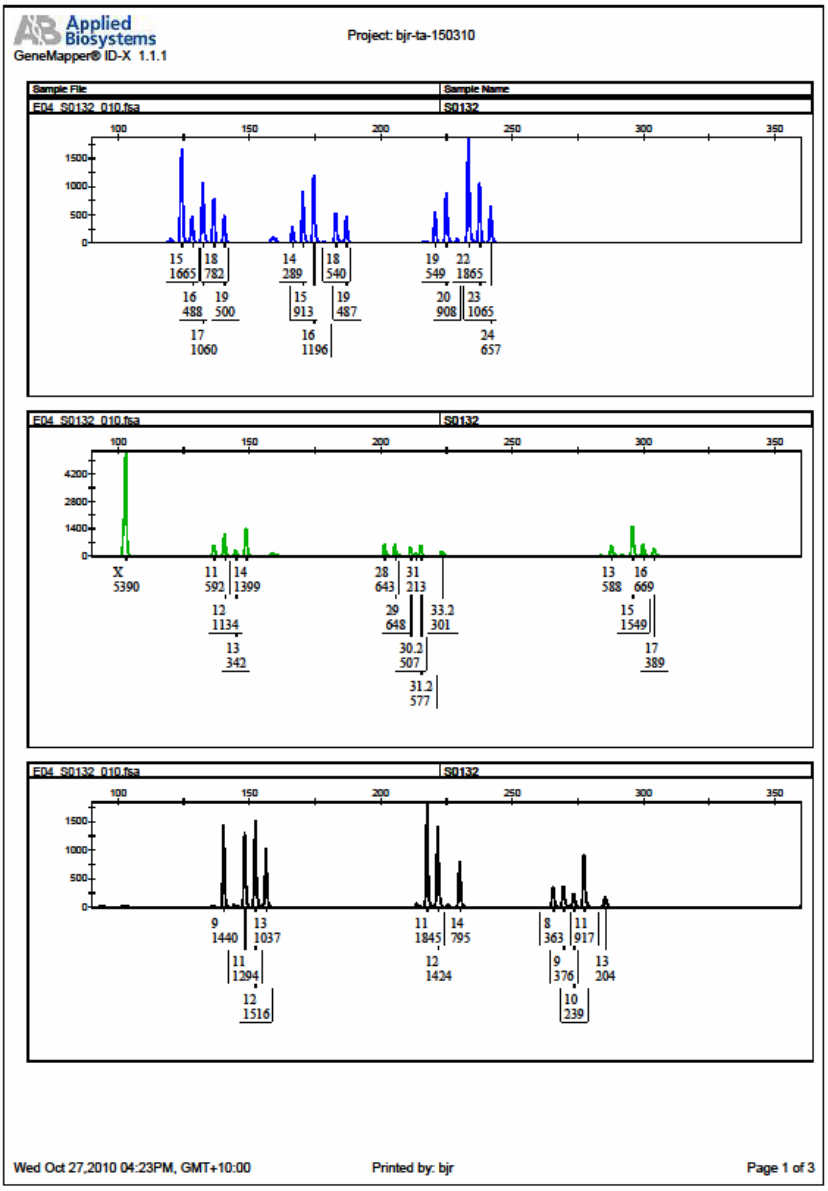
Laboratory	Contributor	LR	Comment
Cybergenetics -TA	A251477 A251972 A251666	**379 billion** **22.9 million** **561 billion**	1% theta

The inferred genotypes for contributor 1 (.729) approach certainty at every locus. For contributor 2 (.059), the inferred genotype approaches certainty at some loci (eg D21 and D18) while other loci display varying degrees of uncertainty. Contributor 3 (.212) inferred genotypes approach certainty at 7 loci. The inferred genotype probabilities are supported by a manual review. The 'certainty' at some loci strengthens the LR while incorporating uncertainty into a lower LR at other loci. This capability is making full use of the sample data and provides an excellent example of the enhanced capability TA would provide in DNA interpretation. TA performs very well when separation between contributors in the MCMC history is clear.

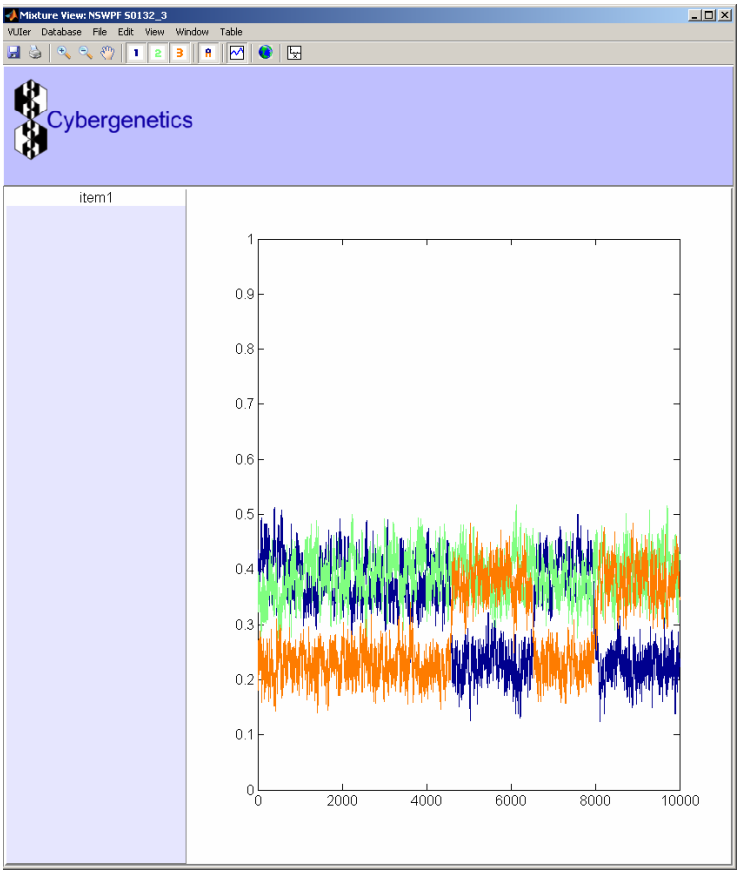




The ratio of contributors in some mixtures can mean it is more difficult to infer genotypes with high probabilities. Consider the following sample (S0132) where 2 contributors have virtually the same weight and the 3rd is similar.



The MCMC history for contributor 2 (green) is converged to a definite weight while contributor 1 and 3 (blue and orange) are crossing over. Contributor 1 (blue) is spending more time at the higher weight and conversely contributor 3 (orange) spends more time at the lower weight causing the inferred genotypes for blue and orange to merge.



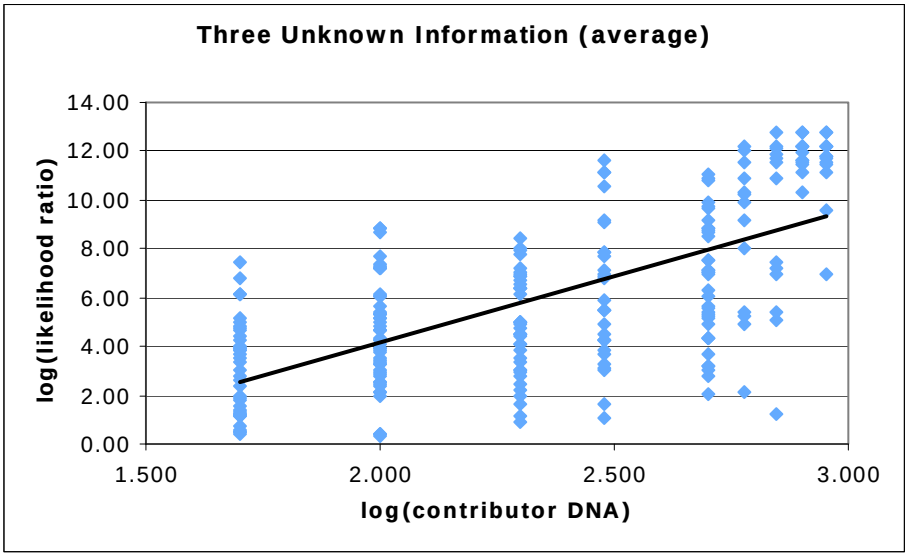
Mixture Variance: NSWPF S0132_3

Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.324	0.087	[0.153, 0.495]
item1	2	0.388	0.038	[0.314, 0.462]
item1	3	0.288	0.081	[0.129, 0.447]

However, despite the challenging nature of this mixture, TA calculates match statistics that are substantially higher than under the current methods.

Contributor	**Current DAL method LR**	**TA LR**
A 251477	986	8.76 thousand
A251666	34,206	694 thousand
A251972	2,873	170 thousand

The following graph using the NSW 3-person mixture data shows that as the contributor DNA amount increases, the match statistic increases (reproduced from Australia TrueAllele Validation study: Exploring each axis in more depth . Cybergenetics April 2011)

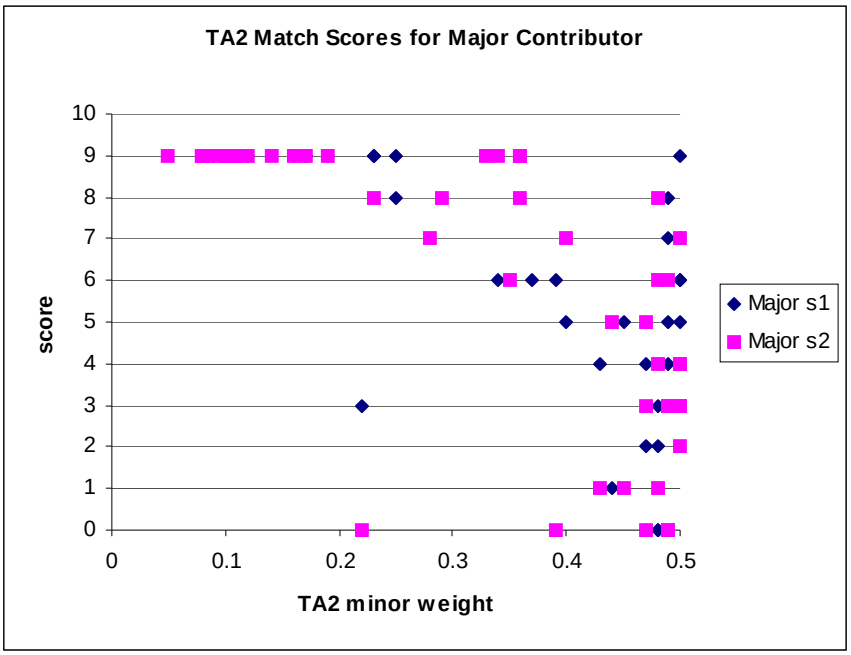


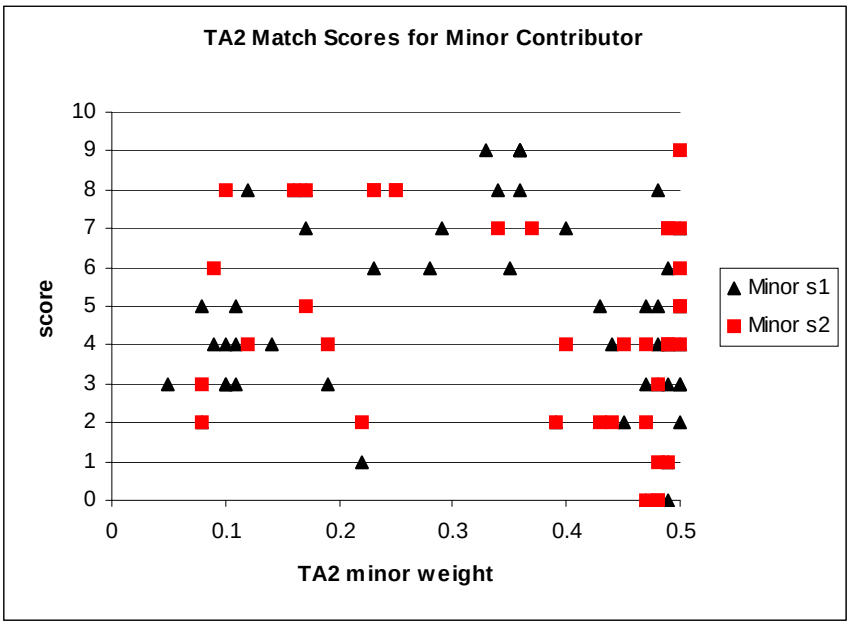
The amount of contributor DNA is the total template amount multiplied by the mixture weight.

4. SPECIFICITY/ACCURRANCY

The requirement for the expert system is that the genotypes of the contributors can be accurately inferred, with uncertainty identified where appropriate depending on the sample data. By using laboratory generated mixtures with known contributors the capability of TA could be assessed. Plots of match scores for the major and minor contributors in two person mixtures were generated. This explored the capability of TA to infer the known genotype with the highest probability.

The number of loci where the known genotype was assigned the highest probability on the distribution list was identified. This was plotted against the contributor weight of the minor component. This allowed an assessment of how well TA performed in respect of inferring the correct genotype





This assessment demonstrated that TA accurately inferred the correct genotype **at all 9 loci** for the major contributor to a mixture when the minor contribution to the mixture was <0.2. As the contribution of the minor contributor increases, the number of loci at which the correct genotype was inferred with the highest probability decreased, but is still reasonable approaching a minor contributor weight of 0.4. Once the minor contributor is close to 0.5, the number of loci with the correct genotype ranges from zero to 9 with a wide spread of scores. This is to be expected with the high level of uncertainty with genotypes in 50:50 mixtures particularly when the total amount of DNA is low. It would be expected and acceptable that uncertainty would occur in the genotype distribution in mixtures of such proportions and it would be unreasonable to expect the highest probability to consistently be assigned to the correct genotype.

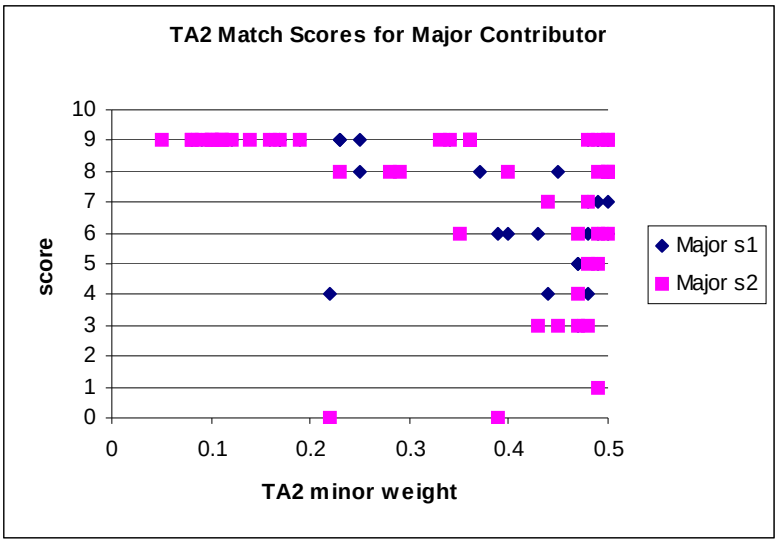
It is clear that TA can accurately resolve 2 person mixtures into major and minor contributors at proportions that could not be resolved under a human review. A human review is restricted by 'rules' as to the required difference between peak heights before they can be separated into individual contributors. TA can apply a continuum of probabilities to genotypes whereas human review requires an all or none separation.

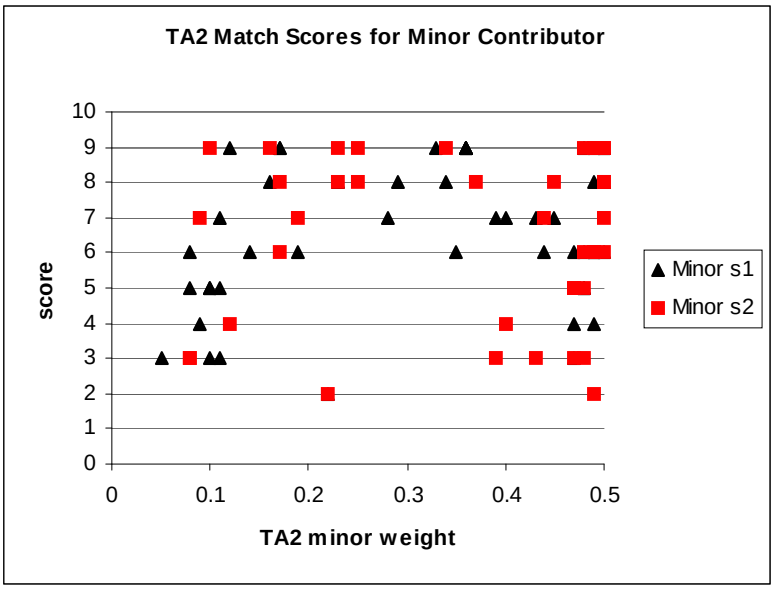
At low template levels the number of loci with the 'correct' genotype assigned the highest probability is reduced, which again is expected given the higher variance around peak height at low template.

When assessing the accuracy in relation to the minor contributor, the data indicates that the most accurate inference of genotypes occurs when the minor contributor is in a small zone around 0.3 of the total weight. At weights above and below this, the genotype of the minor contributor cannot be inferred with the highest probability at many loci.

This sort of assessment of the specificity of TA is of limited value. With mixtures which are hard to resolve such as 50:50 mixtures, it may be that the correct genotype has the second highest probability which may not vary much from the highest when there is uncertainty at the locus.

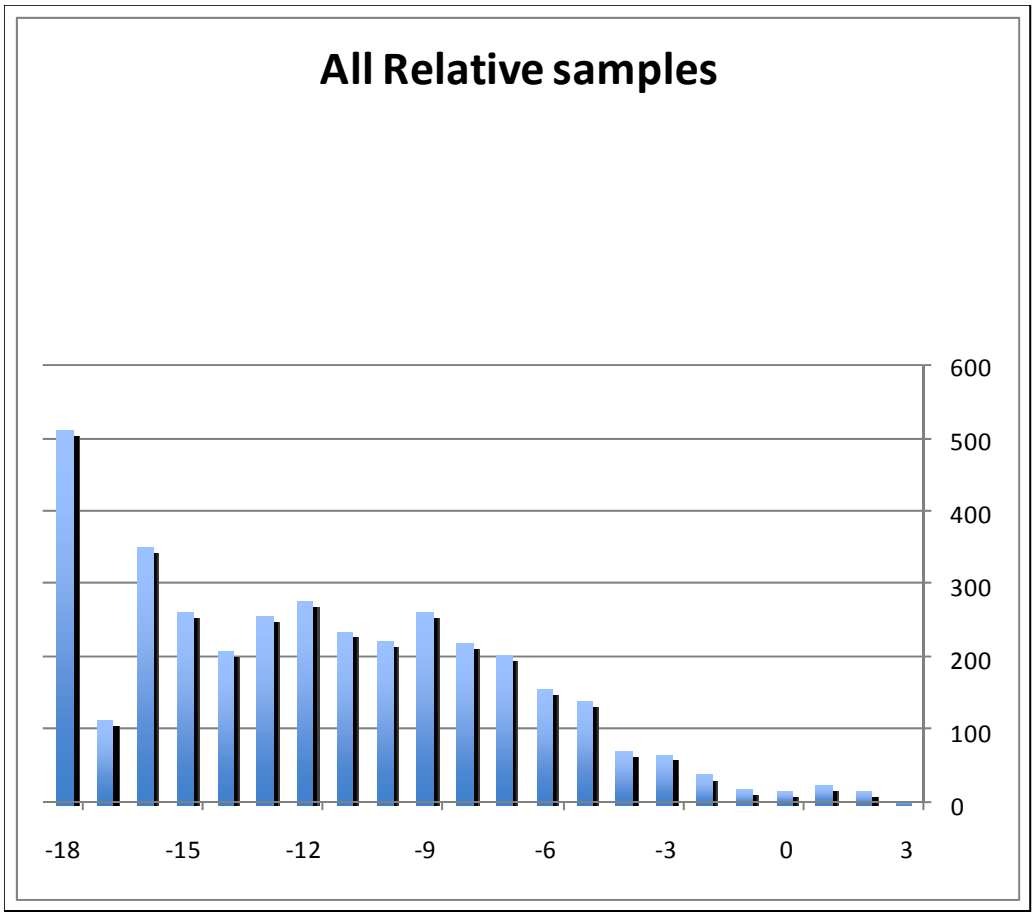
A second match score was generated which considered an inferred genotype probability of >20% for the correct genotype to be sufficiently accurate even though other genotypes may have had a higher probability.





This shows that while TA's assessment of some profiles did not generate the correct highest probability, the probability was reasonably high on the distribution list. It is identifying the correct genotype with good support. However there wasn't a great deal of difference between assigning the highest probability to the known genotype compared to a probability of >20%.

The 15 known contributors to the samples generated in relation to this study did not have LRs>1 for any mixture for which they were not a known contributor. In addition, reference samples from 9 close relatives of the known contributors were matched to all the samples within the study. Only 2 of the relative reference samples had an LR >1. One of these relatives was related to both individuals in a 2 person mixture and 2 of the 3 contributors in a 3 person mixture.



X axis : Log LR **Y axis : count**

Reproduced from Australia TrueAllele Validation study: Exploring each axis in more depth. Cybergenetics April 2011.

5. REPRODUCIBILTY

All of the samples were processed in duplicate and the reproducibility between runs was assessed. A report was provided by Cybergenetics (document 3) documenting the reproducibility across the different study components. It was noted that the reproducibility of duplicate runs decreased as the match scores decreased. Cybergenetics carried out an additional analysis of the reproducibility in stratified ranges which demonstrated the results with higher match scores generally have a greater reproducibility.

Below are their reproducibility results for the log(LR) match scores in the two main test groups, stratified by thousand, million, etc.

The reported log(LR) statistics in each line are:
group mean, within-group standard deviation

Two Unknown

LR< thousand or Log LR <3
1.3076, 0.7264

LR< million or Log LR <6
4.7927, 0.5929

LR< billion or Log LR <9
7.3598, 0.2811

LR> billion or Log LR >9
11.3605, 0.2953

Three Unknown

LR< thousand or Log LR <3
1.6557, 0.4482

LR< million or Log LR <6
4.4084, 0.5093

LR< billion or Log LR <9
7.2898, 0.4468

LR> billion or Log LR >9
11.2864, 0.3083

Occasionally TA generates an outlier result and it is recommended that duplicate runs are carried out to identify this occurrence and if found further runs performed to identify the outlier.

S0026 demonstrates an occasion when the joint LR calculation varies by a significant order of magnitude for the minor contributor (A252034) to a 2-person mixture.

TA1

S0026-2 vs A252034

The match rarity between the evidence and the suspect is 2.72 million. The inferred mixture weight parameters are as follows:

Mixture Variance: NSWPF S0026_2

Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.045	0.025	[0.000, 0.094]
item1	2	0.955	0.025	[0.906, 1.000]

TA2

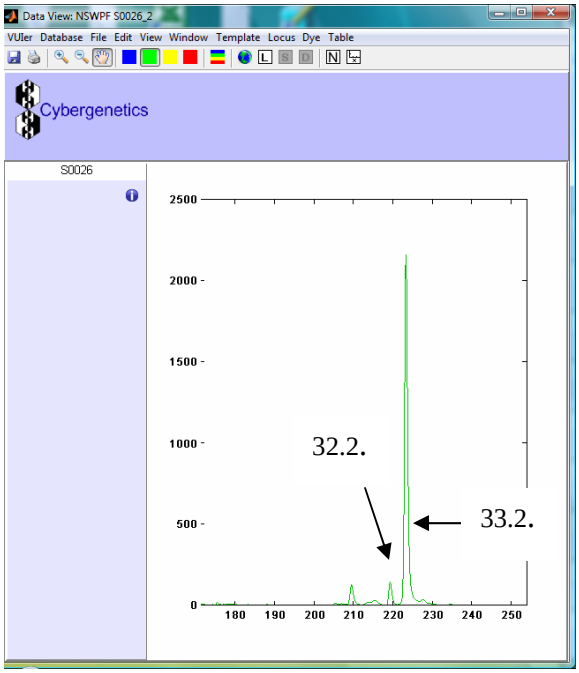
S0026-2 vs contributor 2 vs A252034

The match rarity between the evidence and the suspect is 866. The inferred mixture weight parameters are as follows:

Mixture Variance: NSWPF S0026_2

Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.923	0.019	[0.886, 0.960]
item1	2	0.077	0.019	[0.040, 0.114]

Focusing on a single locus (D21) illustrates the significant variation in the reported probabilities: The correct minor genotype at D21 is highlighted in red



Analysis 1	**D21**
genotype	**Q(x)**
30 , 32.2	0.961
30 , 30	**0.016**
30 , 31.2	0.007
32.2, 32.2	0.006

The true genotype (30,30) of the minor contributor has a probability of 1.6%, while another possible genotype for the minor contributor (30,32.2) has a probability of 96%.

