ANNUAL REPORT SUMMARY FOR 1999
Prepared by the Parentage Testing Standards Committee

PREFACE

This annual report summary contains statistics for 1999 compiled, and when possible, compared to prior years for which data exists. The report is divided into four sections: The first section discusses case volumes reported from accredited labs. The second section details the ongoing trends and changes in technologies used to resolve cases of disputed parentage. In the third section, data submitted by labs that perform immigration testing is summarized. Finally, in the fourth section, mutation rates and other observations of potential interest reported by respondents for 1999 are discussed. Mutations observed for 1999 are added to those reported for prior years for genetic systems detected using both RFLP and PCR methods.

The Parentage Testing Committee hopes this annual report summary is useful to parentage testing laboratories and welcomes your comments and criticisms regarding its contents. If data are not presented that you feel would improve the report, we would appreciate hearing from you.

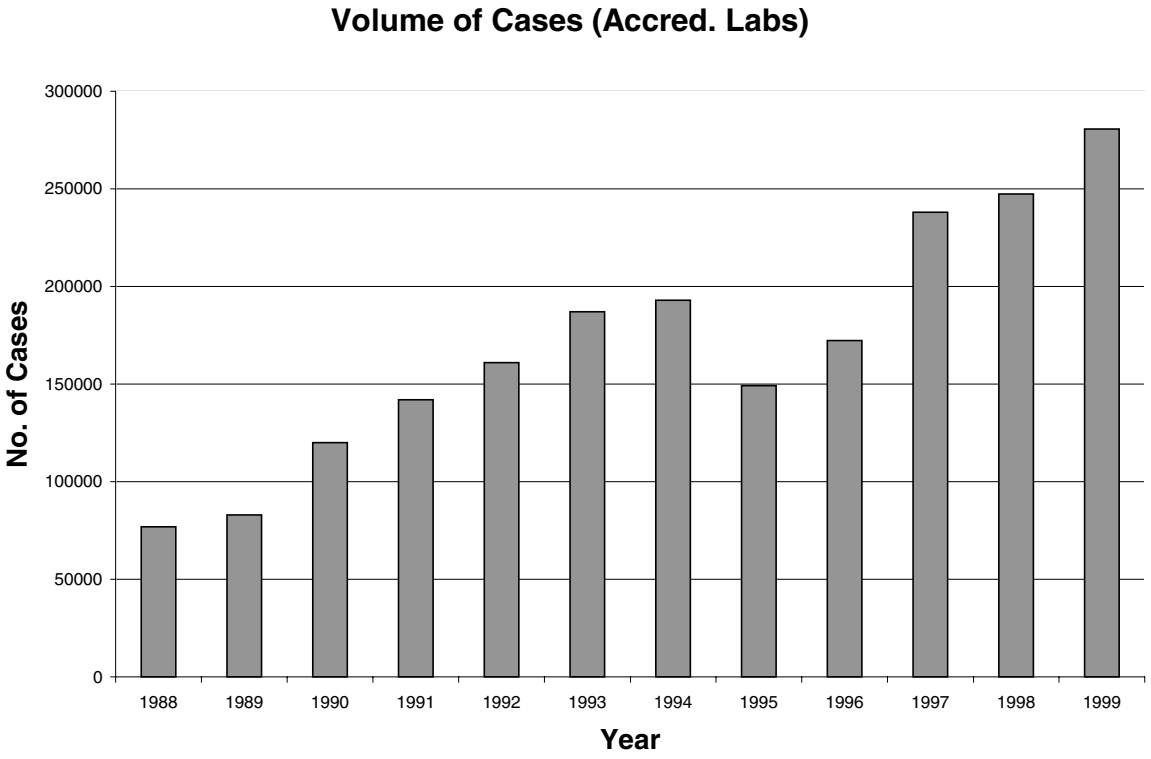
SECTION I: LABORATORY AND VOLUME STATISTICS

The questionnaire was sent to 88 labs (44 US-accredited, 27 US-non-accredited, and 17 foreign-non-accredited). As of July 2000, we had received data from 44 accredited labs (100%), 4 US-non-accredited labs (14%) and 4 foreign labs (24%) for an overall response rate of 59%. Accredited labs should be aware that submission of annual report data is expected by the AABB and is to be submitted by the deadline to ensure as complete and accurate a report as possible. The changes that have occurred in parentage testing labs over the past 5 years are summarized in Table 1.

