RESULTS

1. Study Population

Two hundred and fourteen women from those attending the gynecological clinic, Maharaj Nakorn Chiang Mai Hospital during January to August 1998 were enrolled randomly for this study. They came to visit the clinic for different reasons including routine annual checked-ups and certain degrees of clinical abnormality. Their age groups ranged from <25 to >64 years. The number of women in each age group is shown in Table 2. Women aged between 35 to 49 years formed the majority of those taking part.

Table 2. Age distribution of the study population.

Age group (years)	Number	Percentage
< 25	8	3.74
25 – 29	9	4.21
30 – 34	22	10.28
35 – 39	36	16.82
40 – 44	48	22.43
45 – 49	37	17.29
50 – 54	18	8.41
55 – 59	13	6.07
60 – 64	15	7.01
> 64	8	3.74
Total	214	100.00

The cytological and histological types of these women were later diagnosed and shown in Table 3 and Figure 19. Sixty two women were diagnosed with normal cervix. Based on the Bethesda system, the preinvasive cervical abnormality found in this study was classified as a low grade squamous intraepithelial lesion (LGSIL) and a high grade squamous intraepithelial lesion (HGSIL). These conditions were found in 60 and 44 women, respectively. Among 48 women with invasive carcinomas, the squamous cell carcinoma was found most frequently (40 women or 18.69%) followed by adenocarcinoma (6 women or 2.74%). The small cell carcinoma was found in only 2 women or 0.9%.

Table 3. Cytological and pathological diagnosis of the study samples.

Diagnosis	Number	Percentage
Normal	62	28.97
Preinvasive stages		
LGSIL	60	28.04
HGSIL	44	20.56
Invasive stages		
Squamous cell carcinoma	40	18.69
Adenocarcinoma	6	2.80
Small cell carcinoma	2	0.94
Total	214	100.00