Analysis 2	**D21**
genotype	**Q(x)**
30, 30	**0.615**
30, 31.2	0.172
30, 33.2	0.077
30, 32.2	0.076
29, 30	0.022
30, 34.2	0.021
29, 31.2	0.005
30, 38.1	0.004

The true genotype (30,30) of the minor contributor now has a probability of 61.5%. while the genotype 30, 32.2 is now reduced to 7.6%.

It may be necessary to carry out more analyses to identify outliers. A third run produced another set of probabilities for D21 as follows:

Analysis 3 D21

Genotype	Q(x)
30, 32.2	.7780
30, 30	**.1621**
30, 31.2	.0211
30, 33.2	.0144
32.2, 32.2	.0075
29, 30	.0063
31.2, 32.2	.0042

Many different allele pairings were considered by TrueAllele in the three different runs. Given that there is no change to the data, a similar posterior probability distribution of the unknown minor contributor genotype at each locus would be expected. However, D21 demonstrates a locus where the probability distribution is very different and therefore the LR at this locus is very different.

In this particular instance further replicates did not resolve which was an outlier and also indicates that TA's priors for stutter are so broad that it does not process very low level homozygotes in a way that gives a consistent probability for the correct genotype.

Genotype	Analysis 1 q(x)	Analysis 2 q(x)	Analysis 3 q(x)
30,32.2	.961	.076	.7760
30,30	.016	.615	.1621
LR for D21	**0.261**	**6.2**	**2.5**

This particular example results in joint LRs which are significantly different with one run presenting a match rarity statistic of 866 and in another run 2.7 million. Laboratory policy may consider implementation of protocols to conduct multiple runs to identify and avoid reporting outliers.

6. EASE OF USE/USER SUPPORT

Discussion with Cybergenetics during the course of this evaluation have been constructive and there is opportunity to have input into future versions of TA. In response to discussion, some adjustments to the TA system are already in progress.

- Automated reporting.

- A capability to give a probability as to the number of contributors to the mixture will be included by the end of 2011.

- The function dealing with stutter behaviour will be altered by the end of 2011. This system calibration was a function of previous TA versions however has not been used for a number of years. It is expected that this function will improve performance with low template samples. More focus will be achieved by the addition of more information. This will not be specific to stutter alone, but rather include all lab dependant settings in relation to peak variance.

- Cybergenetics will also assess the features of the system in relation to the high level of 'crossing over' observed in mixtures with similar levels of contributions from each individual. It has been suggested that it might be useful to alert the analyst that there is not enough information in the data to sort the mixture weight out between the contributors rather than just display a lot of crossing over events. It is anticipated that the move to STR kits with additional loci will be helpful in this situation.

- During the early exploration of the review module, it was found to be challenging to reconcile the data with the likelihood function. Newer TrueAllele interfaces are de-emphasising likelihood, and will reserve it primarily for instructional purposes. It is more intuitive to focus on the posterior probability for data interpretation.

7. TIME EFFICIENCES

In recent years laboratories are expanding capacity to analyse DNA samples by embracing liquid handling robots. Expert computer systems such as TA provide the opportunity for automated interpretation of STR data to avoid shifting the bottleneck to the interpretation stage. Many samples of limited complexity will require minimal review that will result in the timely interpretation and reporting of DNA results. Currently a minimum of two experts must review an STR interpretation, which can be a significant time investment. The use of an computer based expert system such as TA could be considered as a primary review with an additional human expert providing the second review. Many samples of limited complexity could be processed with a single review, which would in turn lead to time efficiencies.

Whilst for many 'simple' mixtures a review of the interpretation will not be an arduous task, it will be time consuming for complex and low template DNA samples. Significant numbers of complex mixtures, which under current methods are 'inconclusive', will now require a human review of the TA interpretation. The time impact of this will vary from lab to lab.

It has been identified during this evaluation that analyst review of the data is required at the initial stage prior to interpretation. This review is critical as TA does not identify artefacts and considers all peaks as possible alleles. It was noted during evaluation that artefacts, which are not removed by the analyst, can affect the integrity of the interpretation. This review will be straightforward for experienced analysts.

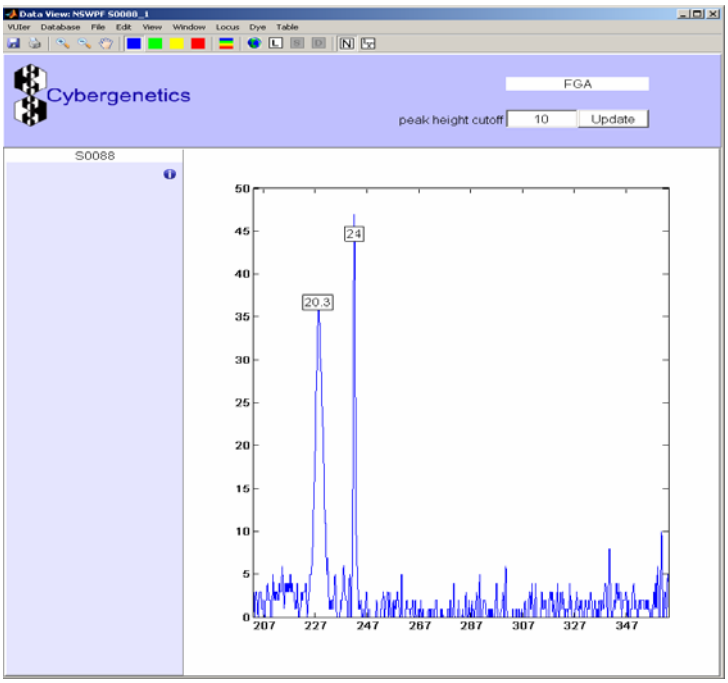
The time required for processing a single mixture is considerable, up to 15 hours for the most complex samples. The time required will double with the implementation of the increased loci in the new kits currently being rolled out in Australia. Joint analysis of multiple amplifications and/or multiple items will increase in proportion to the number of data sets uploaded. Duplicate analysis may be required and on occasion a triplicate analysis may be required to identify outliers. Laboratory policy will dictate the number of analyses required. A single processor channel will be generally limited to the analyses of best 3 mixture samples in a 24 hour period. To process large numbers of complex samples, a considerable number of parallel processor channels will be required.

8. POTENTIAL DIFFICULTIES

8.1 ARTEFACTS

TrueAllele processes raw data files generated during STR profiling. The raw data must be quality checked by the analyst prior to data interpretation. TA will assess **all** peaks as potential contributors to the DNA genotypes and will not disregard, or give less weight, to apparent artefacts that are not identified during the initial data analysis phase.

FGA locus in S0088 gives an example of an artefact peak affecting the genotype probability distribution (the known genotype at this locus is 24,24). In this instance the artefact peak does not overlap an allele in the comparison reference sample and therefore the affect is to reduce the LR at this locus and is therefore conservative. If the artefact peak overlapped it would have the opposite effect.



S0088	Allele pair	Q(x)
FGA	**20.3**, 24	0.582
	24 , 24	0.255
	22 , 23	0.018
	23 , 25	0.012
	22 , 25	0.01
	20 , 25	0.01
	21 , 23	0.01
	22 , 27	0.008

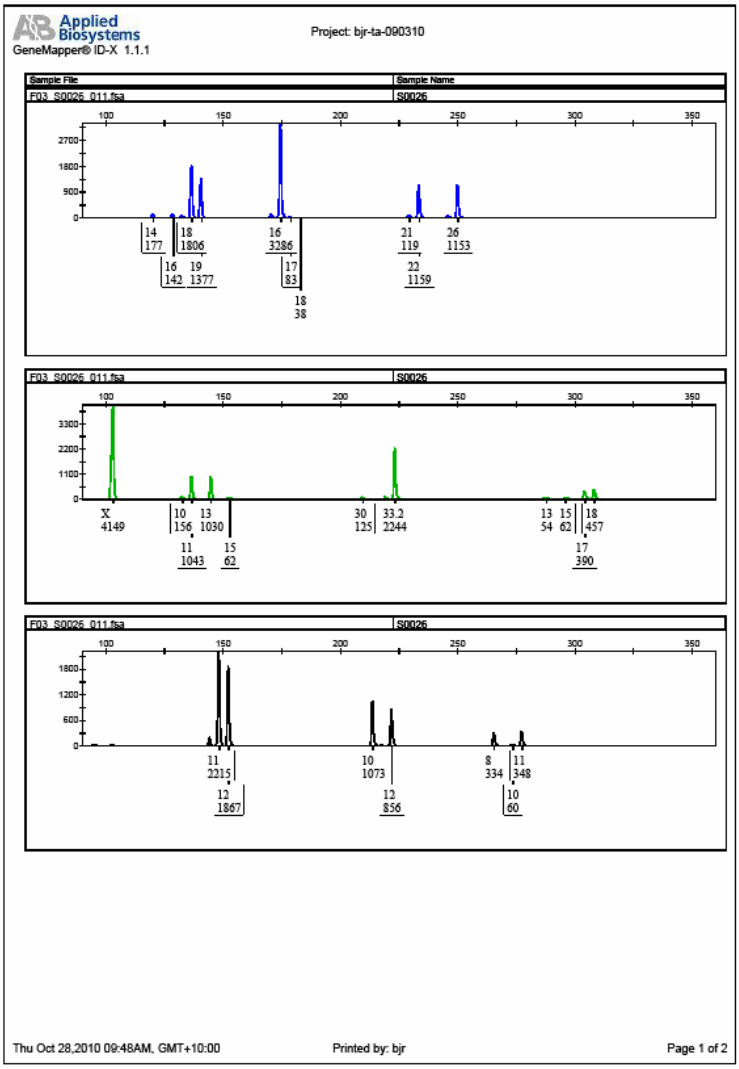
However with mixtures, the inclusion of artefacts may affect the capability of the system to identify the most accurate genotype probability distributions at individual loci which will translate to an effect on the overall LR. This effect will increase in magnitude if artefacts at multiple loci pass through to the interpretation phase.

TA has no inbuilt automatic removal of artefacts however experienced analysts would be expected to assess the epg prior to using TA and identify samples requiring reanalysis or identify the presence the artefacts and therefore this issue should not be a significant problem.

8.2 STUTTER MODELLING

Modelling of stutter raised many questions during the course of this evaluation. When minor components of a mixture are at similar peak heights to stutters, the genotype probabilities are not consistent with what would be expected given a reasonable consideration of stutter contribution.

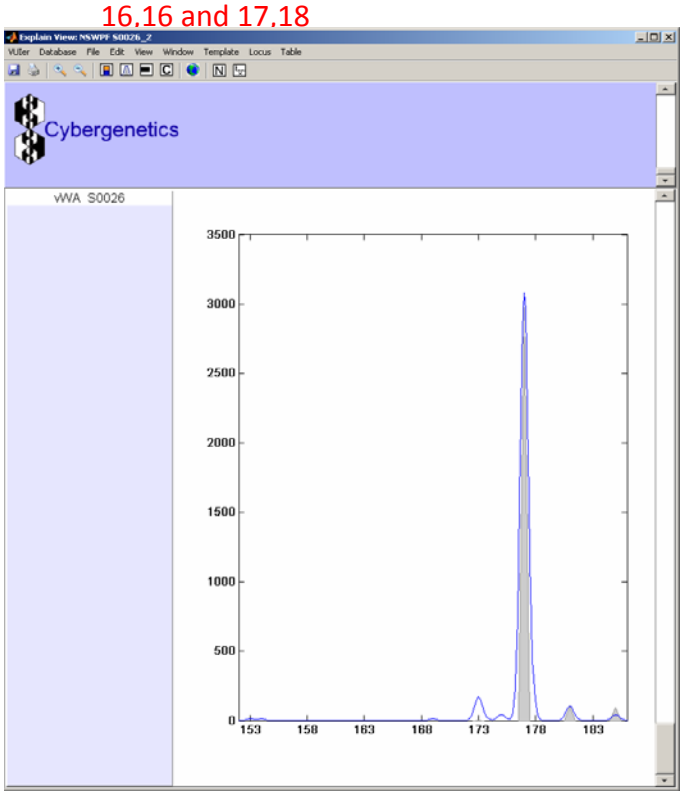
Consider the following examples in sample S0026, a 2 person mixture with the minor contributor at 7%:



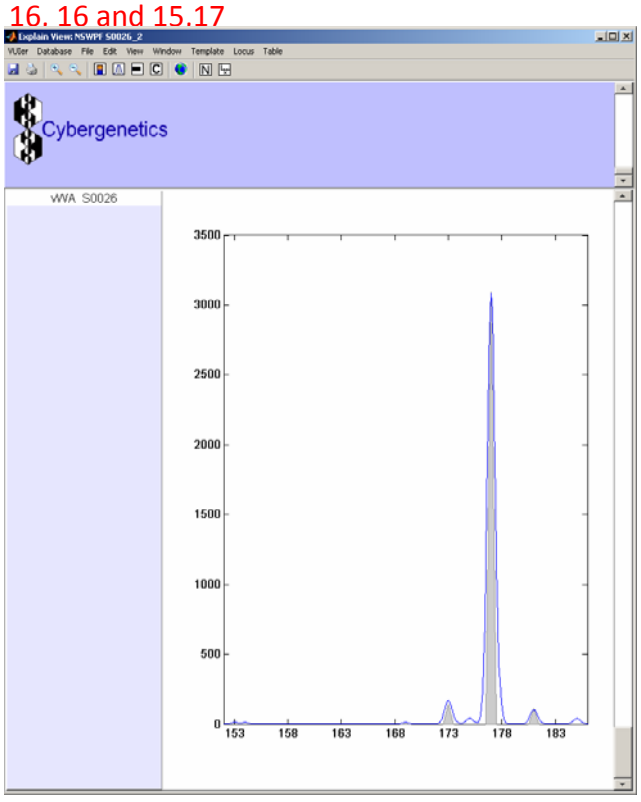
At vWA the probability for the allele pair 15,17 as the minor contributor to this 2 person mixture is 0.977. Given that the 15 peak is 147 RFU on a parent 16 peak of 3286 RFU, it seems that the described probability is underestimating the probability that the 15 peak is stutter. The probability for the correct allele pair 17,18 would have been higher if the 15 had been attributed a high probability as stutter.

The following view shows the pattern expected when the model proposed for vWA is major 16,16 and minor 17,18. There is no attribution of stutter to the 15 allele position on the parent 16 peak which is demonstrated by the absence of grey shading within the peak area in the plot on the left. When the proposed model is changed to major 16,16 and minor 15, 17, as in the plot on the right, the pattern indicates that the peak in the 15 position would be expected to be at the shaded height and therefore a minor contributor of 15,17 provides a good fit for the data because it does not seem to include stutter at the 15 peak. The performance of TA is respect to the determination of genotype probability distributions for minor contributors at levels in the stutter range was considered to be questionable and many examples were seen throughout this study.

16,16 and 17,18



16, 16 and 15,17



When questioned in regard to this stutter modelling, the following response was provided by Cybergenetics:

TrueAllele does not assign a "probability" to the event that a particular peak is stutter. Rather, the entire data pattern is examined relative a proposed peak pattern, with all relevant variables considered (genotypes, mixture weight, stutter, relative amplification, peak variance, etc.). TrueAllele's stutter modeling found some stutter

appearing at this locus, which is why we see genotypes in the posterior distribution that can explain the 15 data peak as (at least in part) a stutter that shadows the large 16 allele peak. Given that the 18 peak's height of 40 rfu is below most human detection thresholds, it is gratifying to see that TrueAllele gave positive probability to allele pairs that included an 18 allele. In particular, the probability of 4% given to 17,18 led to a relatively neutral LR of 0.57. The data indicated that a minor 15,17 allele pair, along with a major 16,16 allele pair, best accounted for the observed data. The computer neither knew nor cared about any comparison genotype; it could only infer from the observed data.

It is disappointing that TA assigned a probability of 4% to the correct minor genotype in this instance. The generation of a low LR is conservative however, this study is attempting to evaluate how accurately TA can infer the correct genotype so the end effect on the LR is of lesser interest.

A similar situation was seen at FGA:
At FGA the probability for the allele pair 21,25 as the minor contributor is 0.959. Given that both 21 and 25 are within levels which would be widely regarded as consistent with stutter, TA seems to assign a high probability to 21,25 as the minor.

The response from Cybergenetics to a query in regard to FGA:

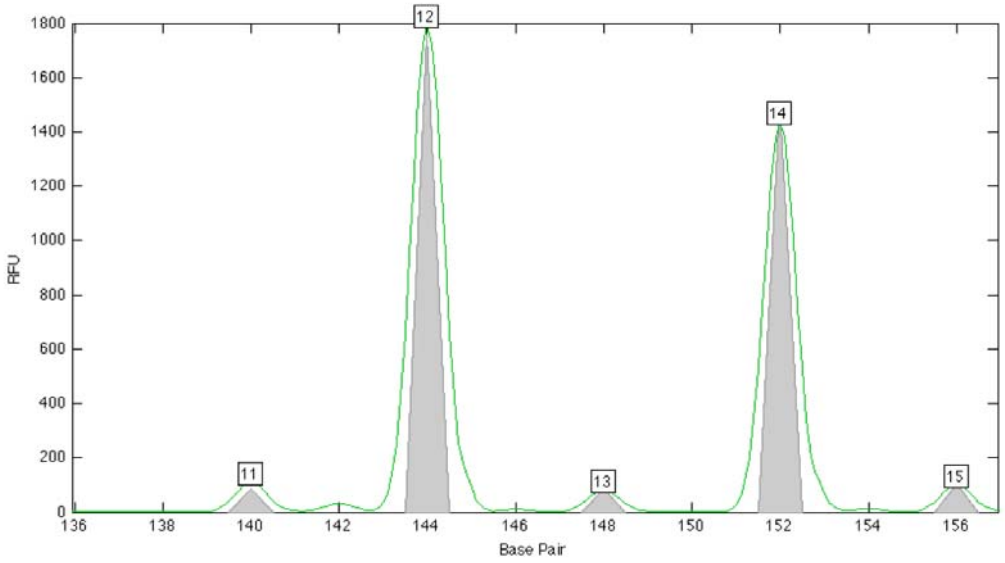
Most solutions here that are "reasonable" to human threshold review ignore the quantitative peak height pattern of the observed data. These qualitative solutions are not all that "reasonable" to quantitative modeling, since the data shows excellent balance between the two major contributor peak heights, and the two "stutter" peaks. To put all of the allele mass on one or the other of these smaller peaks would comprise the observed peak height balance, which is why imbalanced minor genotype possibilities, such as 21,21 (with no allele at all assigned to designation 25) are assigned a lower probability. The 21,21 allele pair was given a posterior probability of 1%, which relative to a prior population probability of 3%, led to a relatively neutral LR of 0.4.

There are other reasonable propositions for alleles pairs at this low contributor level, including for the example the possible masked alleles 22 and 26. A higher level of uncertainty around the genotype probability distribution for the minor genotype at this locus was expected.

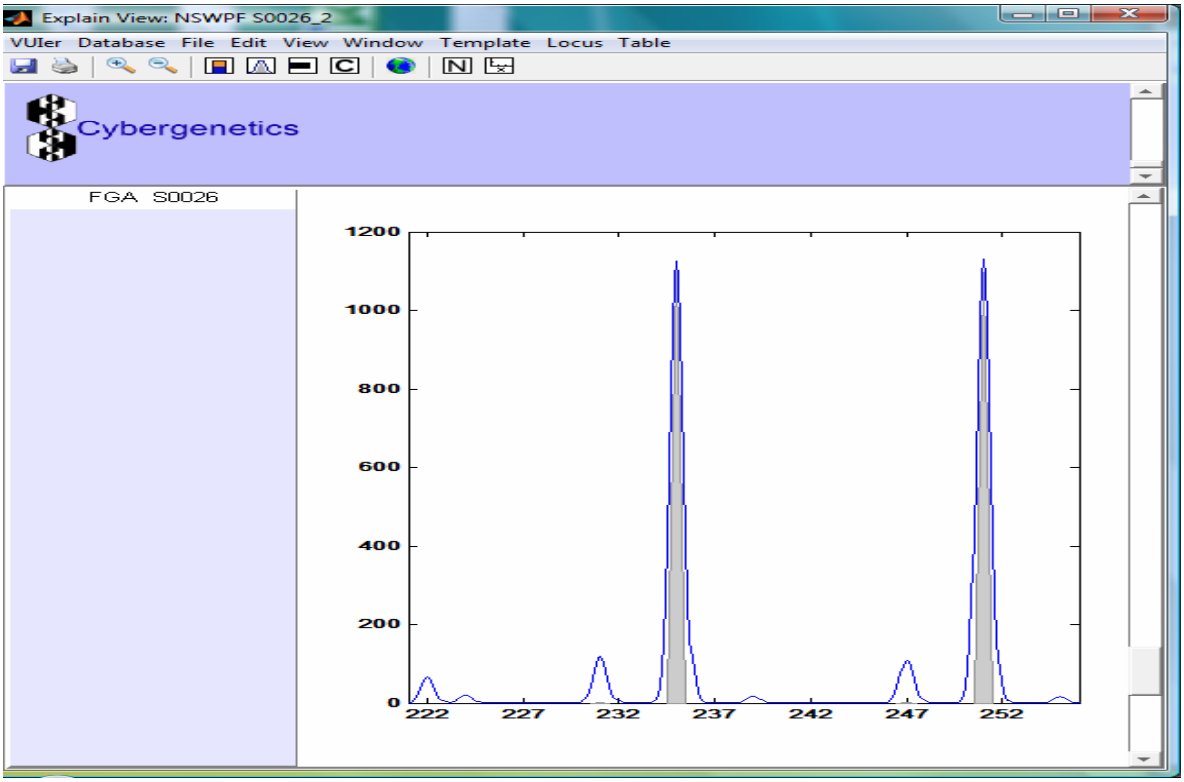
In addition, it seems that there is a lack of consistency in stutter modelling. An example of this is seen in comparison of FGA in sample S0026 to D8 in the Foley case analysed and reported by Cybergenetics. The mixture in the Foley case was recovered from the fingernail of the victim and was of a very similar mixture proportion to S0026 with a minor contributor weight of 7%.

Comparison of the pattern view for D8 (Foley) and FGA (SOO26) shows a different pattern of stutter. In Foley, the data for D8 can be seen to deal with peaks in stutter positions as expected by human review, but it does not seem to be modelling stutter in the same way for S0026.

Extract from Foley presentation showing stutters at 11 and 13 shaded grey in the following graph. The minor genotype was 12, 15.



The following graph shows FGA with genotype 22,26 as the proposed model for both major and minor. If consistent with the behaviour in D8 above, this should result in the peaks in the 21 and 25 position to be patterned as stutters. However, there is negligible shading within the 21 and 25 peaks.



The Cybergenetics response to a query in regard to Foley vs. S0026 was as follows:

This 7% mixture (S0026) *with two unknown contributor genotypes is a very different problem from the far simpler Foley case, where there was only one unknown genotype and a known victim reference profile was available. In Foley, co-ancestry was not considered; in that context, the match scores of the two S0026 repetitions (with theta = 0) were 10^6.2395 (1.74 million) and 10^3.5046 (3,196). In practice, since the larger objectively-inferred LRs tend to be more accurate, we would have repeated the request to confirm the million-fold LR result.*

The Explain window does not use the posterior probability distribution of stutter at a locus. Instead, it shows only an instant snapshot of the state of the memory-less Markov chain. We only use the Explain interface for teaching purposes, and never to assess data or results.