Table 1. Categories of laboratories receiving and responding to the AABB annual survey.

Status	Number receiving Questionnaire	Number Responding	Number not Responding	Number closed or withdrew[1]
Accredited	52(1995)	36(1995)	6(1995)	2(1994-1995)
	48(1996)	37(1996)	5(1996)	4(1995-1996)
	48(1997)	48(1997)	0(1997)	0(1996-1997)
	45(1998)	38(1998)	6(1998)	1(1997-1998)
	44(1999)	44(1999)	0(1999)	0(1999)
Non-Accredited	17(1995)	8(1995)	9(1995)	unknown
	21(1996)	8(1996)	13(1996)	unknown
	13(1997)	4(1997)	9(1997)	unknown
	60(1998)	13(1998)	47(1998)	unknown
	28(1999)	4(1999)	24(1999)	unknown
Foreign	5(1995)	3(1995)	2(1995)	unknown
	5(1996)	2(1996)	3(1996)	unknown

5(1997)	4(1997)	1(1997)	unknown
6(1998)	2(1998)	4(1998)	unknown
17(1999)	4(1999)	14(1999)	unknown



1. During the period from receipt of the Annual Report Survey questionnaire until the deadline for its return.

As shown in Table 1, 100% of all accredited laboratories reported their annual statistics for 1999. The Parentage Testing Committee applauds accredited labs for their efforts to provide the data contained within this report. Only through our combined efforts can the field of Family Relatedness Testing be evaluated annually.Figure 1. Annual testing volumes performed by accredited labs 1988-1999.

A total of 280,510 cases were evaluated by accredited laboratories in 1999; an increase over 1998 of about 11%. US-non-accredited labs responding to the questionnaire performed approximately 0.15% of all cases resolved by accredited labs.

Laboratories responding to the survey can also be categorized in terms of their individual reported case volumes as shown in Figure 2.

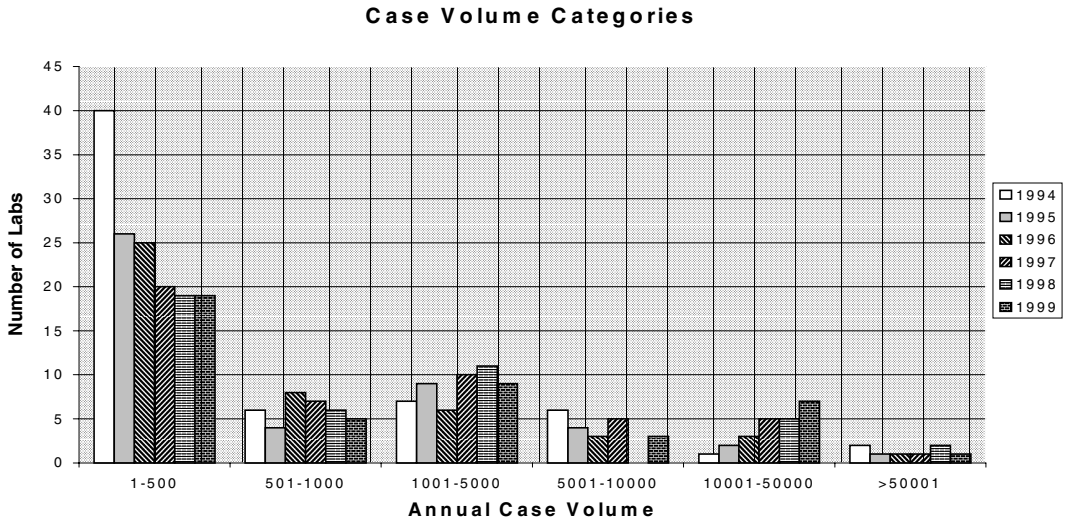


Figure 2. Breakdown of accredited parentage testing laboratories by annual case volumes. Consistent with past years, the most abundant accredited laboratory is one that performs 1-500 cases per year. It is also interesting to note that the number of labs performing 10001-50000 case/year have grown modestly in number (2) and one lab has been lost from the >50,001 cases/year ranking.

The overall exclusion rate for 1998 was 28.2% for accredited labs. Exclusion rates for non-accredited US and foreign labs were slightly less at 22.7% and 20.6% respectively.