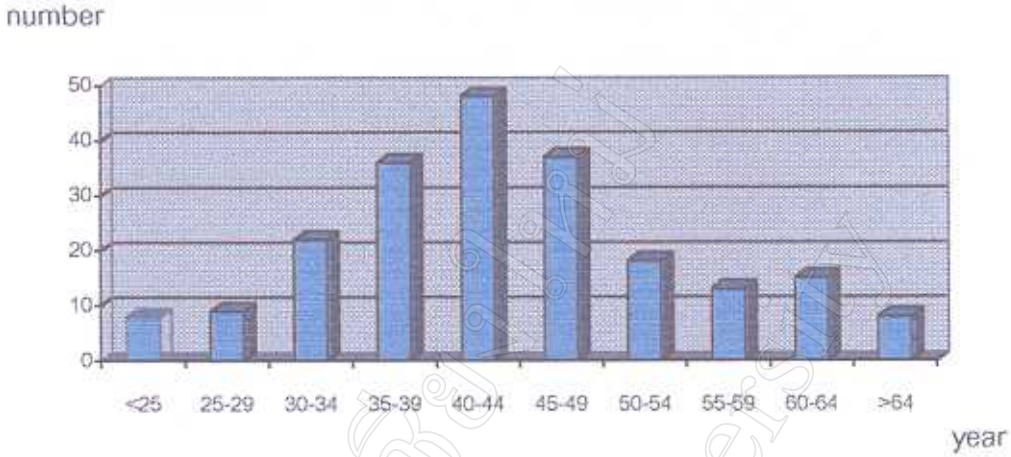

Figure. 18 Bar chart showing the age distribution of the study population

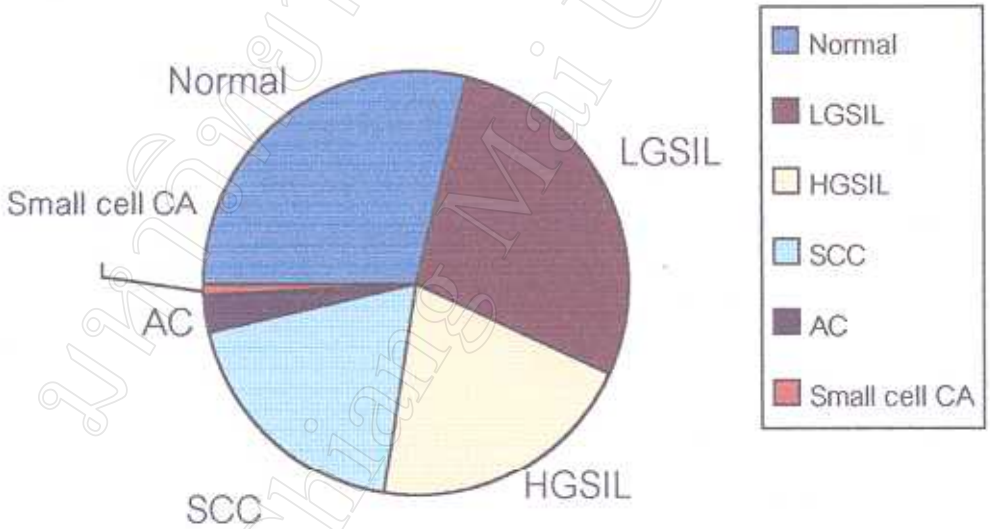

Figure 19. The pie chart showing the cytological and pathological diagnosis of the study samples. (LGSIL = low-grade squamous intraepithelial lesions, HGSIL = high-grade squamous intraepithelial lesions, SCC = squamous cell carcinoma, AC = adenocarcinoma, small cell CA = small cell carcinoma)

When the age group and cyto-histological diagnosis were compared in Table 4, it was found that the squamous cell carcinoma occurred mostly in old age women, with the mean age of 51.6 years. Whereas, the adenocarcinoma was found mostly in women younger than those with squamous cell carcinoma. The mean age of women with adenocarcinoma was 40.67 years. Among the preinvasive group, the LGSIL and HGSIL were found to have a mean age of 43.76 and 42.49 years, respectively.

Table 4. Age group compared with diagnosis of the study group.

Age group (years)	Normal	LGSIL	HGSIL	SCC	AC	Small cell CA
< 25	2	5	1	0	0	0
25 – 29	3	4	1	1	0	0
30 – 34	7	8	5	2	0	0
35 – 39	12	8	10	2	3	1
40 – 44	16	12	12	6	2	0
45 – 49	15	8	7	6	1	0
50 – 54	6	4	6	6	0	0
55 – 59	0	4	1	3	0	1
60 – 64	1	5	1	8	0	0
> 64	0	2	0	6	0	0
Total	62	60	44	40	6	2
Mean age	41.08	43.76	42.49	51.60	40.67	48.5

2. HPV DNA detection

A total of 214 samples were tested for HPV infection. The L1 gene of the HPVs was the target sequence detected by the PCR technique. The positive results showed a single band of amplified products of approximately 250 bp in an agarose gel electrophoresis (Fig. 20). The results were summarized in Table 5. Among those samples, 51 were positive for HPV DNA. The HPV DNA was not found in all samples collected from women with a normal cervix. Sixteen out of 104 samples (15.83%) and 35 from 48 (72.92%), which were collected from women with preinvasive and invasive carcinomas, respectively, were positive for HPV DNA. In the preinvasive groups, the percentage HPV positive in the HGSIL was higher than in the LGSIL, 29.5% and 5.0%, respectively. HPV DNA was detected in all samples with adenocarcinoma while the samples with squamous cell carcinoma and small cell carcinoma were found in 70% and 50%, respectively.

Table 5.. L1 HPV DNA detection.