The TrueAllele version 25 stutter models are the same in this problem as they were in Foley.

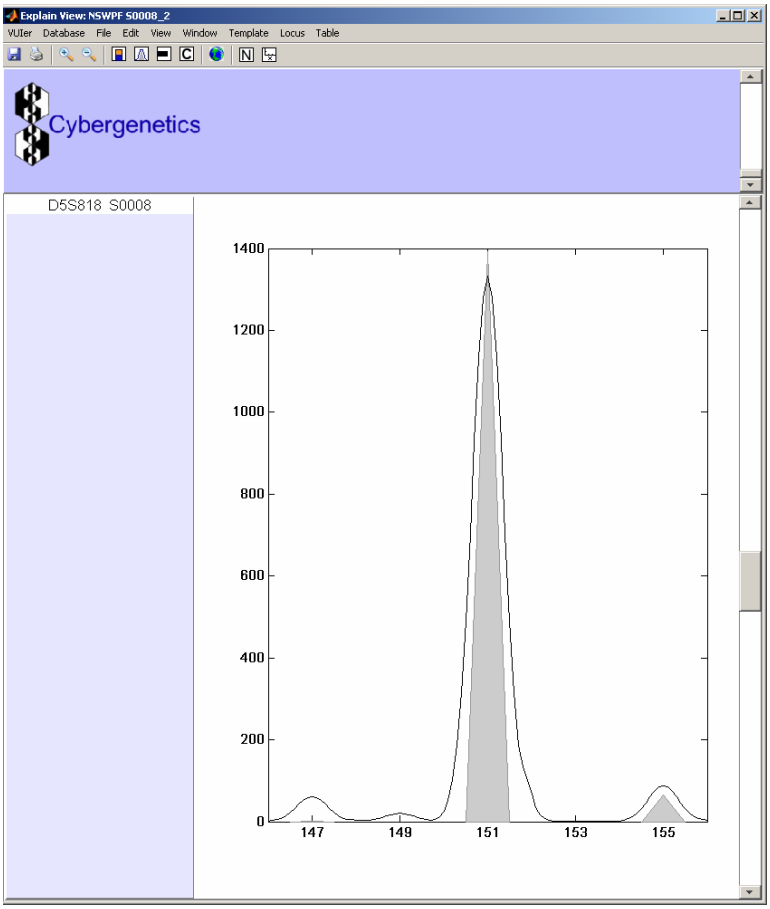
It might be suggested that a major contributor with inferred genotypes of near certainty at all loci simplifies the mixture to a similar degree as a known contributor.

It was also noted that even within the same sample the pattern reflecting the expectation of peak height in stutter positions appears to vary at different loci so that at some loci all of the peak height is attributed to stutter, while at other loci none of the peak height is attributed to stutter. An example of this is seen in 2 person mixture S0008.

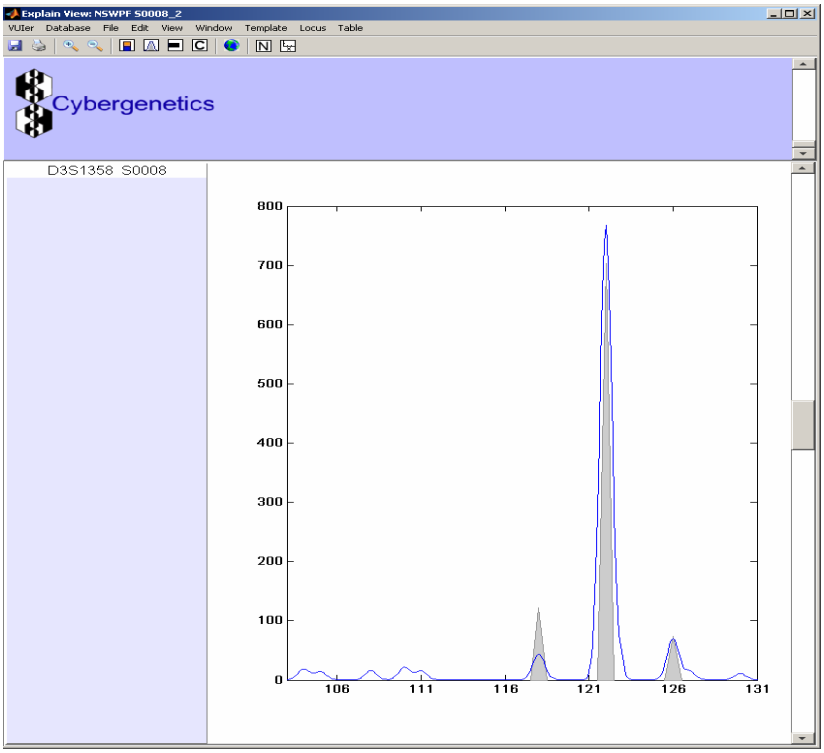
As the genotypes of the constructed mixtures are known, it is possible to identify when peaks are alleles or stutter or a combination of both.

In the following graph, at D5 the proposed model is major 11, 11 and minor 11,12. The view shows the pattern of how this proposed model fits the data

Peak in stutter position is 3.8% on parent 11 peak however TA assigns no discernible stutter and assigns a high probability of 0.929 to the genotype 10,12 but in reality the 10 is all stutter. The correct C2 genotype 12, 12 has a probability of 0.026



In contrast to the stutter modelling at D5, at the D3 locus TA assigns stutter to the peak in the stutter position and even seems to assign excess. The proposed model here for D3 is major 14,14 and minor 15,15. The peak in the stutter position is 5.6% of the parent 14 peak and is typical of a stutter peak and is in fact a stutter peak. TA assigns a high probability of 0.74 to the correct minor genotype 15, 15.



This example illustrates the inconsistent way of handling stutter when the real peaks are of a similar peak height to stutter peaks.

While it is understood that the entire data pattern is examined relative to a proposed peak pattern with a range of variables considered in addition to stutter, TrueAllele might assign more realistic probabilities to the correct genotype if it used stutter modelling parameters determined by laboratory empirical testing.

Discussion around stutter modelling has occurred with Cybergenetics and there is a commitment to restore calibration with more informed priors to deal with stutter and other laboratory dependent parameters by the end of 2011. This is expected to improve the performance of TA in the assessment of low template DNA.

8.3 50:50 MIXTURES

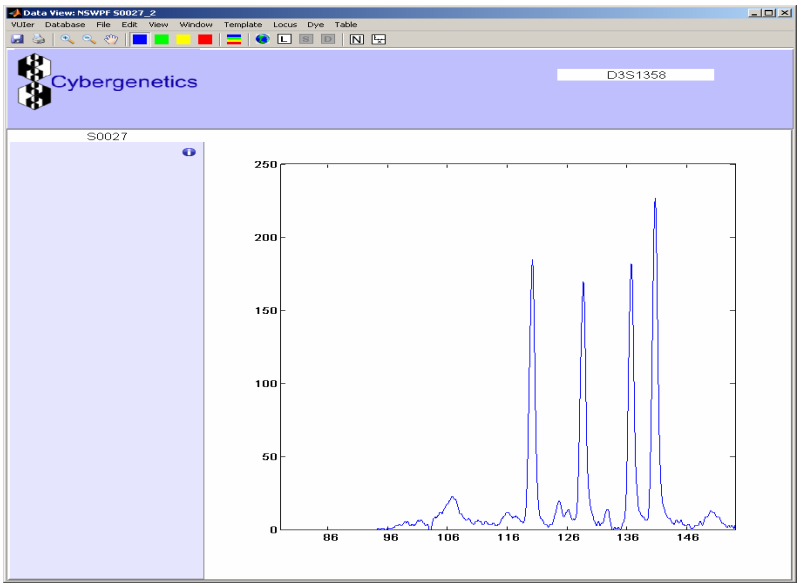
With mixtures where there is little distinction between the level of DNA from two individuals it would be expected that the inferred genotype probabilities would reflect considerable uncertainty, however this does not always seem to occur. Consider the following example (S0027) which illustrates this point:

S0027 TrueAllele mixture weight assessment:

Contributor	Weight	Stdev	95% interval
1	.512	.105	.306-.718
2	.488	.105	.282-.694

In this example it is expected that there would be a relatively even distribution of inferred genotypes especially given the low peak heights. However two genotypes are given a high probability and the other possible genotypes given relatively low probabilities.

D3 has 4 allele peaks in this mixture
14 at 186 rfu, 16 at 171 rfu 18 at 185 rfu 19 at 225 rfu



The l(x) and q(x) values for D3 for both contributors are listed:

D3	L(x)	Q(x)	
16, 18	0.030	**0.443**	
14, 19	**0.788**	**0.351**	
16, 19	0.059	0.072	
14, 18	0.009	0.050	Contributor 2 (.512)
18, 19	0.110	0.046	
14, 16	0.002	0.038	
14, 19	**0.850**	**0.443**	
16, 18	0.021	**0.351**	
14, 18	0.012	0.072	Contributor 1 (.488)
16, 19	0.036	0.050	
14, 16	0.002	0.046	
18, 19	0.080	0.038	

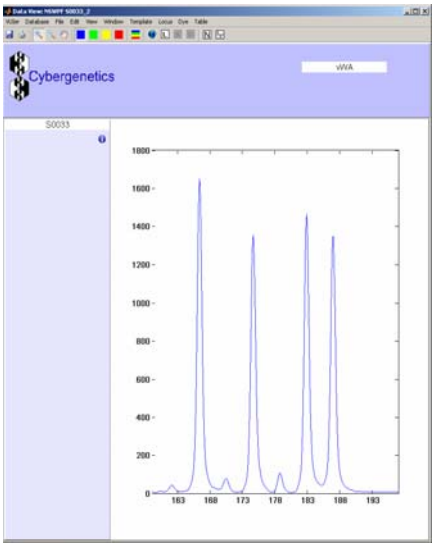
(The known genotype is highlighted in red)

The L(x) is the likelihood function and is said to be a measure of fit and Q(x) is the posterior probability given some prior population frequency R(x).

There were many examples of this throughout the study which are particularly obvious at loci with 4 alleles.

S0033 vWA

The mixture weights are (contributor 1: 0.501, contributor 2: 0.499) and the peak data is 14 1698rfu 16 1405rfu 18 1502rfu 19 1384rfu.



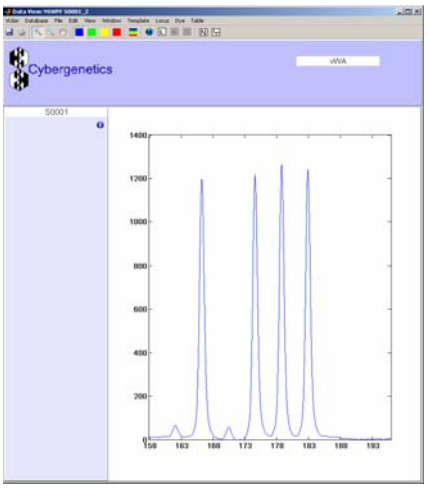
Contributor 2

Allele pair	l(x)	q(x)
16, 18	0.124	0.405
14, 19	0.717	0.387
16, 19	0.049	0.070
14, 18	0.045	0.056
18, 19	0.043	0.045
14, 16	0.023	0.038

(The known genotypes of the contributors are 14,16 and 18,19).

S0001 vWA:

MW Contributor 1: 0.465 Contributor 2: 0.535 and peak data are 14 1242rfu 16 1256rfu 17 1313rfu 18 1280



Contributor 2

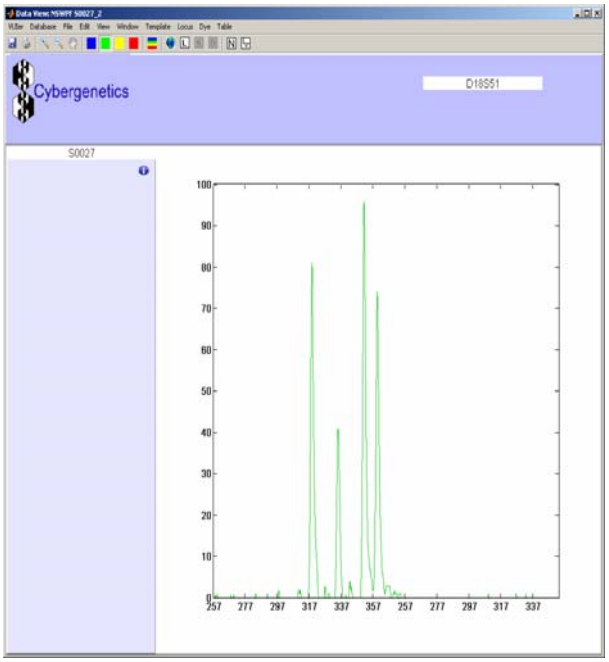
Allele pair	l(x)	q(x)
14, 18	0.803	0.647
16, 17	0.052	0.158
14, 16	0.069	0.074
14, 17	0.043	0.049
17, 18	0.022	0.049
16, 18	0.011	0.023

(The known genotype is highlighted in red).

In these examples it seems that both L(x) and Q(x) have distributions contrary to human review. In particular with this example, genotype 14, 18 is given an unreasonably high probability.

This unbalanced weighting of genotype appears to be somewhat random as on other occasions the genotypes probabilities are far more equally spread. For example D18 (S0027) reports probabilities that are distributed across the 6 genotype options:

D18 has 4 allele peaks in this mixture
13 at 86 rfu, 15 at 43 rfu 17 at 96 rfu 19 at 74 rfu



D18	L(x)	Q(x)	
13, 17	0.220	0.222	
13, 15	0.194	0.206	
15, 18	0.199	0.205	Contributor 2 (.512)
15, 17	0.078	0.129	
17, 18	0.129	0.127	
13, 18	0.173	0.109	

(The genotype of the known contributor is highlighted in red).

A query in regard to this was put to Cybergenetics and the response included the following explanations:

A template mixture weight around 50:50 reflects an average of the separately observed locus mixture weights. While the template average might be 50%, the separate locus weights are usually not this average 50% value. Therefore, there is mixture weight information present at each locus that can help separate out the genotypes, to some extent.

A human might see peaks as having roughly equal peak heights, and assume that there is no further information present. A computer instead has random variables that describe the quantities of DNA present that (with PCR variation and artifacts) can account for the observed data. For example, relative amplification in the PCR can make higher molecular weight peaks amplify less prominently than their true underlying mass.

The computer tries out all possible contributor mass quantities to explain the data. Most of the inferred contributor mass quantities are not in a precise 50:50 ratio at a given locus.

The Explain window is good for teaching, but not as good for examining the data in great detail. This interface only employs a limited number of variables (genotype, mixture weight), and does not provide a full probability model. Similarly, probability should be used for understanding our inferences about belief, rather the mathematical and unintuitive likelihood construct.

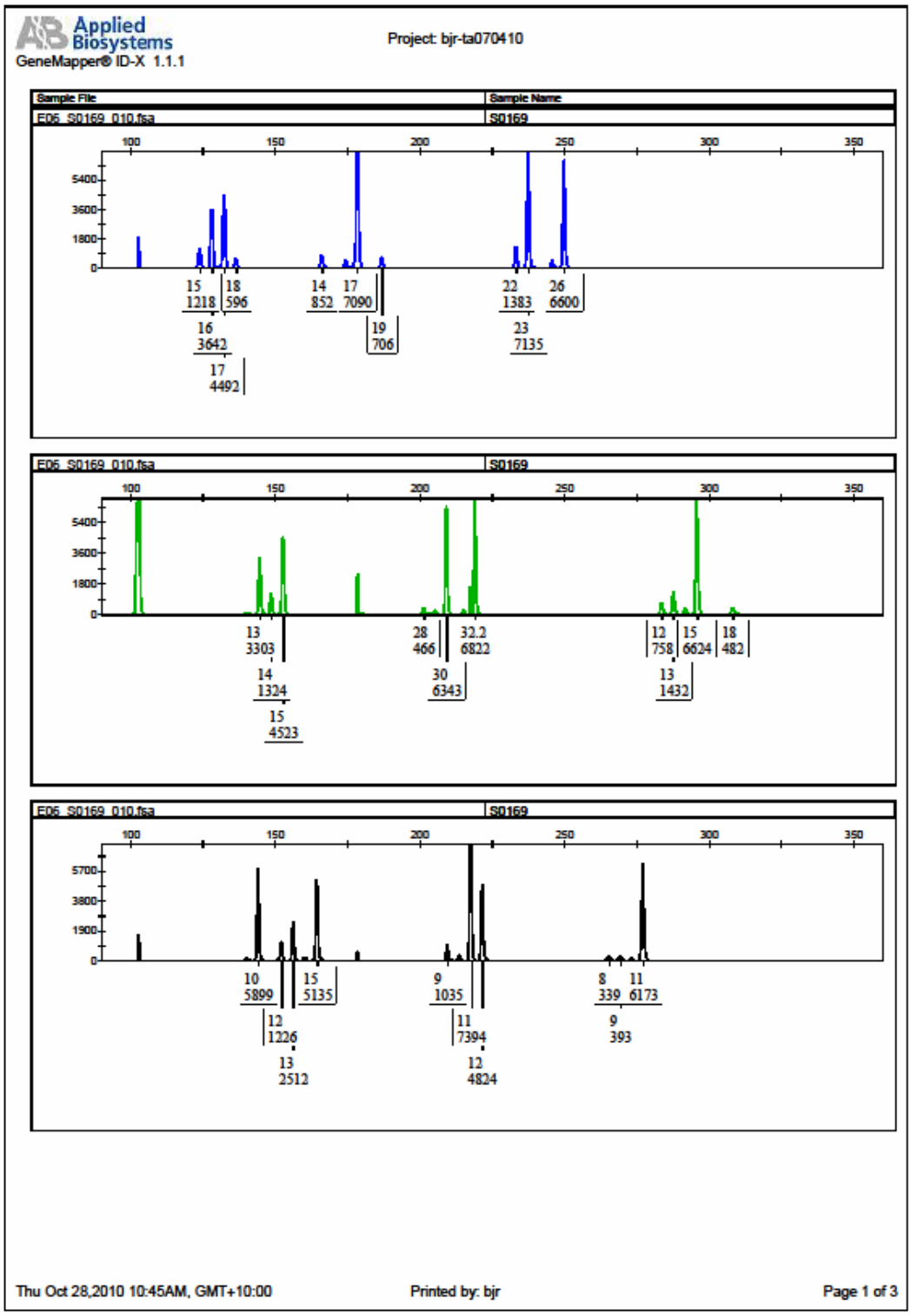
That said, in the S0033 example, the vWA locus data gave support to a higher likelihood assignment for 14,19, once relative amplification (reducing the allele 19 peak height) and stutter (adding to allele 18's peak height) effects were accounted for. What is "intuitive" in one model becomes "unintuitive" in another model.

3 persons mixtures with a major contributor and additional minor components in approximately 50:50 proportions have been identified as an area where TA does not appear to perform well in relation to the minor contributors. S0169 provides an example of an assessment where TA inferred similar mixture weights for both minor contributors:

TrueAllele mixture weight assessement:

Contributor	Weight	Stdev	95% interval
1	.128	.050	0.03 - 0 .226
2	.125	.050	0.027- 0.223
3	.746	.050	0.652- 0.840

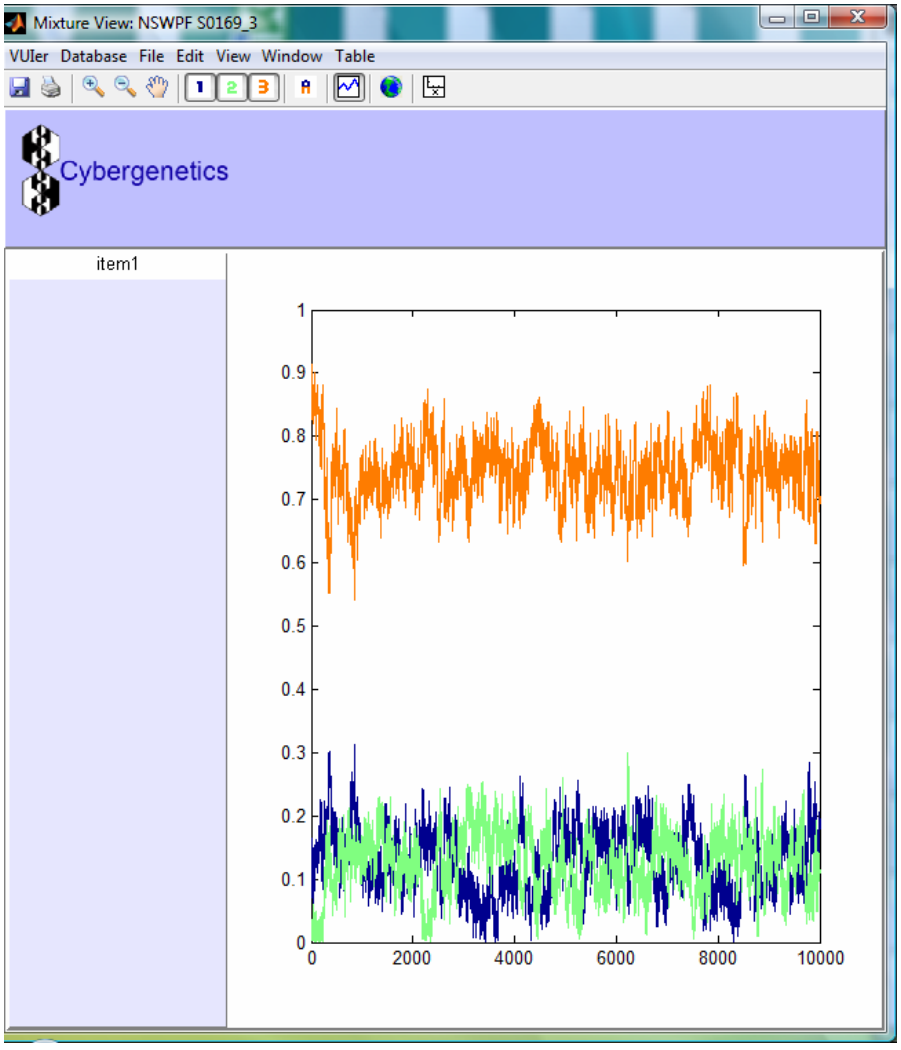
The inference is incorrect for the known contributors but may not be for unknown ones. There may be 3 unknown contributors which could justify the mixture weights as determined. There is significant overlap in genotypes of all contributors at most loci and TA is unable to separate the minors by weight. Only two loci, D3 and D18 provide valid mixture weight indications and these loci suggest that contributor 1 accounts for about twice as much DNA as contributor 2.