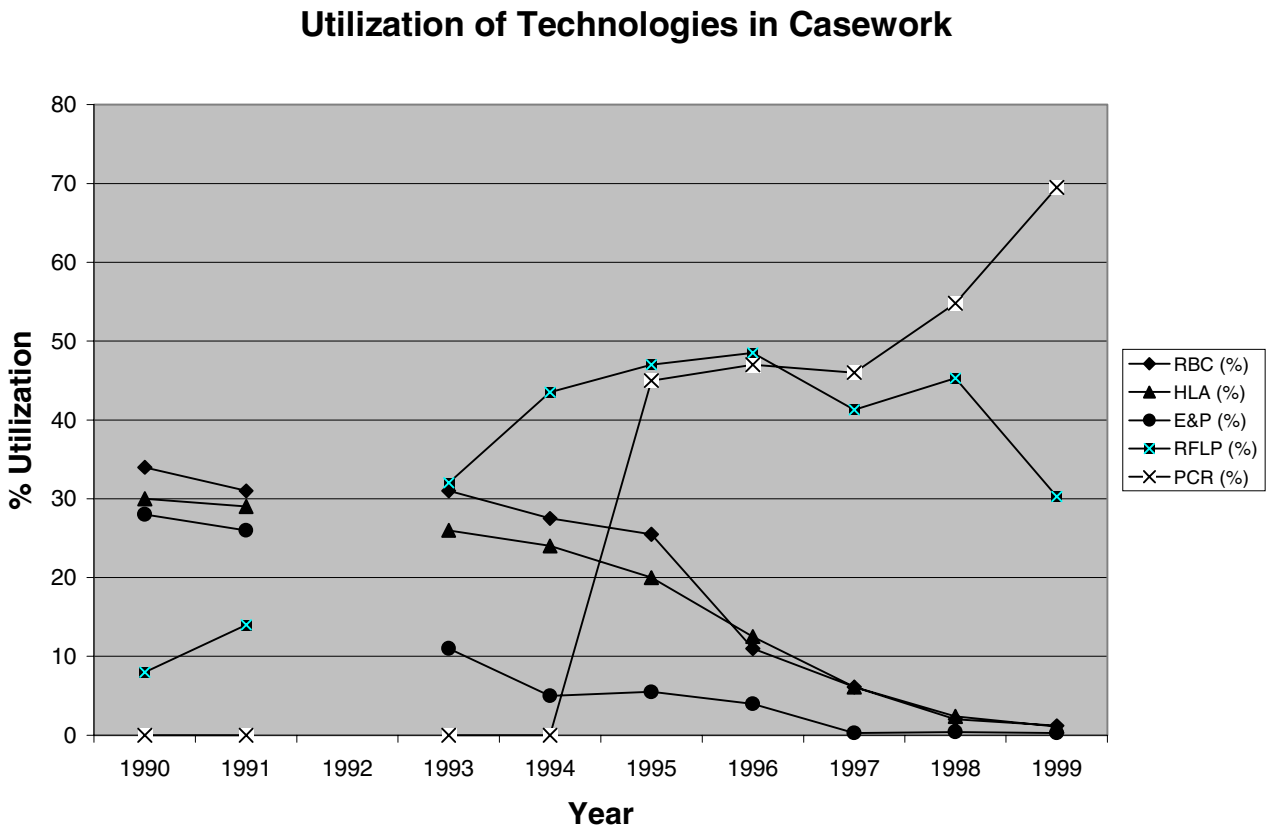
In addition to rates of exclusion, laboratories were asked to provide data regarding the minimum parentage index required to consider a case resolved. Table 2 contains a breakdown of responses from accredited labs that perform RFLP and/or PCR/STR testing. The results in Table 2 show that almost 75% of all parentage testing labs set a paternity index (PI) of 100 as the minimum acceptable level for reporting cases. The second most numerous response was from laboratories that require a minimum PI value of 1000. It was unclear from the responses whether the minima are required for complete trios only or for all cases (i.e. motherless, reconstructions, etc.).

Table 2. Minimum requirements for reporting cases of disputed parentage among accredited labs.

Required Paternity Index	RFLP	PCR/STR
<50	0	0
~100	21	29
~200	1	3
~500	0	1
~1000	5	4
~10000	0	0

The Annual Report questionnaire for 1999 also asked for data regarding referral trends. Among laboratories that responded, 0.85% of all cases evaluated in 1999 represented cases referred from another laboratory while 0.12% of all cases evaluated were referred to another lab. It is unclear why there is a big difference among the numbers of cases referred from a lab versus referred to a lab. Cases referred from a lab may be higher in number because of labs that do no testing in house and did not complete the questionnaire.