	N	L1 HPV positive	percentage
NORMAL	62	0	0.00
PRE-INVASIVE			
LGSIL	60	3	5.00
HGSIL	44	13	29.50
Total	104	16	15.38
INVASIVE			
SCC	40	28	70.00
Adenocarcinoma	6	6	100.00
Small cell carcinoma	2	1	50.00
Total	48	35	72.92
Total	214	51	23.83

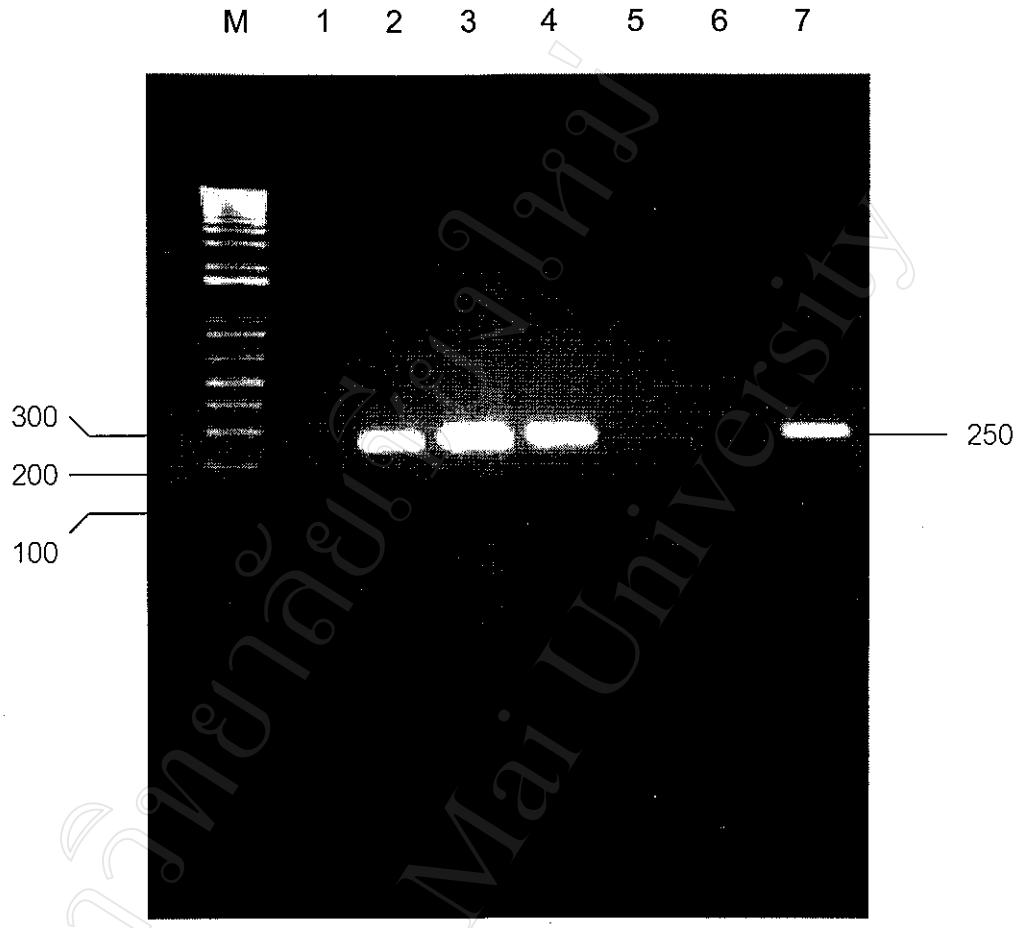

Figure 20. The amplification of L1 HPV DNA from clinical specimen by PCR

Lane M = 1 Kb marker

Lane 1 = negative control

Lane 2 = positive control

Lane 3 = positive sample (sample No. 3)

Lane 4 = positive sample (sample No. 42)

Lane 5 = negative sample (sample No. 18)

Lane 6 = negative sample (sample No. 21)

Lane 7 = positive sample (sample No. 24)

3. HPV Genotyping

All HPV DNA positive samples were analyzed further for HPV genotype distribution by using the RFLP technique. The RFLP patterns of HPVs containing samples are shown in Figures 21 - 28. The patterns obtained from those RFLP were analyzed for HPV specific types by comparing with the RFLP DNA fragment size shown in Table 1. Among those HPV positive samples, 8 different types of HPVs were observed, they were HPV-6, -11, -16, -18, -35, -51, -52 and -59. Unfortunately, there were 4 samples that gave unclear RFLP patterns, which prevented proper identification. It is possible that a mixed infection may have occurred in those cases. However, among the clearly identifed HPV types, HPV16 was the most frequently found (21 out of 47 samples) in both preinvasive and invasive carcinomas, followed by HPV-18 and -35. HPV-18 and -35 were found in an equal number of 7 samples each. In invasive carcinomas, HPV16 was the most prevalent, and they represented 45.7% of all HPV types identified, while HPV-18 and -35 were found in about 14.3% each. The overall HPV genotype distribution is summarized in Table 6.