	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
A251474 C3 (.746)	16,17	17,17	23,26	XX	13,15	30,32.2	15,15	10,15	11,12	11,11
A251973 C1 (.128)	15,17	17,17	23,26	XX	14,15	30,32.2	13,15	10,13	11,11	11,11
A251664 C2 (.125)	16,18	14,19	22,23	XX	13,15	28,32.2	12,18	12,13	9,11	8,9

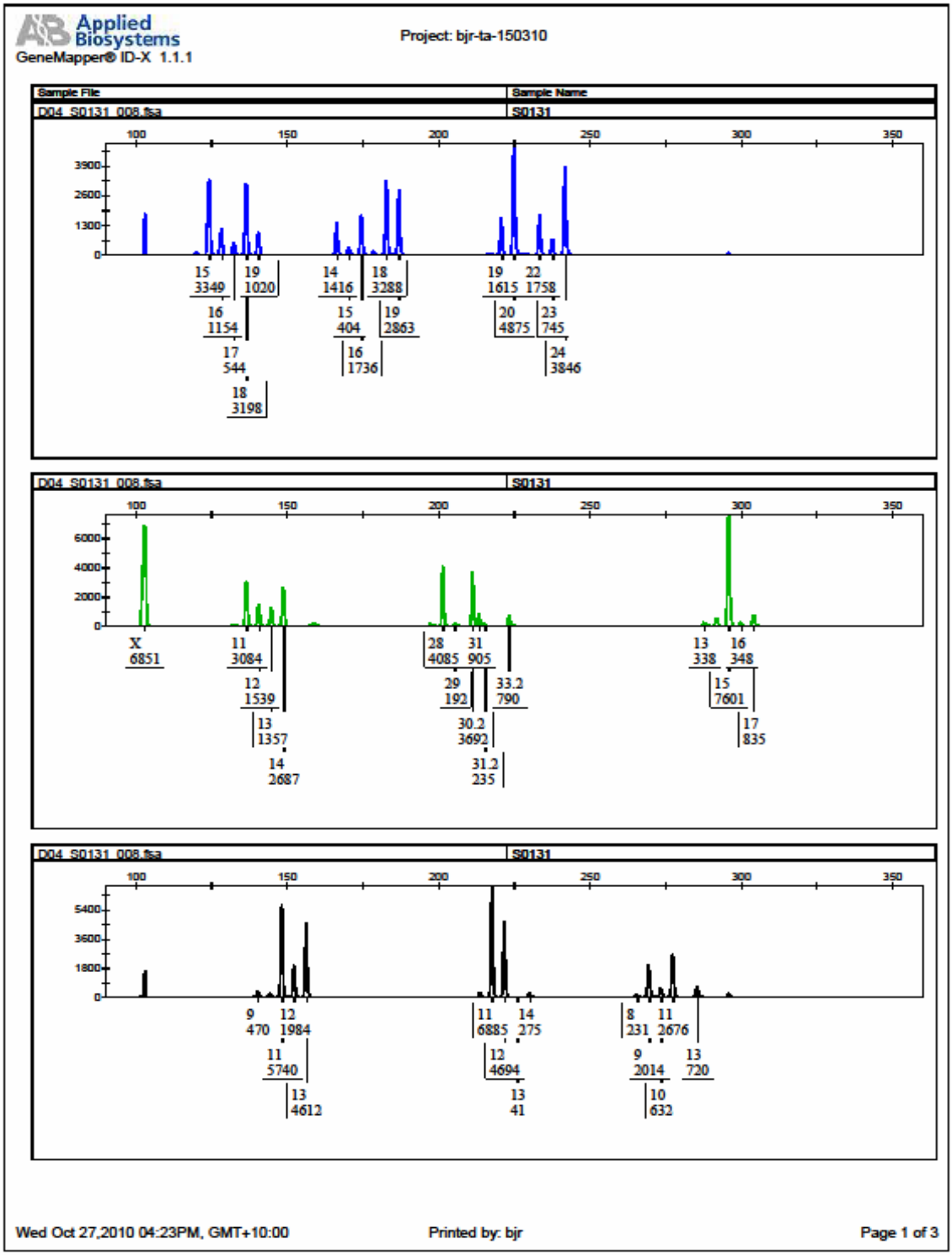
TrueAllele has not performed well with regard to the minor component of this mixture with an LR of 91 and 1.69 for the two known minors. The levels of DNA are high and a basic RMNE calculation places more evidential weight on the data in this instance (approx 1 in 40,000). In contrast, TrueAllele provides virtually no support for inclusion of A251973 (LR of 1.69) and limited support in relation to A251664 (LR of 91).

The inferred mixture weights of contributor 1 and contributor 2 are essentially the same at 0.128 and 0.125, therefore the pattern of genotype probabilities for contributor 1 and 2 are similar. This may lead to a problem when the genotypes of the true minor contributors are not the same as TA infers the same genotype as the most likely for both contributors and doesn't create complimentary pairs. In a 50:50 mixture this will lead to a reduced LR as a result of the uncertainty associated with the allele pairs, however in this example the minor contributors were not meant to be in equal amounts and were notionally 300pg of Contributor 1 and 100pg of Contributor 2. Therefore the inferred genotypes did not reflect Contributor 2 well. The problem in this case may have been affected by excess DNA which can cause a deviation from a linear data pattern. Review of data analysis by experienced analysts may help resolve these issues. The MCMC history shows that TA was unable to settle on separate weights for these contributors.



8.4 NUMBER OF UNKNOWN CONTRIBUTORS TO A MIXTURE

TA will analyse the data under any analyst request. A proposed model that is completely inconsistent with the data is not 'flagged' by TA for review. The following 3-person mixture, S0131, was analysed as a 2-person mixture:



Profiles of the contributors to S0131

	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
A251477 C1 (.729)	15,18	18,19	20,24	XY	11,14	28,30.2	15,15	11,13	11,12	9,11
A251972 C2 (.059)	15,17	15,16	22,23	XY	12,14	29,31.2	13.16	9,12	12,14	8,11
A251666 C3 (.212)	16,19	14,16	19,22	XY	12,13	31,33.2	15,17	11,12	11,11	10,13

TrueAllele mixture weight assessment under 2 person request:

Contributor	Weight	Stdev	95% interval
1	.249	.041	.169 - .329
2	.751	.041	.671 - .831

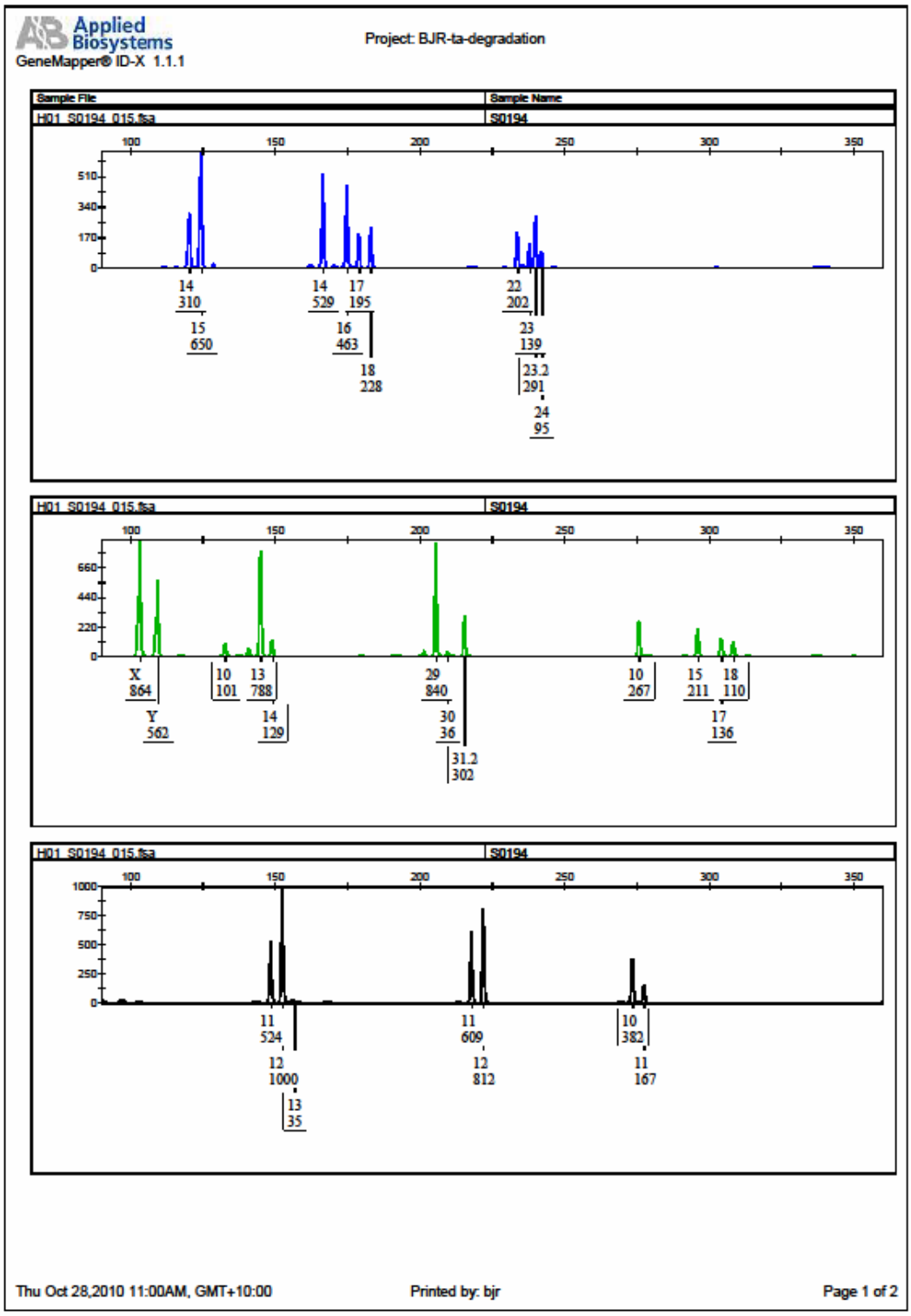
Calculation:

Laboratory	Contributor	LR
Cybergeneticss	1 A251666 2 A251477 1 / 2 A261972	1.4E+10 3.6E+11 <1

TA reported an LR significantly <1 (in the order of 10E-13) for one of the proposed contributors (A261972) under the 2-person request. While a human review would suggest a 3 person analysis and not support an LR <1, in the context of a probabilistic analysis as a 2 person mixture such a minor contributor would be expected to have a very low likelihood but not be absent from the inferred profiles in a TA assessment.

Since TA does not flag this situation, careful review of data and the requests under which they have been processed will be critical to protect against results from erroneous requests. This has been discussed with Cybergenetics and they have indicated that they will incorporate the capability to give a probability to the number of contributors by the end of 2011

S0194 provides a more complex example of the effect the number of unknowns in the request on the inferred genotypes. In this instance it is a 3-person mixture however one of the contributor's DNA has been degraded and is only present at very low levels. This mixture was processed under a 2-person and 3-person request.



Profiles of the contributors to S0194

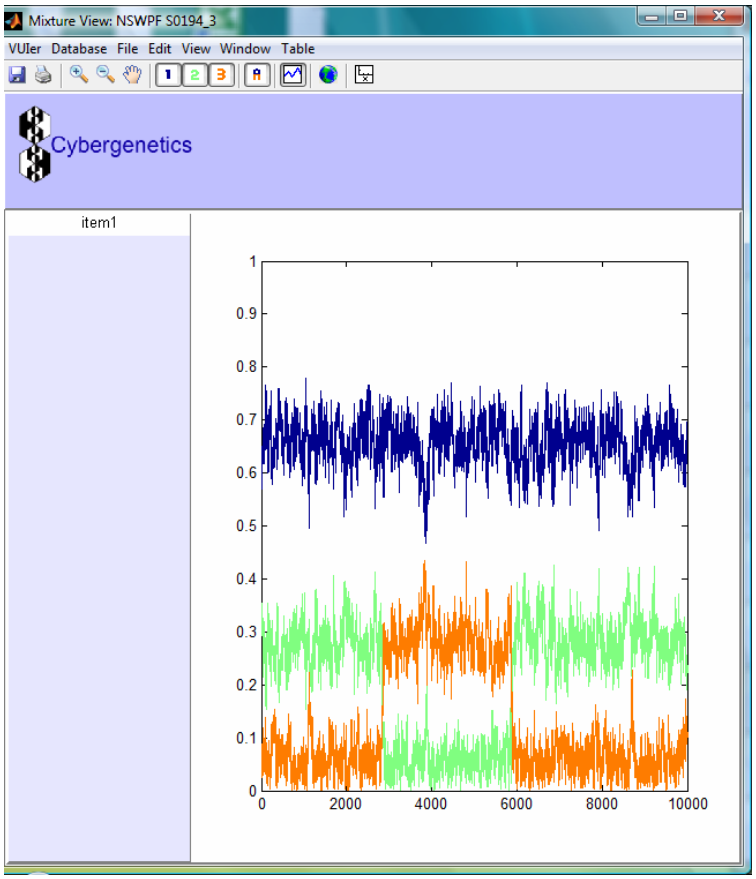
	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
A251475 C1	15,15	14,16	22,23.2	XY	13,13	29,31.2	10,15	12,12	12,12	10,10
A252032 C2	14,14	17,18	23,24	XX	10,14	29,29	17,18	11,11	11,11	11,11
A252033 C3	16,16	17,18	25,25	XX	10,12	30,30	13,16	11,13	11,11	10,12

TA assessment of the mixture weight under a 3- person request appears to overestimate the contribution of the degraded contributor C3 as the EPG does not support a weight of 12% of the total DNA for contributor 3:

Mixture Variance: NSWPF S0194_3

Template	Contrib	Weight	Stdev	95% Interval
item1	1	0.657	0.042	[0.575, 0.739]
item1	2	0.215	0.121	[0.000, 0.452]
item1	3	0.128	0.167	[0.000, 0.455]

This appears to be the result of crossing over and the very minor contributor spending some time at a higher weight as shown in the MCMC history:



This history suggests that about 0.05 would be a more realistic weight for C3. The higher inferred weight for C3 reduces the probability that its alleles may have dropped out and affects the probabilities for the other contributors.

The degraded contributor C3 has an LR of <1.

In the match report TA calculated an LR of 708 million for the major contributor (C1) to this mixture and this is problematic. While such an LR provides extremely strong support for the hypothesis that the DNA originates from A251475, careful examination of the match report

might suggest an alternative identification hypothesis. High probabilities for allele pairs which do NOT match A251475 are seen at five loci. Therefore a reasonable argument might be raised that a relative of A251475 might be **more likely** to be the source of this DNA than A251475, especially since two loci, D18 and FGA, have rare genotypes that provide the bulk of the LR.

S0194_3 vs. A251475

The LR calculation assumes three unknown contributors in the evidence relative to a AU_CAU human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 708 million.

The joint LR is approximately 708 million.
The log(LR) information is 8.85.

			Genotype Probability Distribution			Weighted Likelihood		Likelihood Ratio	
locus	allele pair x	Likelihood l(x)	Questioned q(x)	Reference r(x)	Suspect s(x)	Numerator l(x)*s(x)	Denominator l(x)*r(x)	LR	log(LR)
D13S317	11, 12	0.844	**0.901**	0.1746			0.14747		
	12, 12	0.156	0.099	0.0909	**1**	0.15559	0.01414		
						0.15559	0.16161	0.962	-0.016
D18S51	10, 15	1	1	0.0059	1	0.99979	0.00592	168.818	2.227
D21S11	29, 29	0.627	**0.684**	0.0518			0.03247		
	29, 31.2	0.373	0.316	0.046	**1**	0.37302	0.01715		
						0.37302	0.04962	7.516	0.876
D3S1358	14, 15	0.857	**0.818**	0.0698			0.05983		
	15, 15	0.143	0.182	0.0819	**1**	0.14258	0.01167		
						0.14258	0.07151	1.993	0.3
D5S818	11, 12	0.641	**0.756**	0.2666			0.17097		
	12, 12	0.359	0.244	0.1396	**1**	0.35863	0.05006		
						0.35863	0.22102	1.622	0.21
D7S820	10, 11	0.51	**0.598**	0.1124			0.05734		
	10, 10	0.49	0.402	0.0787	**1**	0.48992	0.03857		
						0.48992	0.09591	5.108	0.708
D8S1179	13, 13	1	1	0.1113	1	1	0.11134	8.981	0.953
FGA	22, 23.2	0.995	0.995	0.0052	1	0.99476	0.00517	191.523	2.282
vWA	14, 16	1	1	0.049	1	1	0.049	20.406	1.31

When this sample was run under a 2-person request the inferred genotype probabilities were generally closer to the known genotypes and as a result the LR increases to 4. 9 billion:

S0194_2 vs. A251475

The LR calculation assumes **two unknown contributors** in the evidence relative to a AU_CAU human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 4.97 billion.

The joint LR is approximately **4.97 billion.**
The log(LR) information is 9.69.

			Genotype Probability Distribution			Weighted Likelihood		Likelihood Ratio	
locus	allele pair x	Likelihood l(x)	Questioned q(x)	Reference r(x)	Suspect s(x)	Numerator l(x)*s(x)	Denominator l(x)*r(x)	LR	log(LR)
D13S317	11, 12	0.761	0.842	0.1746			0.13283		
	12, 12	0.239	0.159	0.0909	1	0.23943	0.02176		
						0.23943	0.15458	1.548	0.19
D18S51	10, 15	1	1	0.0059	1	1	0.00592	168.845	2.227
D21S11	29, 29	0.451	0.514	0.0518			0.02336		
	29, 31.2	0.549	0.486	0.046	1	0.5489	0.02524		
						0.5489	0.0486	11.293	1.053
D3S1358	14, 15	0.589	0.517	0.0698			0.04111		
	15, 15	0.411	0.483	0.0819	1	0.41096	0.03365		
						0.41096	0.07475	5.497	0.74
D5S818	12, 12	0.686	0.558	0.1396	1	0.68607	0.09576		
	11, 12	0.314	0.442	0.2666			0.08368		
						0.68607	0.17944	3.823	0.582
D7S820	10, 11	0.761	0.82	0.1124			0.08559		
	10, 10	0.239	0.18	0.0787	1	0.23858	0.01878		
						0.23858	0.10437	2.285	0.359
D8S1179	13, 13	1	1	0.1113	1	0.99974	0.11131	8.978	0.953
FGA	22, 23.2	0.995	0.995	0.0052	1	0.99467	0.00517	191.527	2.282
vWA	14, 16	1	1	0.049	1	1	0.049	20.406	1.31

This example illustrates that data used by TA need careful review by experienced analysts to make critical decisions as to the appropriated requests to submit to TA. It also illustrates that analyst review is required to protect against reporting a high LR in a situation where an alternative hypothesis is also supported and should be considered further within case context.

TA is by no means a 'push button' system with instant reporting of the generated LR, nor has it ever claimed to be so. In this particular example it is not clear that it is a 3-person mixture although there are indications that this is a possibility. Laboratory policy will be

required to stipulate how to proceed in similar circumstances. The LRs generated by each jurisdiction will not always be standardised using TA as the result depends on the analysis request, the number of contributors and the possibility of degradation (to enable the degradation feature). The anayst must determine all these factors using their individual laboratory guidelines. However TA does provide a significant advance towards standardisation since it would allow all laboratories to provide an LR for this mixture when under current guidelines some laboratories would provide an LR and others would not.

The number of **known** contributors to a mixture and their profiles can be specified under the analysis request. When a single known reference is assumed to have contributed to the mixture there is a considerable information gain in the order of approximately 3 log (LR) units.

9. 'BLACK BOX'

There have been suggestions that TA is 'a black box'. Currently while the mathematics for key variables such as mixture weight, amplification variance, and baseline variance have been disclosed in publications, the handling of other parameters such as stutter, relative amplification of alleles at a locus, and DNA degradation are not disclosed. This makes it difficult to determine how TA handles these issues and it has been noted that TA does not perform very well in relation to some of these on weak samples. Therefore TA has an element of the unknown and more extensive experimentation is required to establish the boundaries.

10. STANDARISATION

There is a national drive to achieve a greater degree of consistency in DNA interpretation. Currently each jurisdiction carries out DNA interpretation following internal guidelines and variations in these guidelines may result in a loss of standardisation. At the current time, there are differences in the interpretation software used, with only some laboratories having the capability to incorporate loci where stochastic effects have resulted in allelic dropout .

While a 'standard' interpretation is generally provided in relation to non- complex data, it is mixtures of a higher degree of complexity that can result in a higher variation in interpretation. The use of an expert system such as TA could provide a mechanism for a more standardised interpretation for complex mixtures. It would certainly be a significant advance towards standardisation over manual approaches. All mixtures, regardless of complexity, can be uploaded and analysed by TA which removes a human decision as to which mixtures are too complex for interpretation.

There is no expectation that identical LRs would be generated given the stochastic nature of the process and replicates should differ by small amounts. However, this study has identified outliers that can generate LRs with significant differences, and laboratory policy will need to identify and protect against outliers.

Different analyst requests in relation to the number of unknown contributors may result in variation. An untested development is to be included in a future version of TA and is expected to provide a probability as to the number of contributors.

Variation in LRs may be introduced if the data analysis stage prior to upload is inconsistent between laboratories. However with experienced analysts and data review it might be reasonable to suggest that the level of variation will be low.

11. RECOMMENDATION/FUTURE DIRECTION

TrueAllele has demonstrated an enhanced capacity for DNA interpretation reaching beyond the scope of current practice within Australian laboratories. It is considered that it would be a powerful interpretation tool, particularly for complex mixtures, increasing the information recovered from the DNA data and moving towards standardisation of DNA interpretation nationally.

To advance understanding of the system, NSW has purchased a small system with 8 parallel processing channels. This will facilitate the continuation of the validation/evaluation trial and allow a full exploration of the capabilities of TrueAllele.

While the system may not perform perfectly in every situation, it represents an opportunity for a significant move forward in respect to DNA interpretation using a continuous probabilistic model.