SECTION II: TECHNOLOGY SUMMARY AND TRENDS



Trends in the use of different technologies for parentage testing are shown for the years 1988 through 1999 in Figure 3.

Figure 3. Utilization of technologies as "routine" by parentage testing laboratories.

For 1999, DNA related technologies were used routinely on about 97% of all cases while serology was used on about 3% of all cases processed. As shown in the figure, the use of enzyme and protein electrophoresis has virtually disappeared in the US, although some European

respondents still rely on this technology. Red cell serology and HLA were each used on approximately 1% of cases in 1999. It should be noted however, that some labs indicating the routine use of red cell antigen testing may actually be performing ABO typing alone as a quick and inexpensive exclusionary tool for their caseload. Finally in 1999, PCR/STR technology began to be the clear choice among parentage testing laboratories for their caseloads. What is seen the Figure 3 is also borne out by the response among accredited labs as to the percentage of cases processed exclusively with PCR/STR technology versus RFLP technology (i.e. 68% versus 30% respectively). 2% of labs reported using a mix of both technologies for processing cases.

Labs were also evaluated for the DNA typing technology used as a function of their case volume. Those results are shown in Table 3.

Table 3. Percentage of cases processed using RFLP or PCR/STR technology based upon laboratory volume.

Volume (cases)	RFLP (%)	PCR/STR (%)
0-500	78	42
501-1000	18	70
1001-5000	61	39
5001-10000	17	83
10001-50000	49	51
>50000	0	100

Among labs using RFLP technology, the most commonly used restriction enzyme is still Hae III. The RFLP systems examined most commonly among RFLP labs are summarized below in Table 4.

Table 4. Top ten RFLP markers used by RFLP laboratories in 1998.

System	% of use[1]
D2S44	84
D10S28	60
D7S467	48
D6S132	44
D4S139	36
D1S339	36
D17S79	28
D4S163	28
D14S13	24
D12S11	24

1. Number of labs reporting they use this genetic marker routinely in casework as opposed to using the marker as a backup system.

The top 3 RFLP markers for 1999 are unchanged from the 1998 report. Beyond the top three, there were changes in the popularity of the other top seven systems with the D5S110 marker disappearing from the top ten list, being replaced by D14S13.

A similar analysis of popular PCR/STR systems is shown in Table 5.

Table 5. Top 15 PCR/STR systems used by parentage testing laboratories in 1999.

System	% of use[1]	System	% of use[1]
1. HUMvWA	94	9. HUMFESFPS	47
2. HUMTHO1	84	10. D3S1358	44
3. HUMCSF1P0	78	11. D18S51	44
4. D7S820	78	12. D8S1179	44
5. HUMTPOX	75	13. FGA	44
6. D13S317	69	14. HUMF13A01	44
7. D16S539	66	15. D21S11	38
8. D5S818	63		

1. Number of labs reporting they use this marker routinely as opposed to using it as a backup system.

In comparing the PCR/STR results for the past several years, it becomes evident that the parentage testing community is standardizing the PCR/STR loci analyzed in cases of disputed parentage, as was the case during the early development of RFLP technology in the late 1980s. Whereas it was possible to list 20 PCR/STR systems used as a primary test panel last year, there was a clear cutoff this year with the 15 listed in Table 5. These loci can be further grouped into commercially available multiplex kits used for parentage testing. The most commonly used kits are the CTTV and GammaSTR kits available from Promega Corp. (Madison, WI) and Profiler Plus from Perkin Elmer (Emeryville, CA). Interestingly, these kits both require the capability for fluorescent detection suggesting that fluorescence based testing methods are in more common use than silver staining to detect STR alleles.

Also evident from responses from parentage testing labs for 1999 was a further increase in the use of diverse sample types for parentage testing as shown in Table 6. Interestingly, for the first time a lab indicated that they did not accept blood as a source of DNA for testing, using only swabs.

Table 6. Acceptable sample types for parentage testing.