Table 6. Genotype distribution as determined by PCR-RFLP

Histologic Type	HPV type									
	6	11	16	18	35	51	52	59	UN	total
PRE-INVASIVE										
LGSIL				1	1		1			3
HGSIL			5	1	1	1	2	1	2	13
total			5	2	2	1	3	1	2	16
INVASIVE										
Squamaous cell CA	3	1	14	2	5		2		1	28
Adenocarcinoma	1		2	2					1	6
Small cell CA				1						1
total	4	1	16	5	5		2		2	35
Total	4	1	21	7	7	1	5	1	4	51

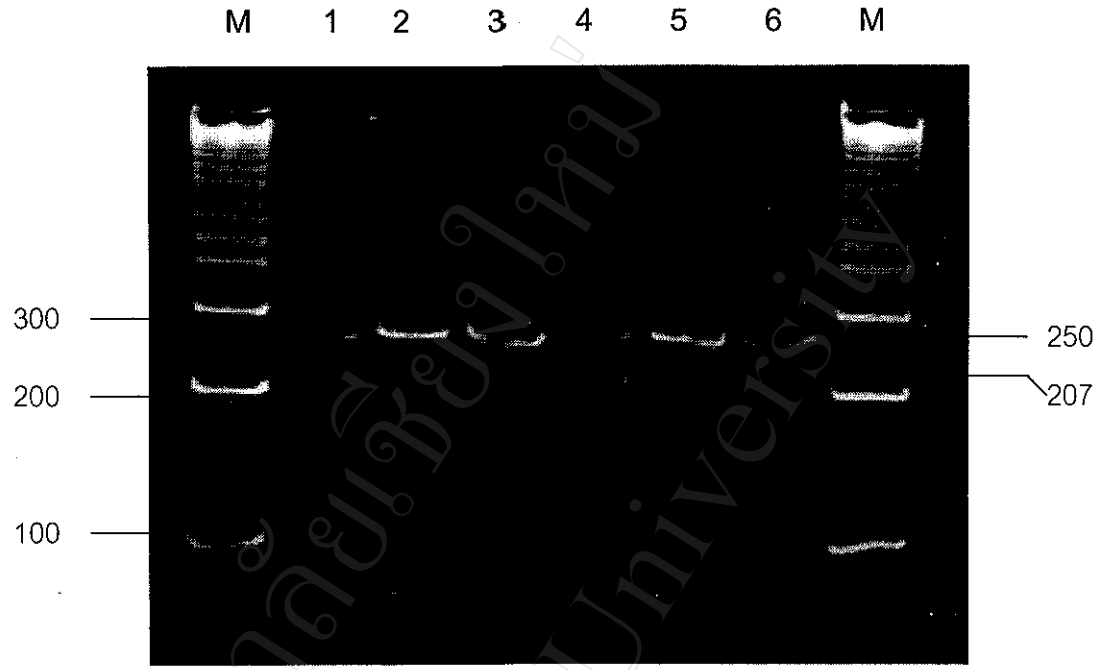

Figure 21. The illustration of the PCR-RFLP pattern of HPV type 6 (sample No. 64)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane 6 = *Eco* RII digested products