Document 1 - Laboratory Generated Samples For Trueallele Analysis

SINGLE SOURCE SAMPLES: (only 1:16 and 1:64 sent for TA analysis)

	dilution target	DNA concentration amped ng	study sample number
A252035		ng	
	neat	1.012	A252035
	1:4	0.253	S0085
	1:8	0.12144	S0086
	1:16	0.06072	S0087
	1:64	0.01518	S0088
A251475			
	neat	1.01625	S0089
	1:4	0.271	S0090
	1:8	0.12195	S0091
	1:16	0.060975	S0092
	1:64	0.01626	S0093
A251670			
	neat	1.05	A251670
	1:4	0.2625	S0094
	1:8	0.13125	S0095
	1:16	0.063	S0096
	1:64	0.01575	S0097
A251972			
	neat	1.088	S0098
	1:4	0.2448	S0099
	1:8	0.1224	S0100
	1:16	0.068	S0101
	1:64	0.01564	S0102
A251469			
	neat	0.915	S0103
	1:4	0.251625	S0104
	1:8	0.1281	S0105
	1:16	0.06405	S0106
	1:64	0.0160125	S0107

Each A number relates to a different person

LABORATORY GENERATED 2 PERSON MIXTURES

person X (PX)	person Y (PY)	total input amount	ratio PX :PY	TA weights	Number of shared alleles	study sample number
A251475	A252032	1ng	1:1	0.48 0.52	1	S0001
	(Aka S0082)		500pg:500pg			
			1:2	0.3 0.7		S0002
			330pg:670pg			
			1:5	0.13 0.87		S0003
			160pg:840pg			
			1:10	0.08 0.92		S0004
			90pg:910pg			
		300pg	1:1	0.44 0.56		S0005
			150pg:150pg			
			1:2	0.28 0.72		S0006
			100pg:200pg			
			1:5	0.14 0.86		S0007
			50pg:250pg			
			1:10	0.09 0.91		S0008
			30pg:270pg			
		150pg	1:1	0.49 0.51		S0021
			75pg:75pg			
			1:2	0.29 0.71		S0022
			50pg:100pg			
		75pg	1:1	0.47 0.53		S0108
			38pg:38pg			
A252034	A251670	1ng	1:1	0.43 0.57	2	S0023
			500pg:500pg			
			1:2	0.26 0.74		S0024
			330pg:670pg			
			1:5	0.11 0.89		S0025
			160pg:840pg			
			1:10	0.04 0.96		S0026
			90pg:910pg			
		300pg	1:1	0.46 0.54		S0027
			150pg:150pg			
			1:2	0.25 0.75		S0028
			100pg:200pg			
			1:5	0.18 0.82		S0029
			50pg:250pg			
			1:10	0.12 0.88		S0030
			30pg:270pg			
		150pg	1:1	0.5 0.5		S0031
			75pg:75pg			
			1:2	0.32 0.68		S0032
			50pg:100pg			
		75pg	1:1	0.34 0.66		S0109
			38pg:38pg			
A251477	A251666	1ng	1:1	0.5 0.5	3	S0033
			500pg:500pg			

person X (PX)	person Y (PY)	total input amount	ratio PX :PY	TA weights		Number of shared alleles	study sample number
			1:2	0.35	0.65		S0034
			330pg:670pg				
			1:5	0.16	0.84		S0035
			160pg:840pg				
			1:10	0.11	0.89		S0036
			90pg:910pg				
		300pg	1:1	0.49	0.51		S0037
			150pg:150pg				
			1:2	0.32	0.68		S0038
			100pg:200pg				
			1:5	0.17	0.83		S0039
			50pg:250pg				
			1:10	0.1	0.9		S0040
			30pg:270pg				
		150pg	1:1	0.47	0.53		S0041
			75pg:75pg				
			1:2	0.36	0.64		S0042
			50pg:100pg				
		75pg	1:1				S0110
			38pg:38pg				
A252033	A252032	**1ng**	1:1	0.32	0.68	**6**	S0043
			500pg:500pg				
			1:2	0.25	0.74		S0044
			330pg:670pg				
			1:5	0.18	0.82		S0045
			160pg:840pg				
			1:10	0.15	0.85		S0046
			90pg:910pg				
		300pg	1:1	0.63	0.3		S0047
			150pg:150pg				
			1:2	0.16	0.84		S0048
			100pg:200pg				
			1:5	0.09	0.91		S0049
			50pg:250pg				
			1:10	0.09	0.91		S0050
			30pg:270pg				
		150pg	1:1	0.32	0.68		S0051
			75pg:75pg				
			1:2	0.22	0.78		S0052
			50pg:100pg				
		75pg	1:1	0.43	0.57		S0111
			38pg:38pg				
A251463	A251472	**1ng**	1:1	0.37	0.63	**10**	S0053
			500pg:500pg				
			1:2	0.5	0.5		S0054
			330pg:670pg				
			1:5	0.22	0.78		S0055
			160pg:840pg				

person X (PX)	person Y (PY)	total input amount	ratio PX :PY	TA weights		Number of shared alleles	study sample number
			1:10	0.17	0.83		S0056
			90pg:910pg				
		300pg	1:1	0.45	0.55		S0057
			150pg:150pg				
			1:2	0.48	0.52		S0058
			100pg:200pg				
			1:5	0.17	0.83		S0059
			50pg:250pg				
			1:10	0.19	0.81		S0060
			30pg:270pg				
		150pg	1:1	0.51	0.49		S0061
			75pg:75pg				
			1:2	0.5	0.5		S0062
			50pg:100pg				
		75pg	1:1	0.49	0.51		S0112
			38pg:38pg				
A251973	A251474	1ng	1:1	0.5	0.5	13	S0063
			500pg:500pg				
			1:2	0.37	0.63		S0064
			330pg:670pg				
			1:5	0.17	0.83		S0065
			160pg:840pg				
			1:10	0.08	0.92		S0066
			90pg:910pg				
		300pg	1:1	0.49	0.51		S0067
			150pg:150pg				
			1:2	0.33	0.67		S0068
			100pg:200pg				
			1:5	0.11	0.89		S0069
			50pg:250pg				
			1:10	0.1	0.9		S0070
			30pg:270pg				
		150pg	1:1	0.48	0.52		S0071
			75pg:75pg				
			1:2	0.18	0.82		S0072
			50pg:100pg				
		600pg	1:1	0.49	0.51		S0073
			300pg:300pg				
			1:2	0.37	0.63		S0074
			200pg:400pg				
			1:5	0.1	0.9		S0075
			100pg:500pg				
			1:10	0.08	0.92		S0076
			60pg:540pg				
		75pg	1:1				S0113
			38pg:38pg				

Each A number relates to a different person

LABATORY GENERATED 3 PERSON MIXTURES

ratio	person X	person Y	person Z	study sample number	Maximum number of alleles
X:Y:Z	target amount	target amount	target amount		
MIX 1	A251666	A251477	A251972		6 alleles at 1 locus and 5 loci with 5 alleles
1:1:1	500pg	500pg	500pg	S0114	
1:1:1	300pg	300pg	300pg	S0115	
1:1:1	100pg	100pg	100pg	S0116	
1:1:1	50pg	50pg	50pg	S0117	
5:5:2	500pg	500pg	200pg	S0118	
2:5:5	200pg	500pg	500pg	S0119	
5:2:5	500pg	200pg	500pg	S0120	
3:1:1	600pg	200pg	200pg	S0121	
1:3:1	200pg	600pg	200pg	S0122	
1:1:3	200pg	200pg	600pg	S0123	
8:1:1	800pg	100pg	100pg	S0124	
1:8:1	100pg	800pg	100pg	S0125	
1:1:8	100pg	100pg	800pg	S0126	
18:1:1	900pg	50pg	50pg	S0127	
1:18:1	50pg	900pg	50pg	S0128	
1:1:18	50pg	50pg	900pg	S0129	
7:3:1	700pg	300pg	100pg	S0130	
3:7:1	300pg	700pg	100pg	S0131	
1:3:7	100pg	300pg	700pg	S0132	
MIX 2	A252032	A252033	A251475		6 alleles at 1 locus and 1 locus with 5
1:1:1	500pg	500pg	500pg	S0133	
1:1:1	300pg	300pg	300pg	S0134	
1:1:1	100pg	100pg	100pg	S0135	
1:1:1	50pg	50pg	50pg	S0136	
5:5:2	500pg	500pg	200pg	S0137	
2:5:5	200pg	500pg	500pg	S0138	
5:2:5	500pg	200pg	500pg	S0139	
3:1:1	600pg	200pg	200pg	S0140	
1:3:1	200pg	600pg	200pg	S0141	
1:1:3	200pg	200pg	600pg	S0142	
8:1:1	800pg	100pg	100pg	S0143	
1:8:1	100pg	800pg	100pg	S0144	
1:1:8	100pg	100pg	800pg	S0145	
18:1:1	900pg	50pg	50pg	S0146	
1:18:1	50pg	900pg	50pg	S0147	
1:1:18	50pg	50pg	900pg	S0148	
7:3:1	700pg	300pg	100pg	S0149	
3:7:1	300pg	700pg	100pg	S0150	
1:3:7	100pg	300pg	700pg	S0151	

ratio	person X	person Y	person Z	study sample number	Maximum number of alleles
X:Y:Z	target amount	target amount	target amount		
MIX 3	**A251973**	**A251474**	**A251664**		**4 or less at all loci**
1:1:1	500pg	500pg	500pg	S0152	
1:1:1	300pg	300pg	300pg	S0153	
1:1:1	100pg	100pg	100pg	S0154	
1:1:1	50pg	50pg	50pg	S0155	
5:5:2	500pg	500pg	200pg	S0156	
2:5:5	200pg	500pg	500pg	S0157	
5:2:5	500pg	200pg	500pg	S0158	
3:1:1	600pg	200pg	200pg	S0159	
1:3:1	200pg	600pg	200pg	S0160	
1:1:3	200pg	200pg	600pg	S0161	
8:1:1	800pg	100pg	100pg	S0162	
1:8:1	100pg	800pg	100pg	S0163	
1:1:8	100pg	100pg	800pg	S0164	
18:1:1	900pg	50pg	50pg	S0165	
1:18:1	50pg	900pg	50pg	S0166	
1:1:18	50pg	50pg	900pg	S0167	
7:3:1	700pg	300pg	100pg	S0168	
3:7:1	300pg	700pg	100pg	S0169	
1:3:7	100pg	300pg	700pg	S0170	
MIX 4	A251475	A252035	A251671		**5 alleles at 1 locus**
1:1:1	500pg	500pg	500pg	S0171	
1:1:1	300pg	300pg	300pg	S0172	
1:1:1	100pg	100pg	100pg	S0173	
1:1:1	50pg	50pg	50pg	S0174	
5:5:2	500pg	500pg	200pg	S0175	
2:5:5	200pg	500pg	500pg	S0176	
5:2:5	500pg	200pg	500pg	S0177	
3:1:1	600pg	200pg	200pg	S0178	
1:3:1	200pg	600pg	200pg	S0179	
1:1:3	200pg	200pg	600pg	S0180	
8:1:1	800pg	100pg	100pg	S0181	
1:8:1	100pg	800pg	100pg	S0182	
1:1:8	100pg	100pg	800pg	S0183	
18:1:1	900pg	50pg	50pg	S0184	
1:18:1	50pg	900pg	50pg	S0185	
1:1:18	50pg	50pg	900pg	S0186	
7:3:1	700pg	300pg	100pg	S0187	
3:7:1	300pg	700pg	100pg	S0188	
1:3:7	100pg	300pg	700pg	S0189	

Each A number relates to a different person

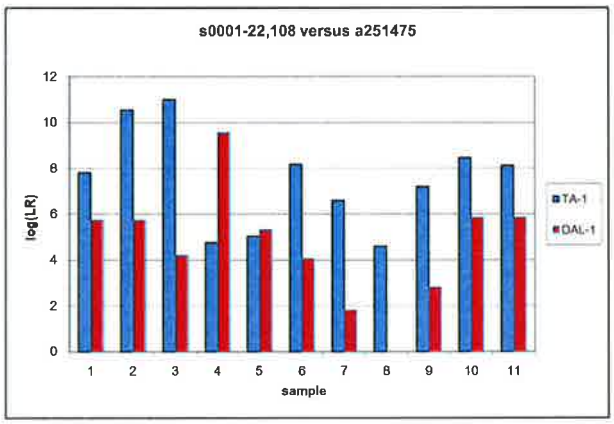
Mix 5

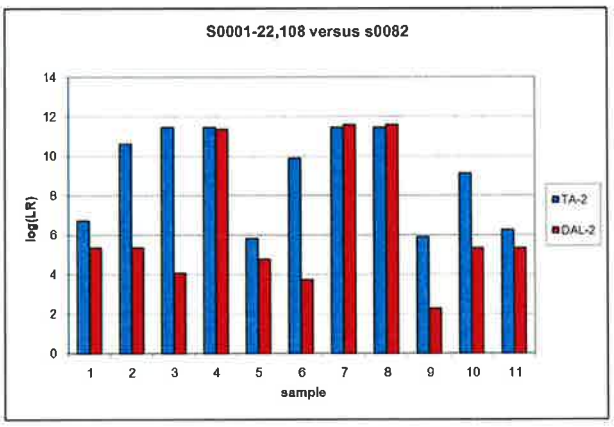
3 person mixture with one contributor's DNA degraded.

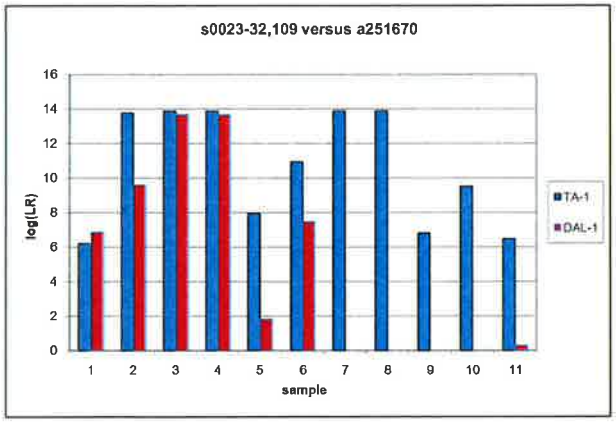
	Mix ratio S0082:A252033:A251475	Degraded contributor	Total input
S0190	1:1:1	S0082	.9ng
S0192	1:1:1	A251475	.9ng
S0193	1:1:1	S0082	.3ng
S0194	1:1:1	A252033	.3ng
S0195	1:1:1	A251475	.3ng
S0196	1:1:1	S0082	.15ng
S0197	1:1:1	A252033	.15ng
S0198	1:1:1	A251475	.15ng
S0199	5:5:2	S0082	1.2ng
S0200	1:1:3	A251475	1ng
S0201	1:8:1	A252033	1ng
S0202	18:1:1	S0082	1ng
S0203	3:7:1	A252033	1ng

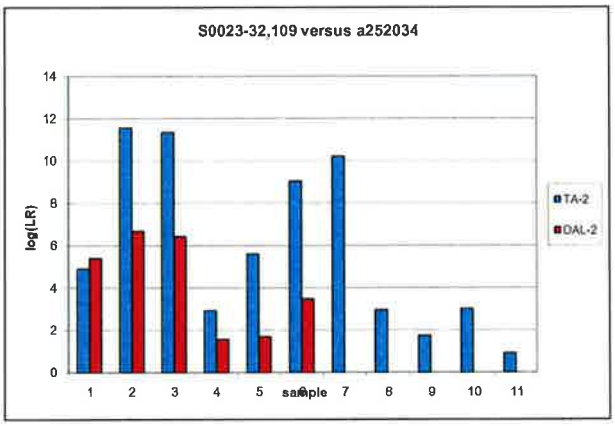
Comparison of information (= log(LR)) by different methods				
system2	FES_TA2 Number of Contributors = 2			
	log(LR)	log(LR)	DAL ref1	DAL ref2
Evidence	A251475	S0082		
S0001_2	7.823	6.745	5.748	5.371
S0002_2	10.547	10.629	5.748	5.371
S0003_2	11.011	11.474	4.207	4.088
S0004_2	4.764	11.474	9.562	11.373
S0005_2	5.044	5.829	5.324	4.785
S0006_2	8.175	9.909	4.049	3.736
S0007_2	6.603	11.474	1.813	11.595
S0008_2	4.6	11.474		11.595
S0021_2	7.207	5.909	2.809	2.279
S0022_2	8.477	9.132	5.859	5.342
S0108_2	8.119	6.265	5.859	5.342
Evidence	A251670	A252034		
S0023_2	6.224	4.911	6.862	5.409
S0024_2	13.773	11.571	9.609	6.697
S0025_2	13.892	11.357	13.686	6.428
S0026_2	13.892	2.937	13.686	1.568
S0027_2	7.954	5.602	1.836	1.689
S0028_2	10.943	9.04	7.486	3.49
S0029_2	13.886	10.225		
S0030_2	13.892	2.975		
S0031_2	6.814	1.746		
S0032_2	9.522	3.027		
S0109_2	6.49	0.922	0.301	
Evidence	A251666	A251477		
S0033_2	7.331	5.957	6.889	5.584
S0034_2	12.696	11.563	9.04	7.911
S0035_2	12.737	9.769	12.863	11.456
S0036_2	12.725	4.899	12.863	9.555
S0037_2	6.379	4.98	6.889	5.584
S0038_2	12.566	11.493	6.889	5.584
S0039_2	12.738	10.575	12.863	9.955
S0040_2	12.734	6.825	12.863	7.726
S0041_2	5.147	3.864	6.889	5.584
S0042_2	6.505	5.601	6.889	5.584
S0110_2	0.134	5.378	1.89	0.954
Evidence	S0082	A252033		
S0043_2	7.372	3.492	6.126	6.273
S0044_2	7.916	0.145	6.126	6.273
S0045_2	6.963	-0.727	10.729	4.215
S0046_2	4.219	-2.493	11.595	3.64
S0047_2	9.021	8.484	5.529	5.675
S0048_2	11.474	10.054	11.595	4.783

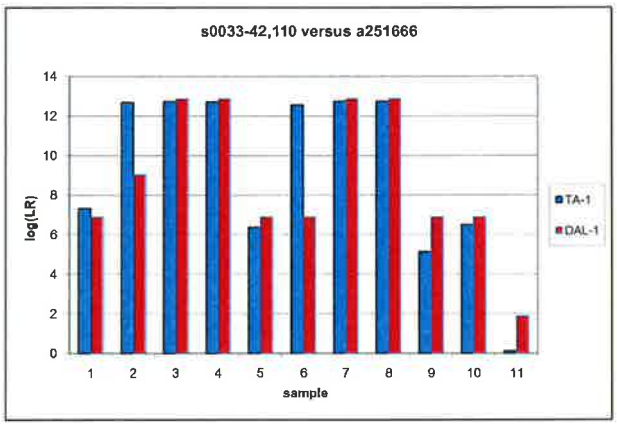
	log(LR)	log(LR)	DAL ref1	DAL ref2
S0049_2	11.474	6.992	11.595	3.942
S0050_2	10.097	0.993	11.595	4.672
S0051_2	10.253	9.39	5.529	5.675
S0052_2	11.469	10.335	11.595	6.442
S0111_2	3.66	2.211	3.644	3.483
Evidence	A251472	A251463		
S0053_2	10.213	9.545	6.58	5.967
S0054_2	7.373	6.225	6.58	5.967
S0055_2	10.945	7.197	10.997	5.967
S0056_2	10.945	8.586	6.07	5.133
S0057_2	8.199	7.163	6.58	5.967
S0058_2	7.975	6.64	6.58	5.967
S0059_2	10.809	5.621	10.253	3.401
S0060_2	10.938	2.58	10.901	3.032
S0061_2	7.285	6.206	6.58	6.346
S0062_2	5.098	3.956	6.046	5.758
S0112_2	-0.49	1.559		
Evidence	A251474	A251973		
S0063_2	10.566	8.727	8.484	7.981
S0064_2	12.72	11.89	8.484	7.981
S0065_2	12.808	7.904	12.103	6.846
S0066_2	12.81	2.074	12.103	2.13
S0067_2	10.718	8.796	8.484	7.981
S0068_2	12.359	10.364	7.348	6.846
S0069_2	12.81	-1.68	12.103	0.903
S0070_2	12.81	-1.94	12.103	
S0071_2	10.123	8.125	6.944	6.444
S0072_2	12.603	5.251	9.585	0
S0073_2	10.624	8.71	7.902	7.399
S0074_2	12.482	11.435	7.902	7.399
S0075_2	12.81	0.541	12.103	1.826
S0076_2	12.81	-2.19	12.103	0.903
S0113_2				
	log(LR)		DAL ref	
Evidence				
S0086_1	12.178		9.525	
S0088_1	7.494		0.602	
S0092_1	12.185		8.575	
S0093_1	5.417		0.845	
S0096_1	13.508		11.979	
S0097_1	5.517		3.498	
S0101_1	11.53		11.67	
S0102_1	2.763		1.613	
S0106_1	11.716		11.946	
S0107_1	5.04		7.216	