Sample Type	Acceptance by Labs (%)
Blood	98
Swabs	93
Bloodstains	73
Tissues	64
Pre-natal	50
Paraffin embedded/fixed tissue	16

In 1999, out of 605494 samples reported processed for DNA, 448443 (74%) represented buccal swabs whereas 157051 (26%) represented blood samples.

SECTION III. TESTS PERFORMED

Laboratories were asked on the questionnaire about the types of family relatedness testing performed. Included among options were whether or not they performed immigration testing, and, if so, how many of those tests involved complete trios, siblings, etc. Among accredited labs, 3.3% of all tests performed in 1999 were identified by respondents as being for immigration purposes. Of the tests designated for immigration purposes, only 15% involved both parents. The fact that about 85% of immigration work involved only one parent emphasizes the need for use of a testing methodology that is sufficiently discriminatory to resolve these cases in a compelling way. Interestingly, of the immigration work performed by accredited labs, the average exclusion rate was only 12.5%. This is very likely because of the prescreening of applicants by the Embassies before testing is ever ordered, thereby raising the prior probability of paternity. Of the respondents that perform immigration testing, about 50% indicated they report paternity and avuncular indices (AI) and a likelihood ratio comparing PI and AI.

SECTION IV. SUMMARY OF MUTATIONS REPORTED

Laboratories were asked to provide a summary of all apparent mutations encountered during 1999, both for RFLP and for PCR/STR systems. The Parentage Testing Committee appreciates the fact that laboratories often have enough trouble processing cases without having to keep track of all the mutation data we request for the Annual Report Summary. However, only through saving data of this type can we ever hope to accurately interpret a mutation when one is encountered in casework and furthermore, a study of these events may yield important clues concerning the dynamic properties of the genome.

Table 7 updates the mutation rates for RFLP systems that appeared in the 1998 Annual Report and also adds several new loci. Not tabulated in the table are mutation rates for markers used so rarely that there are not even 500 meiotic events reported by the lab(s) using them. Should these markers become more widely used, they will appear in future Annual Reports.

Table 7. Apparent mutations summarized for genetic markers analyzed by RFLP mapping.

System	Maternal[1] (%)	Paternal[1] (%)	Null[2] (%)	Multi-Banded
D1S7	9/569(1.58)	11/706(1.56)	1/560(0.18)	0/435(<0.230)
D1S339	174/75969(0.23)	346/94608(0.37)	24/77942(0.03)	33/55493(0.06)
D2S44	283/172742(0.16)	202/187799(0.11)	303/175936(0.17)	210/166131(0.13)
D4S139	35/73061(0.05)	909/94783(0.96)	16/70939(0.02)	706/75263(0.94)
D4S163	4/21669(0.02)	42/41635(0.10)	32/46065(0.07)	16/29731(0.05)
D5S110	120/20644(0.58)	343/19664(1.74)	8/20142(0.04)	372/24399(1.52)
D5SS43	0/525(<0.191)	0/536(<0.187)	UNK.	UNK.
D6S132	10/43779(0.02)	55/63873(0.09)	1/64869 (<0.01)	39/99392(0.04)
D7S21	20/979 (2.04)	41/1317 (3.10)	UNK.	1/1235 (0.081)
D7S22	15/2734 (0.55)	91/3187 (2.86)	UNK.	UNK.
D7S467	17/73133(0.02)	135/111907(0.12)	7/119348(<0.01)	39/99392(0.04)
D10S28	278/152273(0.18)	148/151695(0.10)	45/106785(0.04)	27/108383(0.03)

D12S11	5/12302(0.04)	13/16040(0.08)	3/13267(0.02)	3/10444(<0.01)
D14S13	19/30596(0.06)	108/33085(0.33)	3/21391(0.01)	119/26343(0.45)
D16S309	0/176(<0.06)	2/2129(0.09)	UNK.	UNK.
D16S85	0/518(<0.19)	2/542(0.55)	0/676 (<0.148)	0/676 (<0.148)
D17S26	60/63059(0.10)	157/65205(0.24)	3/21165(0.01)	32/55997(0.06)
D17S79	7/14828(0.05)	20/19672(0.10)	11/8795(0.13)	14/16032 (0.087)

1. The data under these column headings refers to: number of inconsistencies/number of total meioses expressed as a percentage within the parentheses.
2. Null alleles are assumed to exist in cases of paternal or maternal exclusion due to non-matching homozygous banding patterns when there is otherwise overwhelming evidence in favor of paternity or maternity.