Lane M = 1 Kb DNA marker

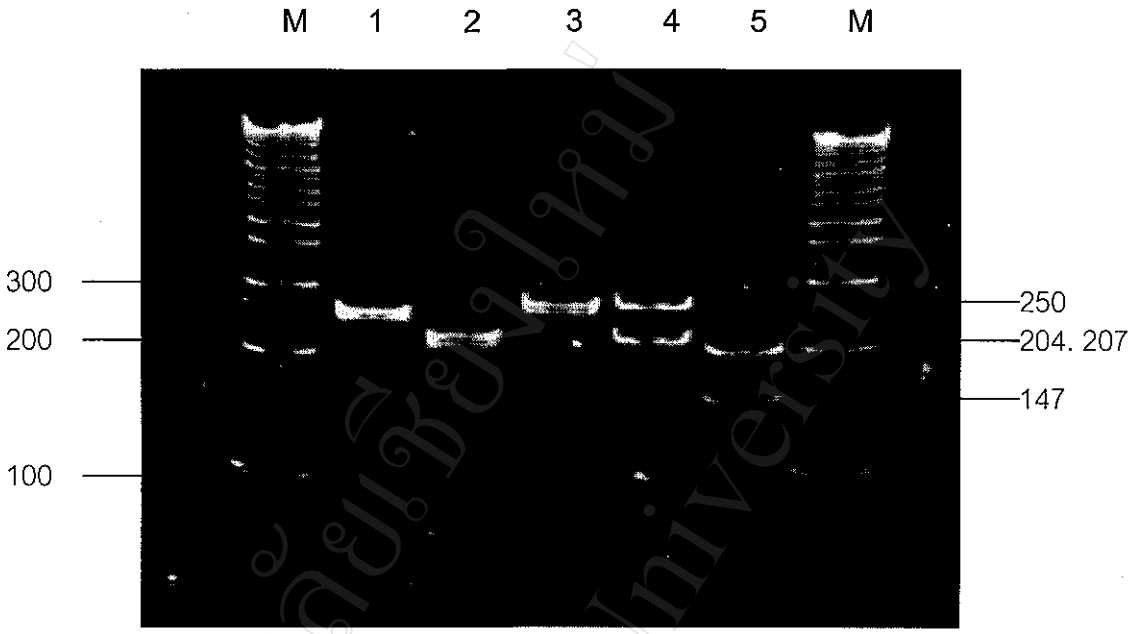

Figure 22. The illustration of the PCR-RFLP pattern of HPV type 11 (sample No. 38)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane M = 1 Kb DNA marker