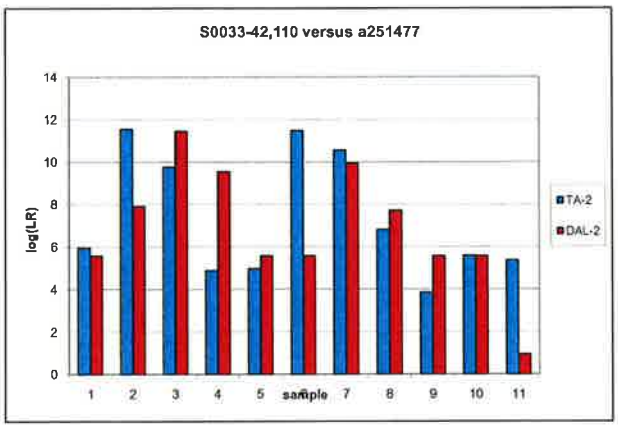

s0001-22,108 versus a251475
log(LR)
sample
TA-1
DAL-1

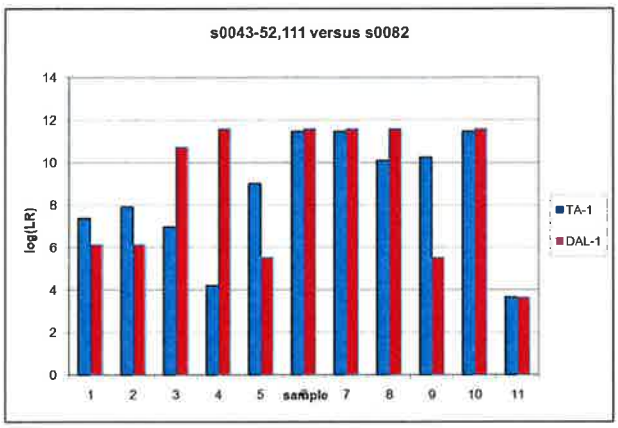

S0001-22,108 versus s0082
log(LR)
sample
TA-2
DAL-2

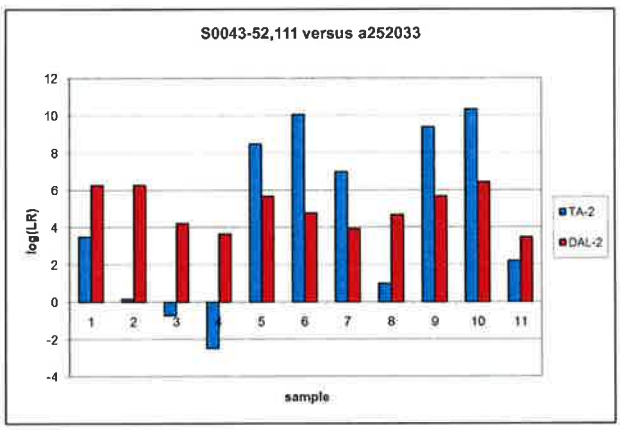

s0023-32,109 versus a251670
log(LR)
sample
TA-1
DAL-1

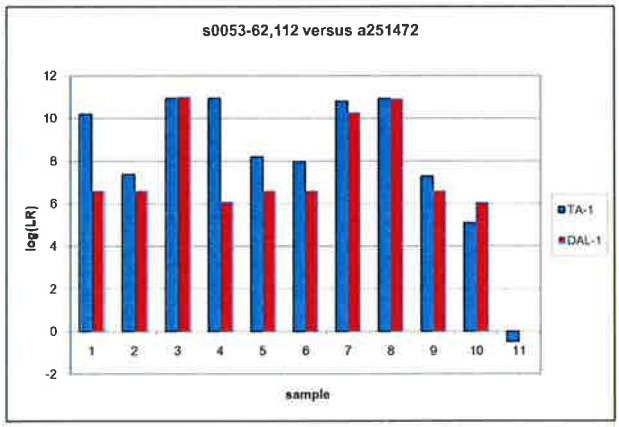

S0023-32,109 versus a252034
log(LR)
sample
TA-2
DAL-2

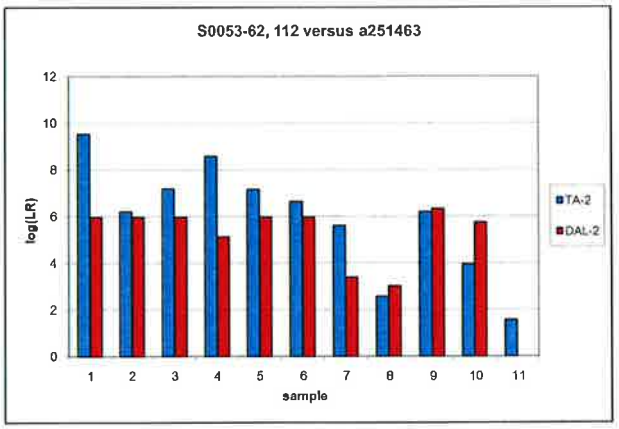

s0033-42,110 versus a251666
log(LR)
sample
TA-1
DAL-1

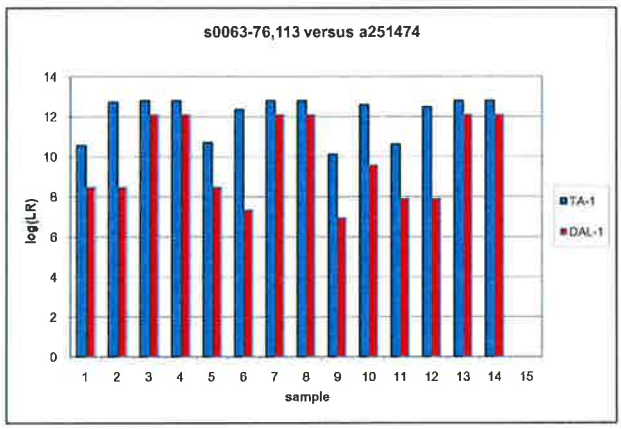

S0033-42,110 versus a251477
log(LR)
sample
TA-2
DAL-2

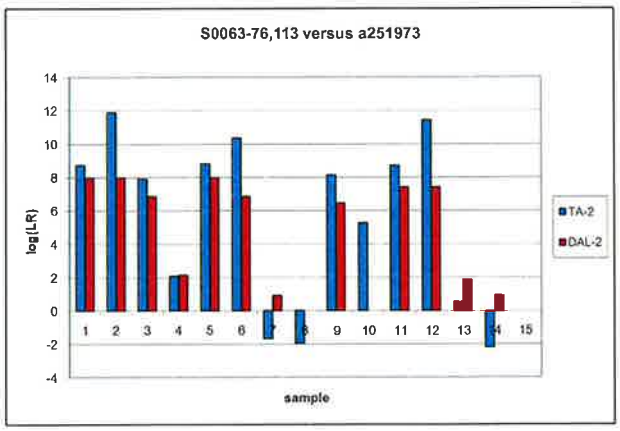

s0043-52,111 versus s0082
log(LR)
sample
TA-1
DAL-1

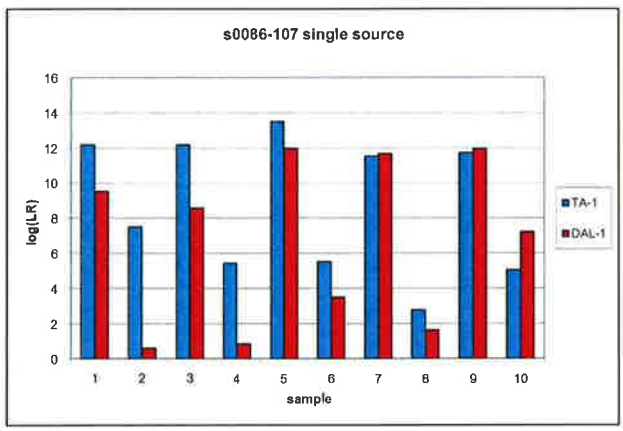

S0043-52,111 versus a252033
log(LR)
sample
TA-2
DAL-2


s0053-62,112 versus a251472
log(LR)
sample
TA-1
DAL-1

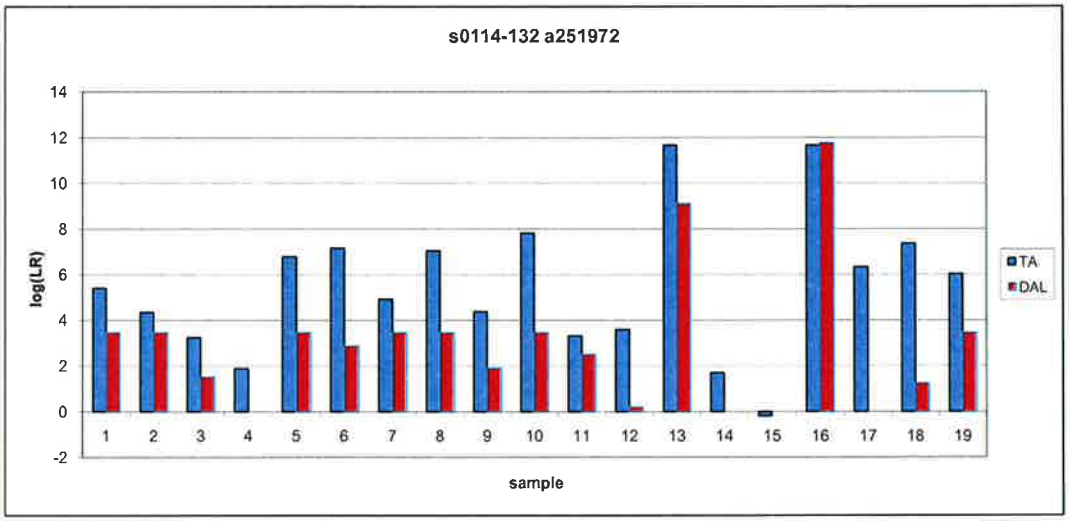

S0053-62, 112 versus a251463
log(LR)
sample
TA-2
DAL-2

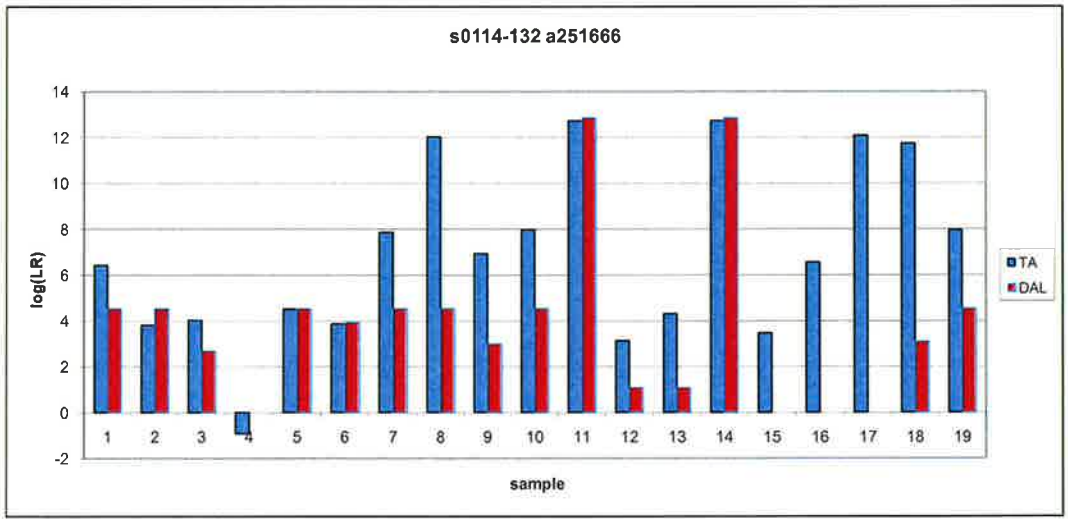

s0063-76,113 versus a251474
log(LR)
sample
TA-1
DAL-1

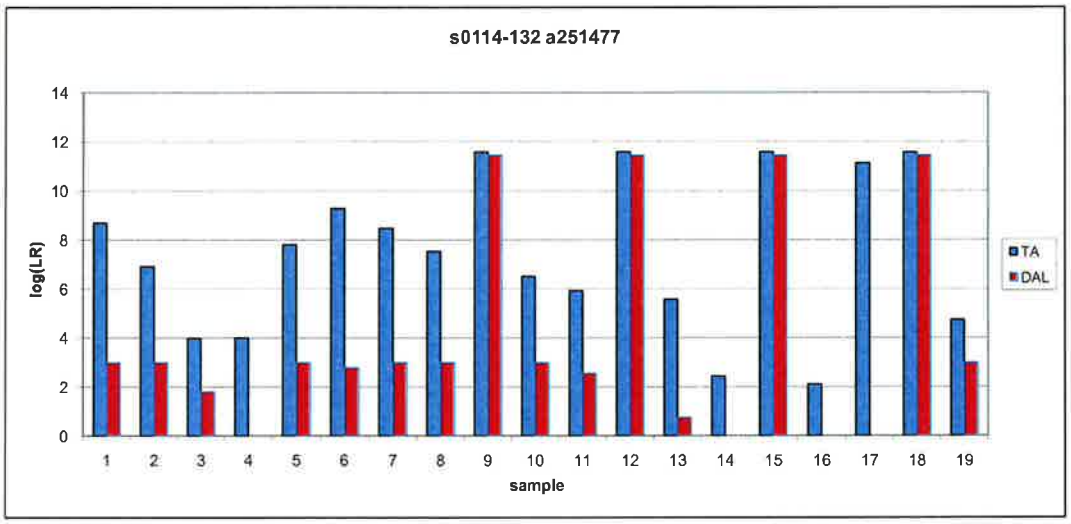

S0063-76,113 versus a251973
log(LR)
sample
TA-2
DAL-2


s0086-107 single source
log(LR)
sample
TA-1
DAL-1

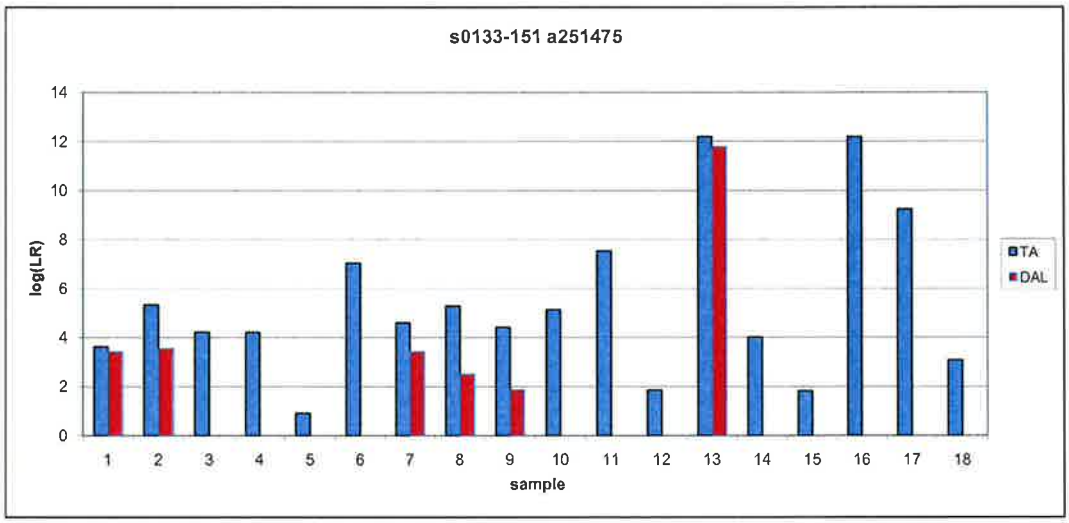
system2	FES_TA2	1% theta				
		TrueAllele			DAL	
Evidence	A251972	A251666	A251477	A251972	A251666	A251477
S0114_3	5.411	6.431	8.693	3.458	4.534	2.994
S0115_3	4.353	3.808	6.921	3.458	4.534	2.994
S0116_3	3.242	4.03	3.994	1.519	2.684	1.796
S0117_3	1.89	-0.911	4.012			
S0118_3	6.783	4.52	7.801	3.458	4.534	2.994
S0119_3	7.163	3.865	9.274	2.861	3.923	2.776
S0120_3	4.926	7.872	8.474	3.458	4.534	2.994
S0121_3	7.05	12.038	7.531	3.458	4.534	2.994
S0122_3	4.372	6.93	11.584	1.903	2.994	11.456
S0123_3	7.815	7.978	6.511	3.458	4.534	2.994
S0124_3	3.314	12.737	5.927	2.501	12.863	2.55
S0125_3	3.591	3.133	11.584	0.197	1.082	11.456
S0126_3	11.66	4.313	5.567	9.111	1.082	0.745
S0127_3	1.709	12.738	2.426		12.863	
S0128_3	-0.185	3.466	11.584			11.456
S0129_3	11.669	6.554	2.095	11.768		
S0130_3	6.34	12.085	11.131			
S0131_3	7.36	11.749	11.579	1.242	3.081	11.456
S0132_3	6.028	7.967	4.739	3.458	4.534	2.994

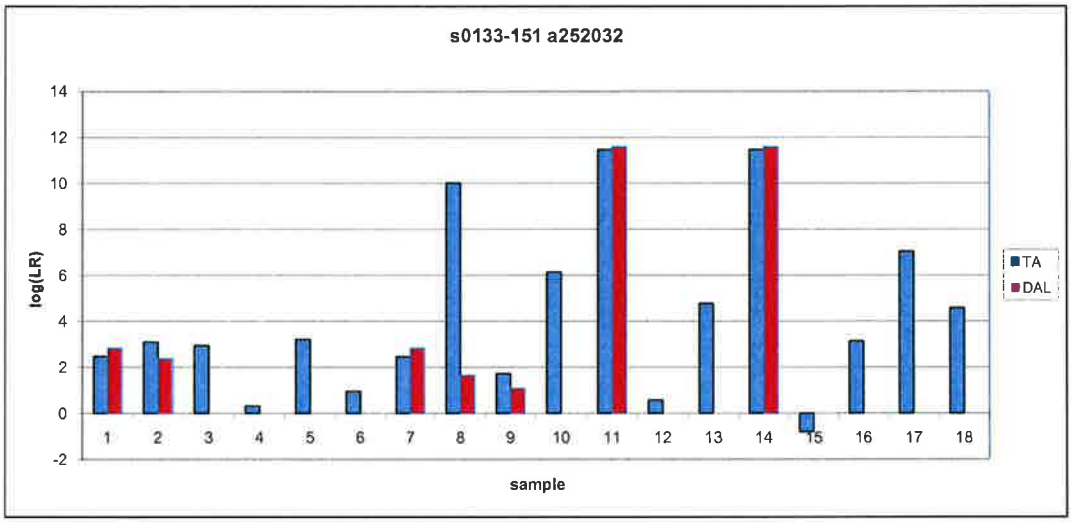

s0114-132 a251972
log(LR)
sample
TA
DAL

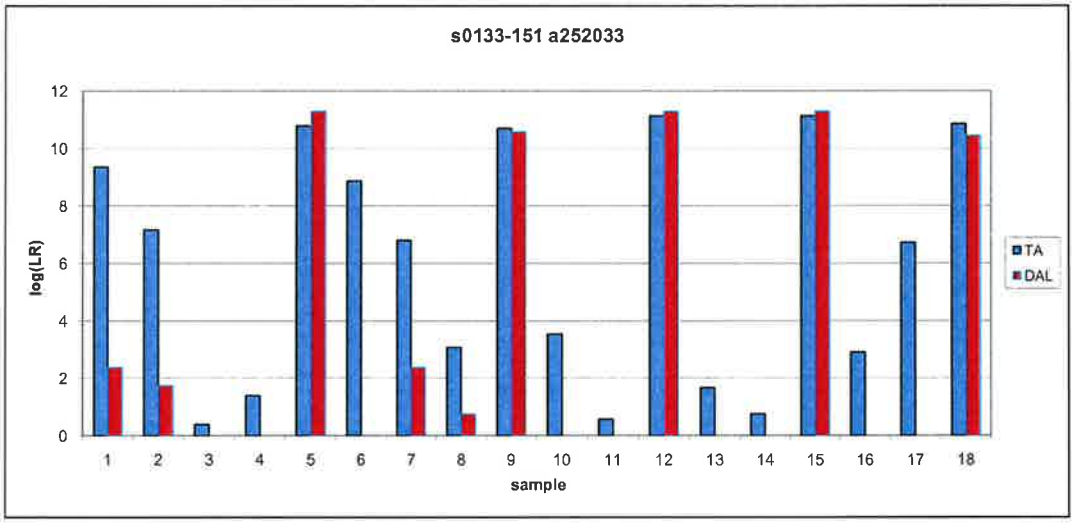

s0114-132 a251666
log(LR)
sample
TA
DAL


s0114-132 a251477
log(LR)
sample
TA
DAL

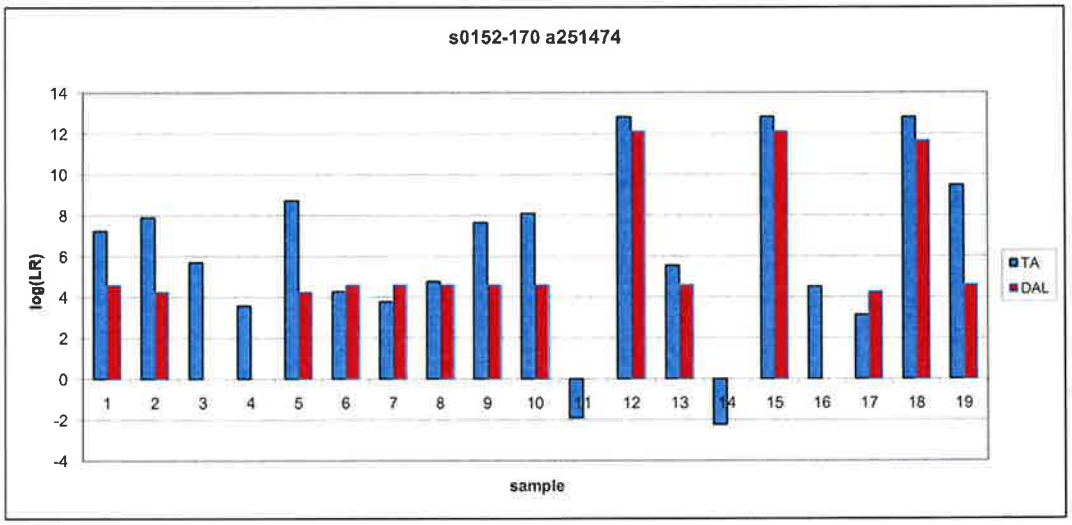
system2	FES_TA2	1% theta				
Evidence	A251475	S0082	A252033	A251475	S0082	A252033
S0133_3	3.625	2.477	9.361	3.419	2.835	2.375
S0134_3	5.336	3.101	7.171	3.54	2.372	1.738
S0135_3	4.229	2.938	0.388			
S0136_3	4.207	0.324	1.387			
S0137_3	0.906	3.206	10.79			11.283
S0138_3	7.037	0.963	8.869			
S0139_3	4.601	2.462	6.807	3.419	2.835	2.375
S0140_3	5.276	10.013	3.076	2.506	1.653	0.75
S0141_3	4.418	1.719	10.693	1.86	1.079	10.57
S0142_3	5.135	6.139	3.538			
S0143_3	7.537	11.473	0.569		11.595	
S0144_3	1.855	0.569	11.133			11.283
S0145_3	12.197	4.771	1.671	11.782		
S0146_3	4.01	11.474	0.759		11.595	
S0147_3	1.828	-0.81	11.133			11.283
S0148_3	12.199	3.132	2.906			
S0149_3	9.242	7.039	6.72			
S0150_3	3.074	4.587	10.849			10.444

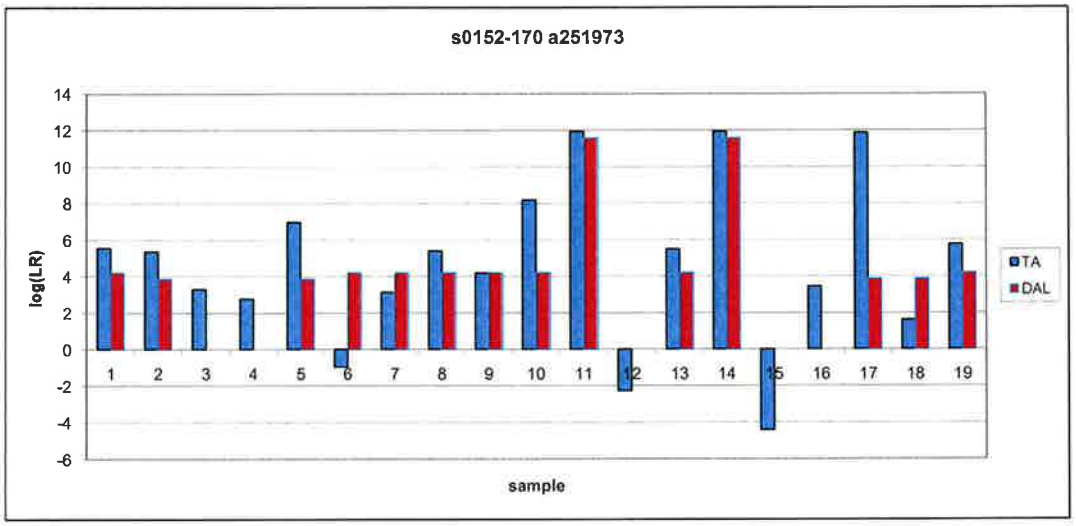

s0133-151 a251475
log(LR)
sample
TA
DAL

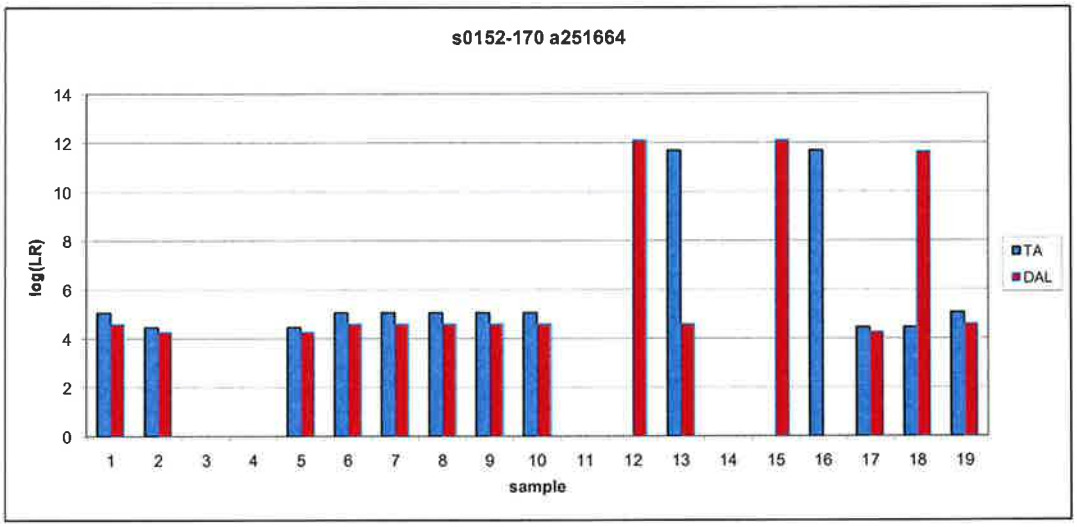

s0133-151 a252032
log(LR)
sample
TA
DAL


s0133-151 a252033
log(LR)
sample
TA
DAL

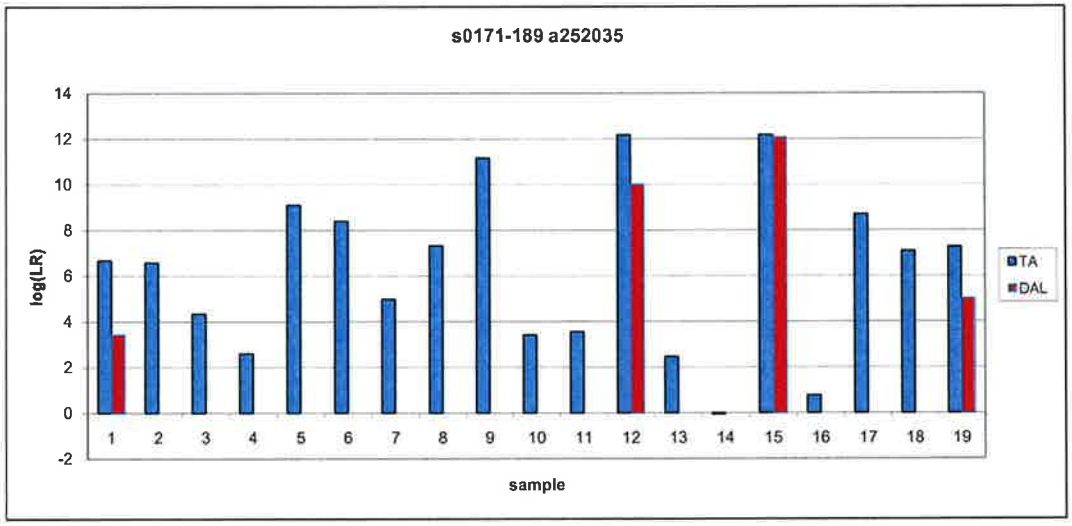
system2	FES_TA2	1% theta				
		TrueAllele			DAL	
Evidence	A251474	A251973	A251664	A251474	A251973	A251664
S0152_3	7.238	5.557	7.655	4.588	4.209	5.06
S0153_3	7.892	5.353	3.629	4.255	3.877	4.459
S0154_3	5.7	3.294	4.626			
S0155_3	3.582	2.761	5.16			
S0156_3	8.711	6.958	4.343	4.255	3.877	4.459
S0157_3	4.263	-0.956	5.461	4.588	4.209	5.06
S0158_3	3.76	3.136	5.13	4.588	4.209	5.06
S0159_3	4.766	5.395	1.816	4.588	4.209	5.06
S0160_3	7.636	4.176	3.393	4.588	4.209	5.06
S0161_3	8.086	8.175	10.658	4.588	4.209	5.06
S0162_3	-1.907	11.943	5.138		11.587	
S0163_3	12.809	-2.279	6.066	12.103		
S0164_3	5.54	5.49	11.568	4.588	4.209	11.683
S0165_3	-2.234	11.942	5.753		11.587	
S0166_3	12.81	-4.403	3.853	12.103		
S0167_3	4.515	3.441	11.75			11.683
S0168_3	3.129	11.866	4.792	4.255	3.877	4.459
S0169_3	12.803	1.612	3.419	11.637	3.877	4.459
S0170_3	9.474	5.764	11.713	4.588	4.209	5.06