Most RFLP systems for which there were large numbers of meiotic events reflected in the 1998 Annual Report did not change appreciably in their mutation rates.

Table 8. Apparent mutations summarized for genetic systems analysed by PCR.

System	Maternal[1] (%)	Paternal[1] (%)	Null[2] (%)	Multi-Banded (%)
D1S80	4/14052(0.03)	75/199543(0.04)	2/60372 (<0.01)	UNK.
D1S2131	0/1212(<0.08)	3/1240(0.24)	UNK.	UNK.
D1S533	UNK.	6/3830(0.16)	UNK.	UNK.
D2S548	1/1212(0.08)	0/1240(<0.08)	UNK.	UNK.
D3S1358	0/4889(<0.02)	9/8029(0.11)	UNK.	UNK.
D3S1744	4/4434(0.09)	61/11093(0.55)	0/5901 (<0.02)	2/5305 (0.04)
D3S2386	0/1212(<0.08)	1/1240(0.08)	UNK.	UNK.
D5S818	22/60907(0.04)	194/130833(0.15)	3/74922(<0.01)	UNK.
D7S820	14/50827(0.03)	193/131880(0.15)	1/42020(<0.01	1/406(0.25)
D8S306	1/1212(0.08)	3/1240(0.24)	UNK.	UNK.
D8S1179	5/6672(0.08)	29/10952(0.27)	UNK.	UNK.
D9S302	5/2663 (0.19)	12/2611 (0.46)	4/5274 (0.08)	0/5274 (<0.02)
D10S1214	28/2903(0.97)	114/2938(3.88)	UNK.	UNK.
D12S1090	9/4894(0.18)	108/11957(0.90)	0/5865 (<0.02)	0/7987 (<0.02)
D13S317	33/59500(0.06)	106/69598(0.15)	52/62344(0.08)	UNK.
D13S764	(0/1212(<0.08)	0/1240(<0.08)	UNK.	UNK.
D14S297	0/1212(<0.08)	0/1240(<0.08)	UNK.	UNK.
D16S539	12/42648(0.03)	40/48760(0.09)	3/52959(<0.01)	0/1165 (<0.09)
D17S5	0/228 (<0.44)	7/6568 (0.11)	UNK.	UNK.
D17S1185	0/1212(<0.08)	0/1240(<0.08)	UNK.	UNK.
D18S51	8/8827(0.10)	29/9567(0.26)	UNK.	UNK.
D18S535	1/2676 (0.04)	2/2624 (0.08)	0/5300 (<0.02)	0/5300 (<0.02)
D18S849	0/4281(<0.03)	15/9594(0.16)	0/5904 (<0.02)	0/8818 (<0.02)
D19S253	6/1212(0.50)	5/1240(0.40)	UNK.	UNK.
D21S11	12/6754(0.18)	17/6980(0.24)	1/203(0.49)	UNK.
D21S1437	0/1212(<0.08)	1/1240(0.08)	UNK.	UNK.

D22S445	2/1212(0.17)	1/1240(0.08)	UNK.	UNK.
D22S683	2/2670 (0.08)	9/2625 (0.34)	0/5295 (<0.02)	0/5295 (<0.02)
ACTBP2	0/330 (<0.30)	330/51610(0.64)	UNK.	UNK.
CYP19	6/343 (1.75)	205/177210(0.12)	321/47259 (0.68)	UNK.
CYAR04	2/3539 (0.06)	UNK.	UNK.	UNK.
FGA	7/8253(0.01)	555/189973(0.29)	2/1104(0.18)	UNK.
HUMCSF1P0	14/47843(0.03)	311/243124(0.13)	2/42020(<0.01)	UNK.
HUMFESFPS	2/8658(0.02)	71/131536(0.06)	1/3411 (0.029)	0/58828 (<0.010)
HUMF13A01	0/6827(<0.01)	33/58925(0.06)	0/1037 (<0.10)	0/1291 (<0.08)
HUMF13B	1/4206(0.02)	6/15280(0.04)	UNK.	UNK.
HUMLIPOL	0/3889(<0.03)	4/5957(0.07)	2/1222 (0.16)	0/1102 (<0.10)
HUMTHO1	5/42100(0.01)	12/74426(0.02)	2/7983(0.03)	0/2646 (<0.040)
HUMTPOX	2/28766(0.01)	10/45374(0.02)	11/43704(0.03)	13/42020(0.03)
HUMvWA31	20/58839(0.04)	851/250131(0.34)	7/42220(0.02)	1/6581 (0.02)

1. The data under these column headings refers to: number of inconsistencies/number of total meioses expressed as a percentage within the parentheses.
2. Null alleles are assumed when cases of paternal or maternal exclusion occur due to non-matching homozygous banding patterns in cases in which there is overwhelming evidence in favor of paternity or maternity.