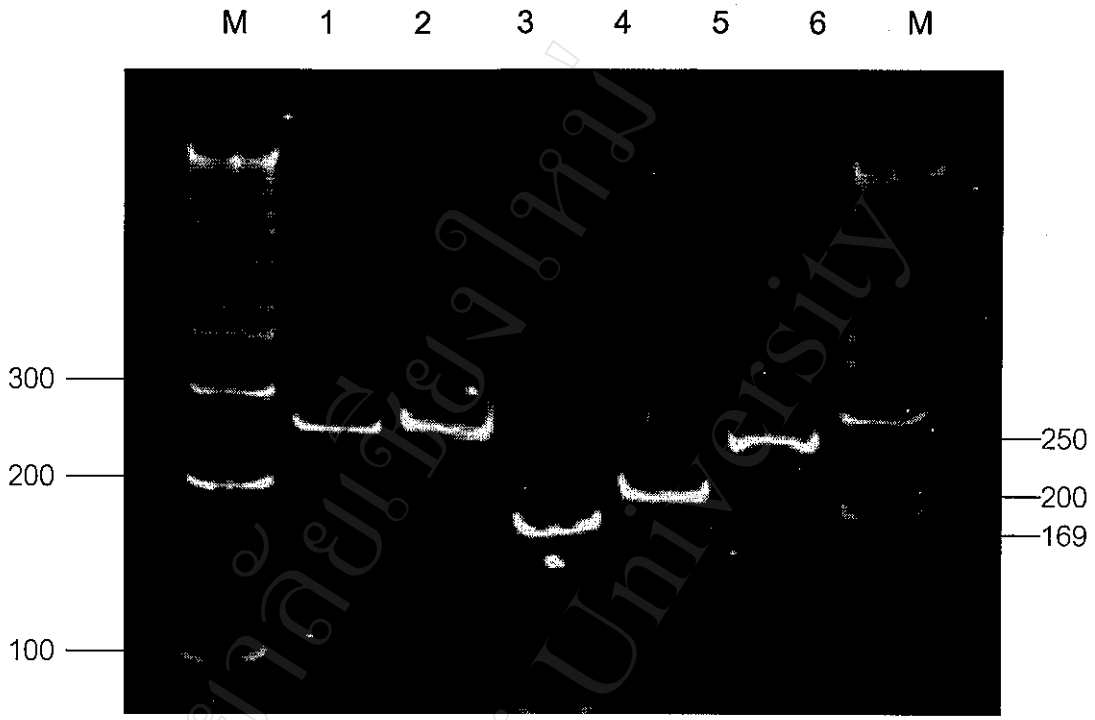

Figure 23. The illustration of the PCR-RFLP pattern of HPV type 16 (sample No. 51)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane M = 1 Kb DNA marker

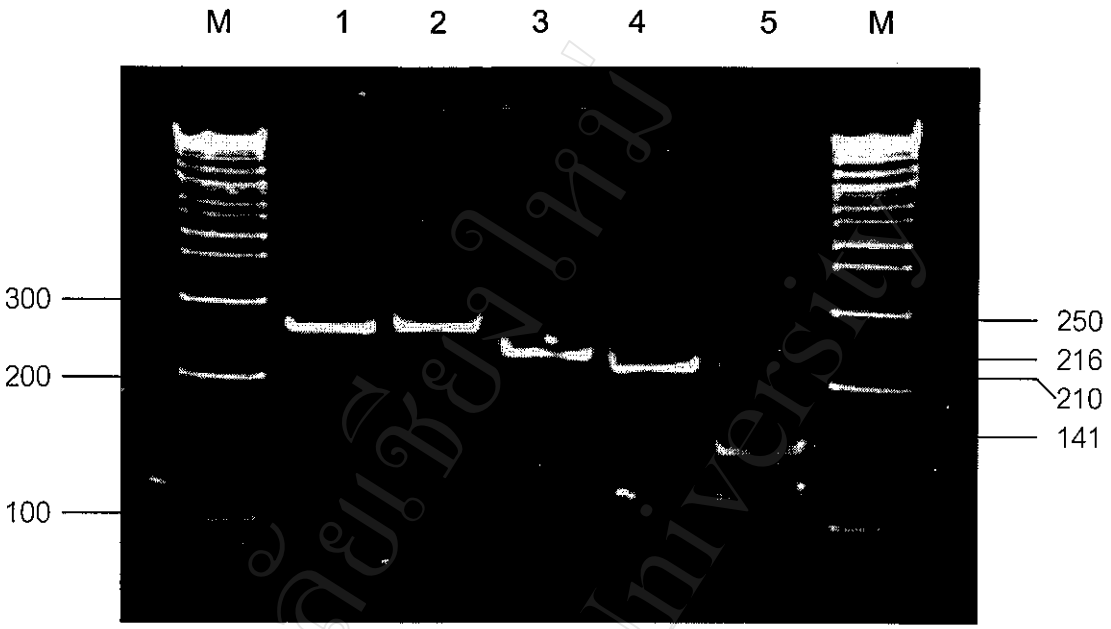

Figure 24. The illustration of the PCR-RFLP pattern of HPV type 18 (sample No. 42)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane M = 1 Kb DNA marker