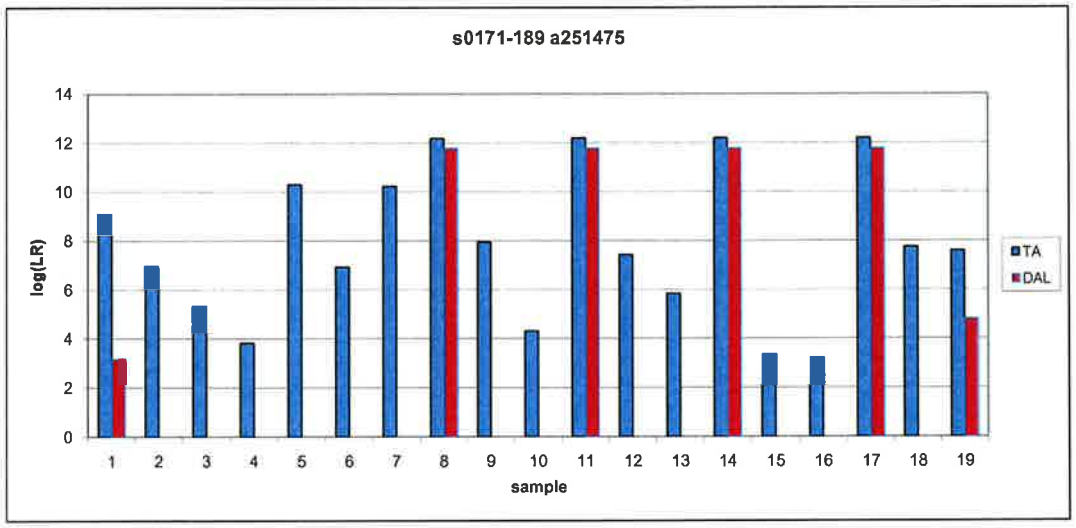

s0152-170 a251474
log(LR)
sample
TA
DAL

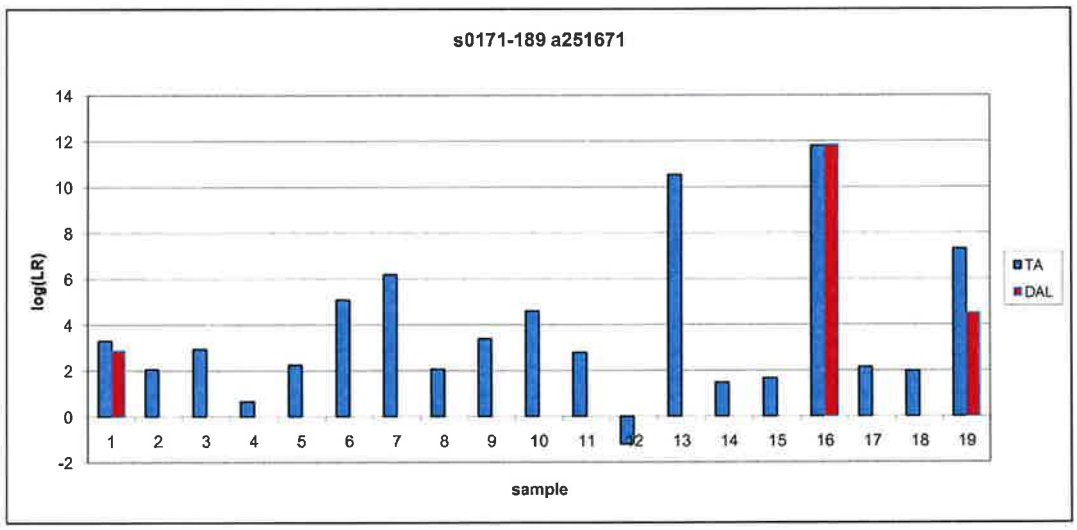

s0152-170 a251973
log(LR)
sample
TA
DAL


s0152-170 a251664
log(LR)
sample
TA
DAL

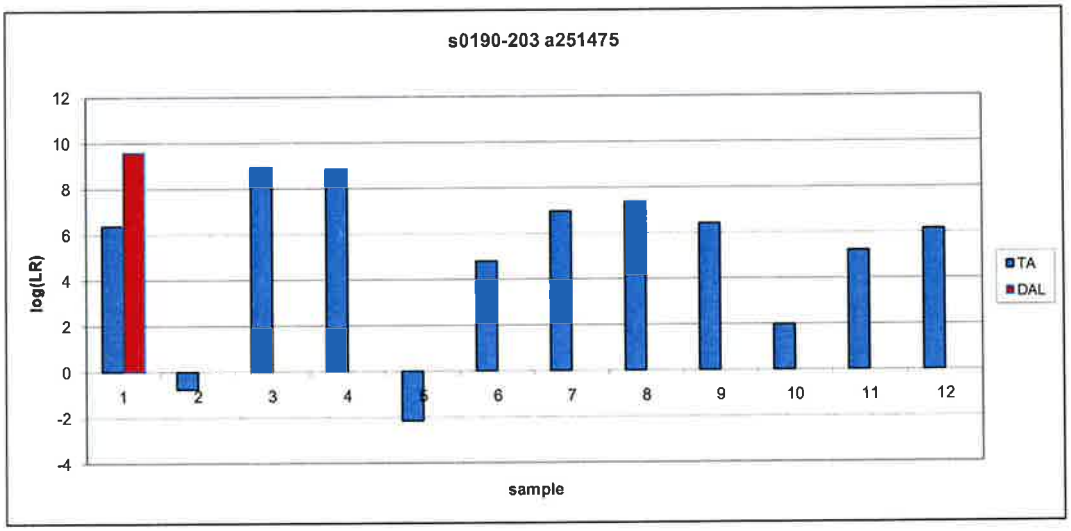
system2	FES_TA2	1% theta				
		TrueAllele			DAL	
Evidence	A252035	A251475	A251671	A252035	A251475	A251671
S0171_3	6.68	9.057	3.31	3.447	3.154	2.872
S0172_3	6.593	6.952	2.049			
S0173_3	4.347	5.355	2.954			
S0174_3	2.6	3.829	0.659			
S0175_3	9.105	10.301	2.248			
S0176_3	8.409	6.93	5.091			
S0177_3	4.975	10.228	6.193			
S0178_3	7.326	12.186	2.064		11.782	
S0179_3	11.155	7.943	3.39			
S0180_3	3.408	4.307	4.605			
S0181_3	3.551	12.199	2.79		11.782	
S0182_3	12.18	7.428	-1.209	10.009		
S0183_3	2.466	5.847	10.544			
S0184_3	-0.056	12.199	1.48		11.782	
S0185_3	12.18	3.364	1.659	12.076		
S0186_3	0.764	3.223	11.804			11.835
S0187_3	8.696	12.197	2.148		11.782	
S0188_3	7.084	7.747	1.983			
S0189_3	7.269	7.599	7.307	5.008	4.811	4.502

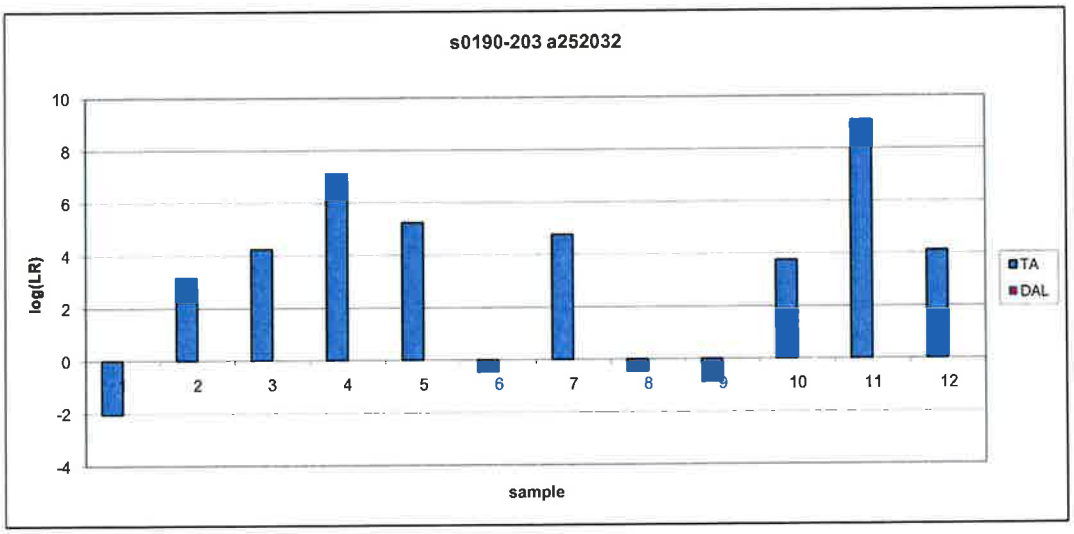

s0171-189 a252035
log(LR)
sample
TA
DAL

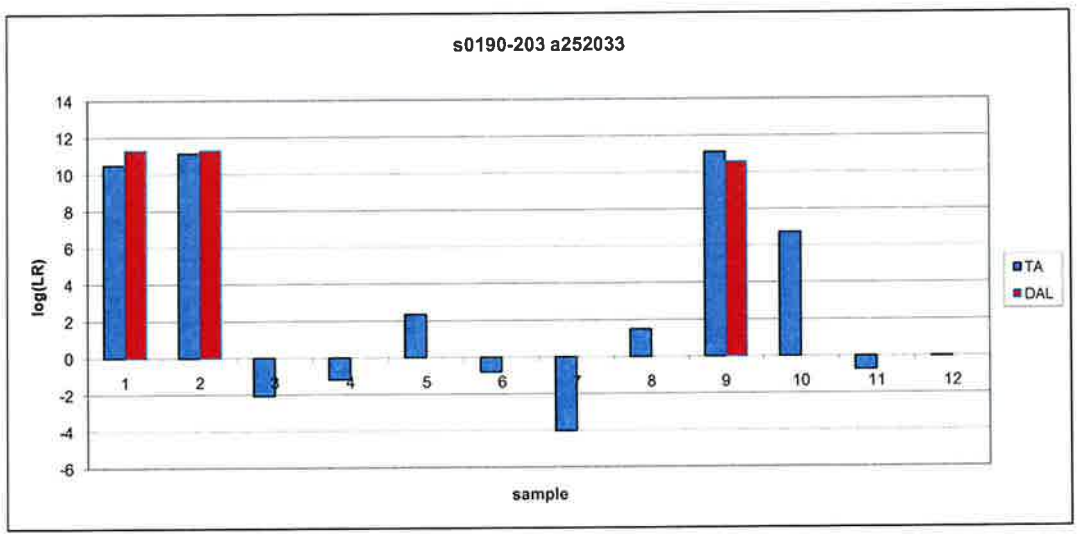

s0171-189 a251475
log(LR)
sample
TA
DAL


s0171-189 a251671
log(LR)
sample
TA
DAL

system2	FES_TA2	1% theta				
		TrueAllele			DAL	
Evidence	A251475	S0082	A252033	A251475	S0082	A252033
S0190_3	6.383	-2.038	10.495	9.592		11.283
S0192_3	-0.761	3.162	11.132			11.283
S0193_3	8.937	4.245	-2.066			
S0194_3	8.85	7.139	-1.191			
S0195_3	-2.163	5.247	2.336			
S0196_3	4.801	-0.473	-0.81			
S0197_3	6.96	4.774	-4.021			
S0198_3	7.375	-0.476	1.488			
S0199_3	6.421	-0.868	11.097			10.57
S0200_3	1.982	3.766	6.73			
S0202_3	5.214	9.099	-0.741			
S0203_3	6.154	4.114	-0.047			


s0190-203 a251475
log(LR)
sample
TA
DAL


s0190-203 a252032
log(LR)
sample
TA
DAL


s0190-203 a252033
log(LR)
sample
TA
DAL

NSW TrueAllele FES Validation Study
Cybergenetics
6 March 2011 - UPDATE

Summary

This update document describes seven validation components explored by Cybergenetics in the NSW TrueAllele FES validation study using the DNA likelihood ratio (LR) as a standard information measure.

For the first six validation components, we examined TrueAllele® efficacy and reproducibility using the additive log(LR) measure. These features were quantified by mean and within-group standard deviation [1] of the positive log(LR) match values. We provide a bar chart for each component that shows the duplicated inferred genotype results, with the matches sorted by descending information.

With mixture items, all positive match results (major, minor, other) are reported. Therefore, there are more matches than there are items.

The seventh component ("Relatives") examines negative TrueAllele match results. A bar chart shows the log(LR) match results, with the matches sorted by descending information.

All LR DNA match statistics are reported at a 1% theta value, to account for co-ancestry.

Study Components

Two contributor mixtures
Three contributor mixtures
Three contributor mixtures with one degraded contributor
Joint amplifications
Joint items
Using a known reference
Relatives

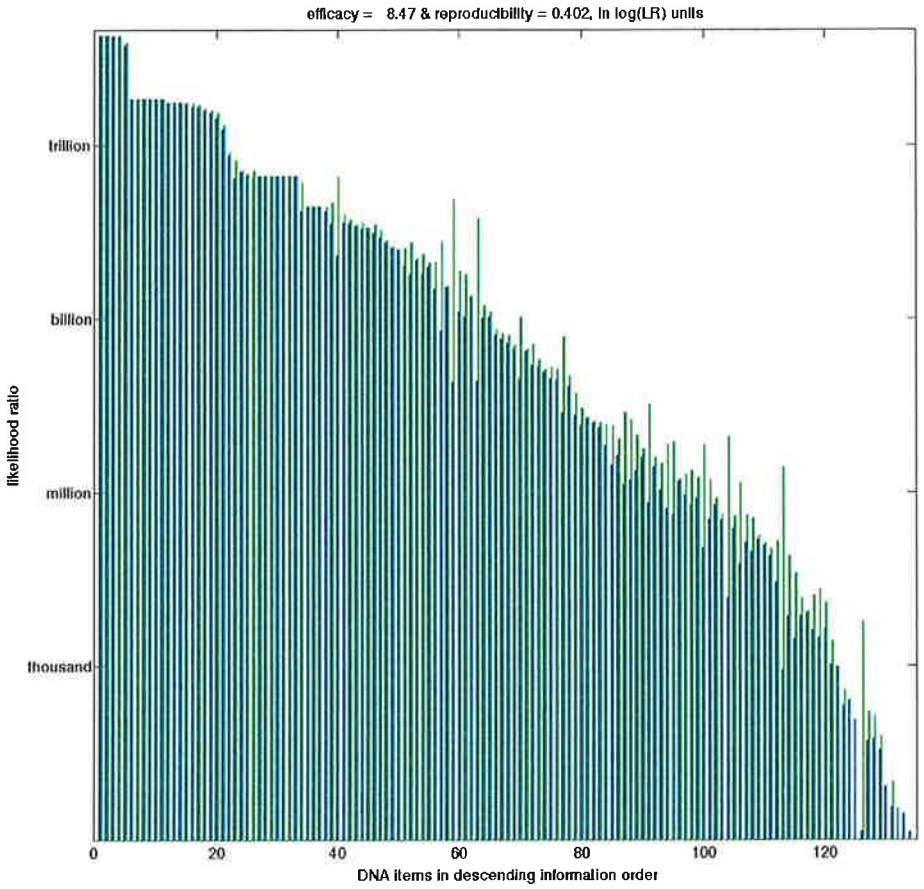
References

[1] Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences*. 2011;56(November):in press.
(Available at: http://www.cybgen.com/information/publications.shtml)

Two contributor mixtures

We analyzed and interpreted apparent two person mixtures in the TrueAllele system in duplicate as "two unknown" genotype requests. The replicated LR results were previously provided in spreadsheet "2unk_results.xls".

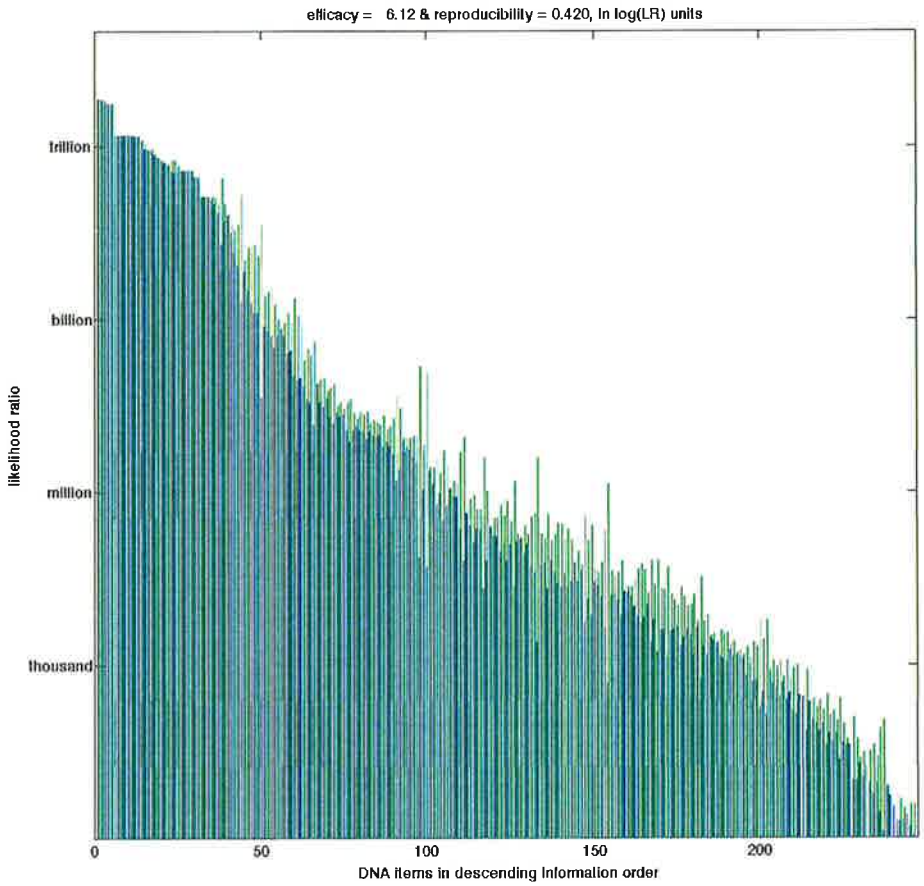
In additive log(LR) units, the information efficacy was 8.47, and the reproducibility was 0.402. In multiplicative LR units, these numbers correspond to factors of 292 million (efficacy) and 2.53 (reproducibility).



Three contributor mixtures

We analyzed and interpreted apparent three person mixtures in the TrueAllele system in duplicate as "three unknown" genotype requests. The replicated LR results were provided in spreadsheet "3unk_results.xls".

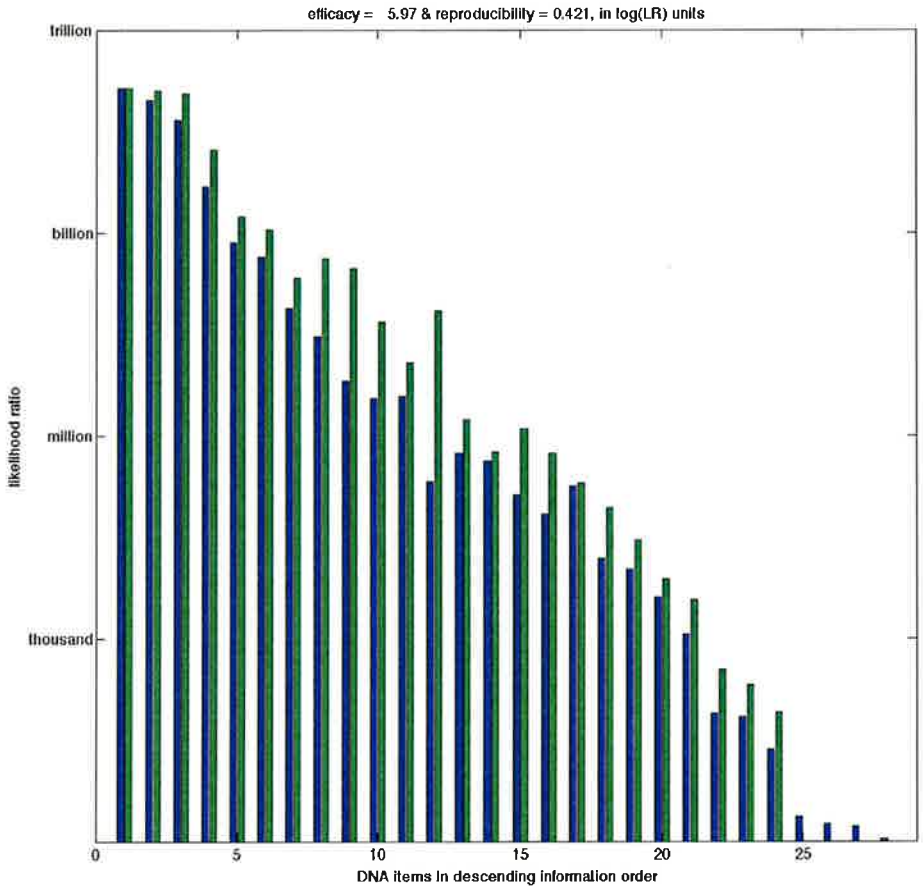
In additive log(LR) units, the information efficacy was 6.12, and the reproducibility was 0.420. In multiplicative LR units, these numbers correspond to factors of 1.31 million (efficacy) and 2.63 (reproducibility).



Three contributor mixtures with one degraded contributor

We analyzed the three contributor mixture data a second time on items that appeared to contain degraded DNA. We had the TrueAllele system rerun items 190-203 twice again, this time with the degraded feature turned on. The replicated LR results were reported in "3unk_dgrd_results.xls", showing some information gain when modeling degraded DNA.

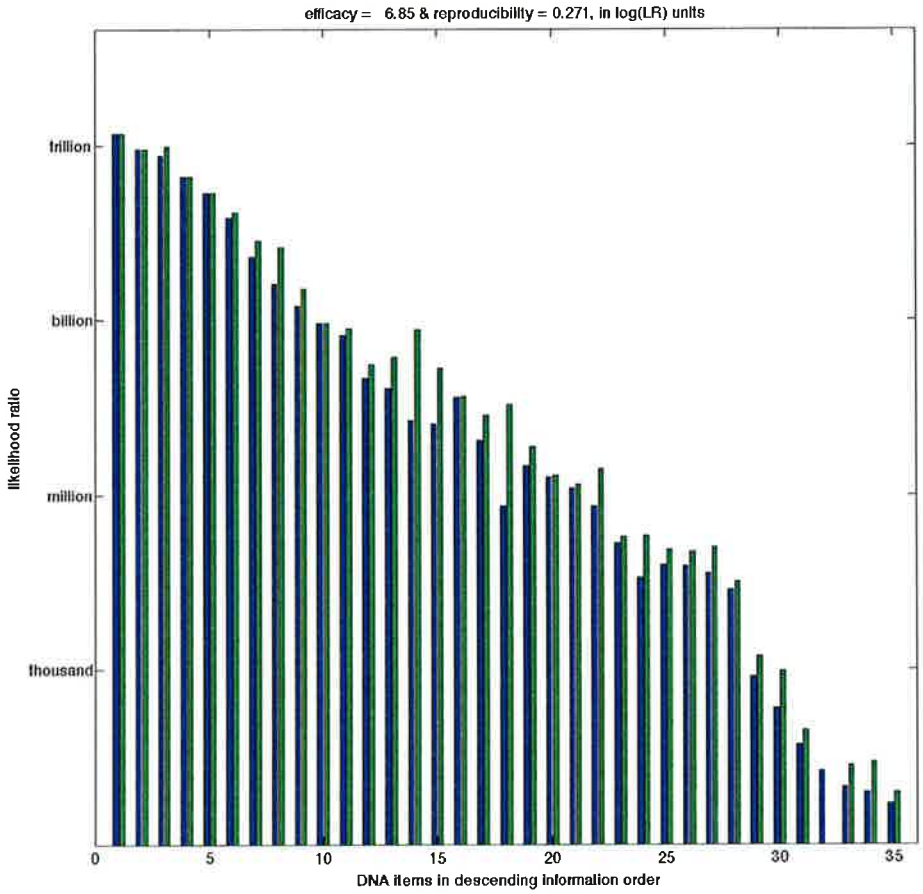
In additive log(LR) units, the information efficacy on this degraded subset was 5.97, and the reproducibility was 0.421. In multiplicative LR units, these numbers correspond to factors of 934 thousand (efficacy) and 2.64 (reproducibility).