A total of 1670 apparent mutations were reported for PCR/STR systems for 1999. As was observed for RFLP systems, some PCR/STR systems retained a fairly constant mutation rate when the data for 1999 were added while for others the mutation rate underwent a more profound change. The PCR/STR system with the highest mutation rate of any DNA marker was still D10S214 which exhibited a mutation rate in the paternal lineage of almost 4%!

An analysis of the characteristics of PCR/STR mutations for which details were provided by respondents is summarized in Table 9.

Table 9. Repeat characteristics for PCR/STR mutations.

# of Repeats from OG[1]	Male (%)	Female (%)	Total (%)
0.5 repeat	1/490 (0.2)	0/139 (<0.2)	1/629 (<0.2)
1 repeat	455/490 (92.9)	127/139 (91.4)	582/629 (92.5)
2 repeats	24/490 (4.9)	8/139 (5.8)	32/629 (5.1)
3 repeats	6/490 (1.2)	1/139 (0.7)	7/629 (1.1)
4 repeats	8/490 (1.6)	1/139 (0.7)	9/629 (1.4)
≥5 repeats	1/490 (0.2)	0/139 (<0.7)	1/629 (<0.2)

1. The change in repeat number accompanying a mutation was felt by respondents to be clear for all mutations listed above.

It is clear from Table 9 that the majority of mutations observed by PCR/STR laboratories involved the addition or deletion of a single repeat from the allele in question. In addition to the

number of repeats an allele changes by as a result of a mutation, the type of change that occurred was also requested and the responses tabulated. Out of 211 mutations involving PCR/STR loci in which respondents gave the direction of change associated with the presumed mutation, there were 104/211 (49.3%) that involved increases in allele size and 82/211 (38.9%) that involved a decrease in size. There were 25/211 (11.9%) of mutations where the direction of change was unclear. Thus, whereas some loci may preferentially gain or lose repeats as a result of mutation, when all PCR/STR loci are considered together, there appears to be a reasonable loss/gain balance.

When loci exhibiting more than 10 mutations were analyzed for a possible imbalance in the addition or deletion of repeats from the suspected parental gene, the results in Table 10 were obtained.

Table 10. Analysis of mutations at PCR/STR loci.

Locus	Number gains/losses	Difference
D5S818	24/9	2.7X
D3S1744	8/7	~even
D12S1090	17/13	~even
D13S317	12/20	0.60X
HUMvWA	14/12	~even

As was discussed in the 1998 Annual Report Summary, the data in Table 10 suggest there may be a predisposition for certain loci to mutate in a specific way, either adding repeats or deleting them from the obligate parental allele. Of further interest is the observation that loci that appeared to exhibit a bias in the characteristics of mutations in the 1998 data, appeared to be more balanced in the characteristics of mutations observed in 1999. For example, there was a 2 fold excess of mutations adding repeats to D3S1744 alleles reported with the 1998 data whereas there were approximately equal numbers of mutations adding and deleting repeats reported for 1999. Other PCR/STR loci appear to exhibit a consistent pattern of mutations for both 1998 and 1999 (D12S1090 for example).

SUMMARY

The data submitted by parentage testing laboratories for 1999 continues and extends data submitted for prior years. The field continues to grow with an volume increase of about 11% in for 1999 and the most common laboratory performing parentage testing is still the small lab performing less than 500 cases/year. DNA typing is still the principal method for processing cases, but PCR/STR methods have taken the clear lead as the method of choice for the first time. Finally, knowledge of mutation rates and characteristics continues to grow with the 1999 data. Interestingly, some loci have been in use for so long that rates are based upon almost 1000 mutations out of 250,000 meiotic events (see HUMvWA for example)!

The Parentage Testing Committee thanks the parentage testing community for providing the information contained within this report. Through our combined efforts, we continue to add knowledge not only to the field of parentage testing, but to the field of human genetics as well.