Joint amplifications

Fifteen duplicate amplifications of validation samples were run (a) individually and (b) jointly using amplifications from the same sample. The replicated LR results were provided in spreadsheet "joint_amp_results.xls".

In additive log(LR) units, the information efficacy was 6.85, and the reproducibility was 0.271. In multiplicative LR units, these numbers correspond to factors of 7 million (efficacy) and 1.86 (reproducibility).

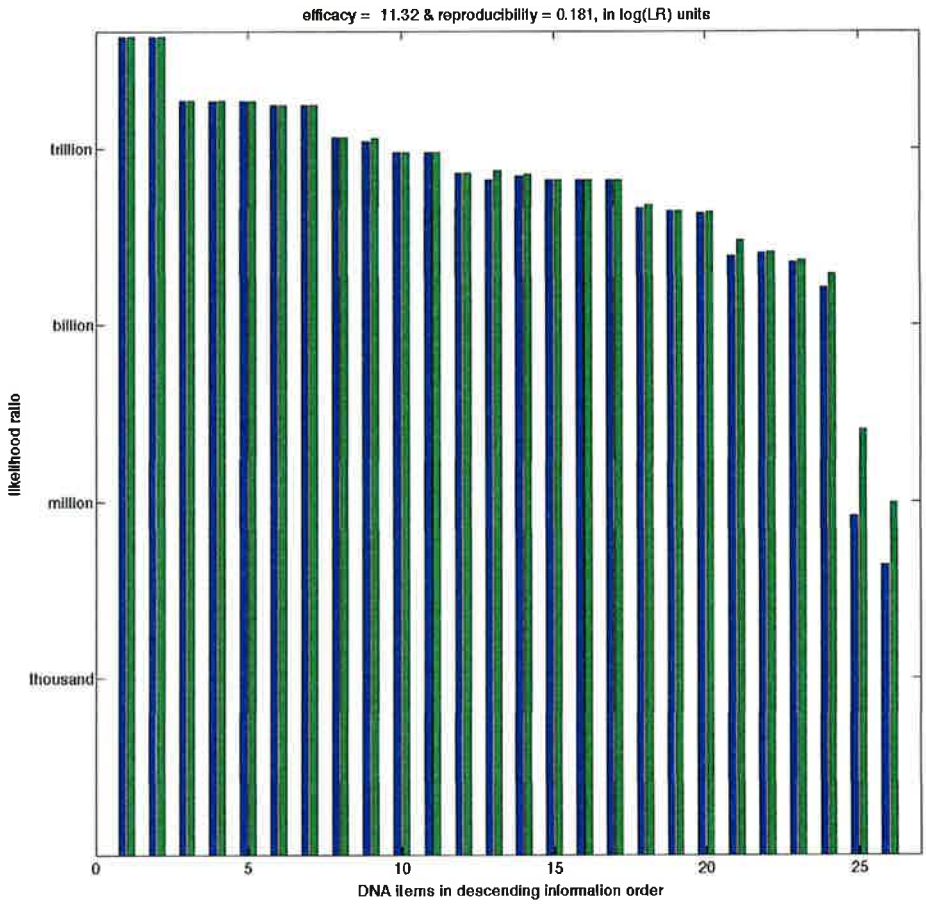


Joint items

Thirteen two unknown requests were run in TrueAllele on multiple mixture items. The goal was to determine whether joint computer interpretation of two different evidence items could extract more identification information than separate interpretations of the same items. We chose items that had (a) the same contributors, but (b) dissimilar mixture weights (for example, combining a 90:10 mixture with a 50:50 mixture).

The replicated LR results provided in "joint_item_results.xls" showed that combining evidence items can increase both information yield and reproducibility.

In additive log(LR) units, the information efficacy was 11.32, and the reproducibility was 0.181. In multiplicative LR units, these numbers correspond to factors of 207 billion (efficacy) and 1.52 (reproducibility).

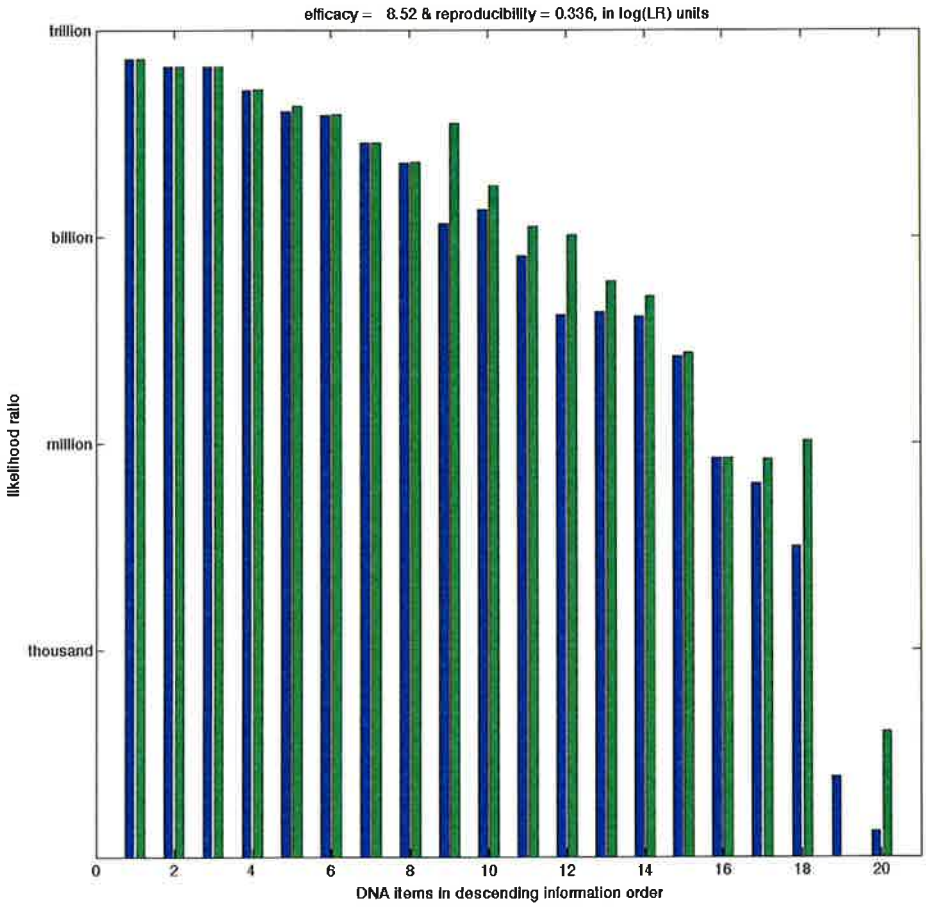


Using a known reference

There were twelve requests that had either a very minor contributor or were a 50:50 mixture. We reran these items in TrueAllele using a known "victim" reference. The duplicate LR results were given in spreadsheet "mixture+vic_results.xls".

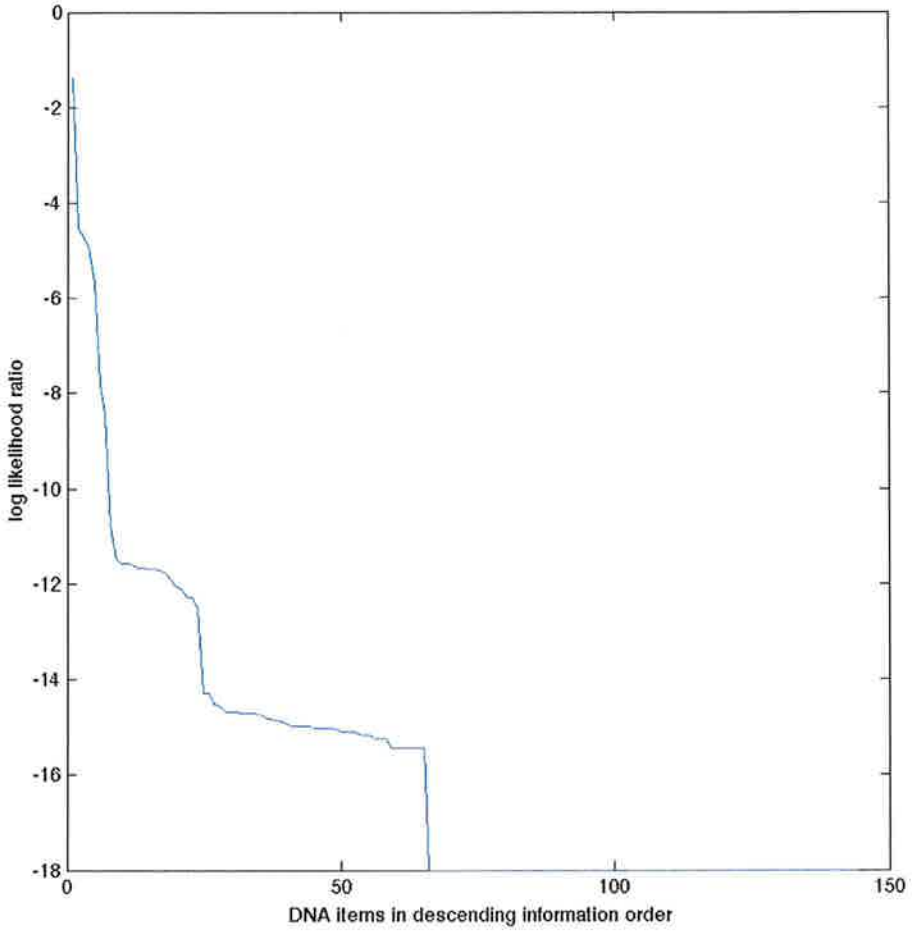
These computer experiments demonstrated that TrueAllele can extract more identification information from an evidence item when using a victim reference.

In additive log(LR) units, the information efficacy was 8.52, and the reproducibility was 0.336. In multiplicative LR units, these numbers correspond to factors of 335 million (efficacy) and 2.17 (reproducibility).



Relatives – Nine relatives of the mixture contributors were analyzed as reference samples and compared to the validation mixture samples. The negative log(LR) match results were reported in spreadsheet "match_vs_relatives.xls."

LR-based pairwise comparisons of mixture genotypes with relatives did not produce any spurious "partial match" with a positive match score. The average log(LR) score was -15.79, which is less than one in a quadrillion.



Document 4 – Mathematical Model

TrueAllele (TA) uses vectors to describe its variables and the key random variables inferred by TA are Q, $\mathbf{w}$, σ^2 and τ^2. The following definitions are used

Q represents a genotype for a contributor
w is the global template mixture weight vector
σ^2 is the global peak height variance inferred over all loci
τ^2 is the global baseline variance inferred over all loci

ψ^2 is the global locus weight variance used to select a trial locus weight from the global weight w
$\mathbf{w}_l$ is a vector of proposed contributor weights at locus l given current values for w and ψ^2
m_l is the proposed total peak height at locus l for the current MCMC cycle
$\mathbf{d}_l$ is a vector that represents the observed peak heights at locus l
$\mathbf{d}_{l,n}$ is the n^{th} observed peak height at locus l in vector $\mathbf{d}_l$
$\mathbf{g}_{k,l}$ is an indicator vector for the genotype of the k^{th} contributor at locus l
μ_l is the proposed vector of peak heights at locus l given current values for m_l, $w_{k,l}$ and $g_{k,l}$

Genotype inference

The probability distribution of any random variable can be determined by Bayes theorem, which decomposes the calculation into a *prior* probability and a *likelihood* function. At each locus l, there is a fixed, finite set of possible genotypes X and a set of observed peak heights $\mathbf{d}_l$. Suppose that Q is a questioned genotype of one of the (1, 2, 3 or more) contributors to DNA mixture evidence.

The prior genotype probability $\Pr\{Q = x\}$ is our belief that questioned genotype Q has genotype x in set X before we examine the evidence data. TrueAllele sets the prior for genotype x to the genotype frequency determined by the product rule on an internal population database.

The likelihood function assesses how well a genotype candidate explains the observed data. The likelihood is larger when the peak heights are better accounted for by the peak height pattern predicted for the genotype. For the n^{th} peak height observation $d_{l,n}$ at locus l, the likelihood function for genotype Q is the probability $\Pr\{d_{l,n} \mid Q = x,...\}$ of the data conditioned on genotype value x, where "..." denotes the other model variable values (including genotypes for the other contributors).

Combining the prior genotype probability together with the N independent locus peak height likelihoods, the posterior genotype probability is computed using Bayes theorem as the product of prior probability and joint likelihood

$$(1) \qquad \Pr\{Q = x \mid d_{l,1}, d_{l,2}, ..., d_{l,n}, ...\} \propto \Pr\{Q = x\} \cdot \prod_{n=1}^{N} \Pr\{d_{l,n} \mid Q = x, ...\}$$

The posterior for a genotype at a locus depends on the peaks at the locus and the locus mixture weight.

The proportionality "$\propto$" indicates that the product is normalized by dividing by the total probability $\sum_{x \in X} \Pr\{Q = x\} \cdot \prod_{n=1}^{N} \Pr\{d_{l,n} \mid Q = x, ...\}$, after considering all possible genotypes $x \in X$, in order to produce a genotype probability distribution that adds up to one.

TrueAllele models the quantitative data at STR locus l (of L loci) using several variables and describes these as vectors where appropriate. The observed quantitative peak heights at locus l are held in data vector $\mathbf{d}_l$ and the individual peaks are addressed as $d_{l,i}$. With K contributors to the data, we represent the k^{th} contributor genotype parameter at locus l as an indicator vector, $\mathbf{g}_{k,l}$, where the DNA indicators sum to 1. Hence a heterozygote genotype is represented in vector $\mathbf{g}_{k,l}$ by two 0.5 entries and a homozygote by a single 1 entry; all other vector entries are 0.

Mixture weight inference

There is an amount of DNA from each contributor present in an evidence mixture sample. The proportions of each contributor in the sample form the DNA template mixture weight vector, whose components add up to one at each locus. The peaks at each locus give an estimate of the mixture weight and these are assumed to be conditionally independent, given the template weight.

The dependence of the observed peak height data on mixture weight can be expressed through the likelihood function $\Pr\{d_j \mid W = w,\ldots\}$, where, w is a mixture weight vector, and "..." includes genotype and other values.

Combining these independent likelihood values together with a prior probability $\Pr\{W = w\}$ using Bayes theorem gives the posterior probability distribution

$$\Pr\{W = w \mid d_1, d_2, \ldots, d_i, \ldots\} \propto \Pr\{W = w\} \cdot \prod_{i=1}^{I} \Pr\{d_i \mid W = w, \ldots\} \tag{2}$$

This can be solved using MCMC computation. TA refines mixture weight equation (2) with a hierarchical model to allow each locus to have a different mixture weight based on the global DNA template weight w.

The mixture weight parameter at locus l is a vector $\mathbf{w}_l$ whose K contributor components sum to 1, so that $\sum_{k=1}^{K} w_{k,l} = 1$. The total amount of DNA (total peak heights) at locus l is given by parameter m_l and this is allowed to vary. The quantitative linear model at locus l has an expected peak height vector μ_l given by

$$\mu_l = m_l \cdot \sum_{k=1}^{K} w_{k,l} \cdot g_{k,l} \tag{3}$$

A hierarchical model of mixture weight at every locus provides a better fit to the data so TA draws each individual locus weight $\mathbf{w}_l$ as a hierarchical prior from a common mixture weight $\mathbf{w}$ using a truncated (simplex) multivariate normal distribution as

$$w_l \sim N_{[0,1]^{K-1}}\left(w, \psi^2 \cdot I\right) \tag{4}$$

where the covariance is an identity matrix scaled by a mixture variance ψ^2.

Data uncertainty inference

The variation in PCR amplification and template sampling are sources of uncertainty affecting the observed peak heights. It is known that the variation in peak height y decreases as the amount of DNA template increases so TrueAllele scales peak variance with the peak height as $y \cdot \sigma^2$ to account for stochastic effects.

There is also a signal detection variance or instrument baseline noise. TrueAllele models this baseline variation by a constant background variance τ^2, which also helps account for drop out of alleles. These two independent variance components, $y \cdot \sigma^2$ and τ^2, have probability distributions that are determined by a prior and a likelihood. Both priors $\Pr\{\sigma^2 = s^2\}$ and $\Pr\{\tau^2 = t^2\}$ are modeled using an inverse gamma distribution and the likelihood $\Pr\{d_j \mid \sigma^2 = s^2, \tau^2 = t^2, ...\}$ of observing peak heights d_j at locus j describes the probability of the independent data peak heights given the data uncertainty variances and other parameters (genotype, mixture weight, ...).

The prior variance probability is combined with the likelihoods of the J independent quantitative peak results. Bayes theorem then produces the posterior probability variance distributions

$$\Pr\{\sigma^2 = s^2 \mid d_1, d_2, ..., d_i, ...\} \propto \Pr\{\sigma^2 = s^2\} \cdot \prod_{i=1}^{I} \Pr\{d_i \mid \sigma^2 = s^2, ...\}$$

(5)

$$\Pr\{\tau^2 = t^2 \mid d_1, d_2, ..., d_i, ...\} \propto \Pr\{\tau^2 = t^2\} \cdot \prod_{i=1}^{I} \Pr\{d_i \mid \tau^2 = t^2, ...\}$$

These equations can be solved using Metropolis-Hastings statistical search.

PCR is a stochastic process that yields a variable amount of product DNA. Allele dropout can occur when either a visible peak falls below a defined threshold or does not amplify at all. TA uses probability modeling with a peak height dependent variance and a peak independent variance to account for these events.

Therefore a covariance matrix Σ_l for peak heights at locus l is defined as

(6) $$\sum_l = \sigma^2 \cdot V_l + \tau^2$$

where σ^2 is amplification dispersion, τ^2 is the instrumental detection variation, and V_l is a diagonal matrix of peak heights. All allele pairs are tried for each contributor at each locus.

TA models the observed peak height vector, $\mathbf{d}_l$, at each locus using a truncated ($\geq \mathbf{0}$) multivariate normal distribution of expected peak heights, μ_l, as

(7) $$d_l \sim N_+(\mu_l, \Sigma_l)$$

With very low peak heights the locus independent variance τ^2 ensures that equation (7) assigns a non-zero probability to all genotypes even when their alleles show no peaks in the epg. This ensures that alleles at the locus will have some probability even when they have a zero indicator flag in vector **g**. However, this probability will be very low for a significant allele peak when it is not included in a

proposed/trial vector **g**. In this way some uncertainty is given to peaks that may have dropped out but it is unclear how effective this is with the fairly vague priors used.

To infer the posterior probability q(x) for genotype $\mathbf{g}_{k,l}$, the joint probability distribution in equation (1) is formed over all the relevant random variables. The likelihood function elements $\Pr\{d_{l,i} \mid g_{k,l} = x,...\}$ are given by equation (7) and the prior probabilities are given in equations (4) and (8).

$$
\begin{aligned}
g_{k,l} &\sim \begin{Bmatrix} f_i^2, & i = j \\ 2f_i f_j & i \neq j \end{Bmatrix} \\
w &\sim Dir(1) \\
m_l &\sim N_+(5000, 5000^2) \\
\sigma^{-2} &\sim Gam(10,20) \\
\tau^{-2} &\sim Gam(10,500) \\
\psi^{-2} &\sim Gam(1/2, 1/200)
\end{aligned}
\tag{8}
$$

TA uses the product of population allele frequencies as the prior probability $\Pr\{\mathbf{g}_{k,l} = x\}$ for genotype x. The template mixture weight **w** is assigned a uniform prior probability over the K contributor simplex. The locus mass m_l prior is a (nonnegative) truncated normal distribution on feasible total peak rfu values at the locus. The data variation parameters σ^2 and τ^2 have inverse gamma prior probability distributions, as does the mixture variance ψ^2.

TA's model includes additional variables such as PCR stutter, relative amplification, DNA degradation, and dye separation but the mathematical implementation of these has not been exposed.

Statistical calculation

Our goal is to determine uncertain genotype Q, described by its posterior probability $q(x)$ for each contributor at every locus. The posterior probability distributions of the key random variables Q, **w**, σ^2 and τ^2 were described in equations (1), (2) and (5) and the joint probability distribution over all the data and variables is used to compute Q.

The joint probability distribution is fully specified as the product of the likelihood and prior distributions, given in equations (6) and (7). Using a metropolis-Hastings sampler, we iteratively draw from the posterior probability distributions of variables $\{\mathbf{g}_{k,l}\}$, $\{\mathbf{w}_l\}$, $\{m_l\}$, **w**, σ^2, τ^2 and ψ^2 using MCMC computer methods. After an initial burn in phase, the Markov chain locates the plausible values for all parameters and then samples from the joint posterior probability distribution. Marginalizing these posterior samples for each genotype variable $\mathbf{g}_{k,l}$ at locus l for contributor k, we obtain the desired posterior probability functions $q(x)$ for genotype Q at locus l.

Match strength

Once a questioned genotype Q and its posterior probability q(x) has been inferred from the evidence using the CYB population database, it is compared with a known genotype S from a target reference population R in order to assess match strength. This assessment is done using an LR that uses the relationship $q(x) = \lambda_Q(x) \cdot r_{CYB}(x)$ to factor out prior CYB beliefs. TrueAllele treats the LR as the gain in identification information resulting from having observed evidence data.

The LR can be computed as a ratio of probability-weighted likelihoods

$$LR = \frac{\sum_{x \in X} \lambda_Q(x) \cdot s(x)}{\sum_{x \in X} \lambda_Q(x) \cdot r(x)} \tag{9}$$

In LR equation (9), $\lambda_Q(x)$ is the likelihood function of questioned evidence genotype Q. The prior probability functions r(x) and s(x) are for genotype vectors R and S, respectively.

The logarithm of the LR is a standard measure of information and TrueAllele uses the base ten logarithm $\log_{10}(LR)$ as its information measure.

A comment on TA priors

The priors used by TA are generally vague and the final values are determined from the epg data and not from laboratory experiments. This works for many samples and it is Cybergenetics view that the data dominates the prior. However, this may not always be sufficient to cover the range of possible contributors. In the draft paper TrueAllele Mixture Validation it was stated that

"In principle, more informative priors could be obtained through a laboratory-specific calibration. A Bayesian framework, though, permits the use of generic prior probabilities. Therefore, such calibration is not necessary here, and was not done in this study.

In forming a posterior probability, the likelihood function addresses the observed data, whereas the prior probability does not. With STR data, the likelihood component typically overwhelms the prior contribution. For example, in the two unknown contributor case item 2A, the prior amplification variance σ^2 had a mean value of 2. But after likelihood examination of the STR peak height data, the average σ^2 parameter value increased to 8.61."

A comment on TA coancestry implementation

TA uses an internal database, designated CYB, to infer genotype distributions and has to remove this prior belief when it has to use another reference population or include coancestry θ. Equation (9) is exact when the target population is used to infer genotypes and θ is not included. Under these conditions it can be shown that the LR in equation (9) is equivalent to $LR = \frac{\Pr(E \mid S+U)}{\Pr(E \mid 2U)}$ when the probability of E given each valid genotype combination is included. However, in my view it is an approximation when another population is the target and/or θ is included. In particular it does not take account of the coancestry effect of other contributors nor those of tested non contributors. Coancestry is implemented using the Balding and Nichols equation and incorporated into the r(x) for each inferred genotype.