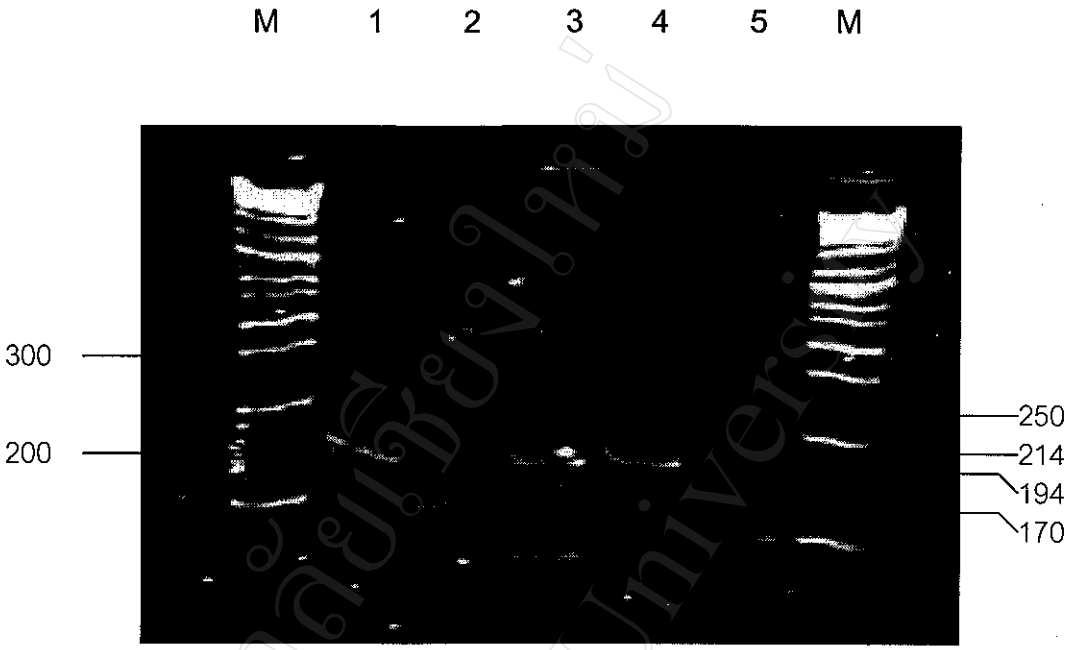

Figure 25. The illustration of the PCR-RFLP pattern of HPV type 35 (sample No. 28)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane M = 1 Kb DNA marker

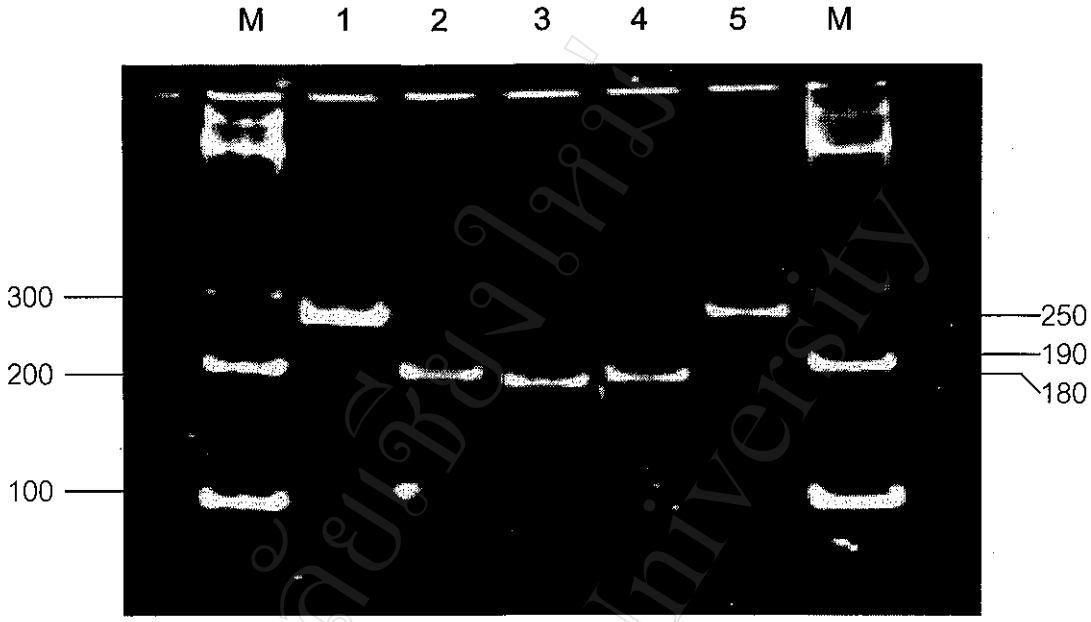

Figure 26. The illustration of the PCR-RFLP pattern of HPV type 52 (sample No. 119)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane M = 1 Kb DNA marker

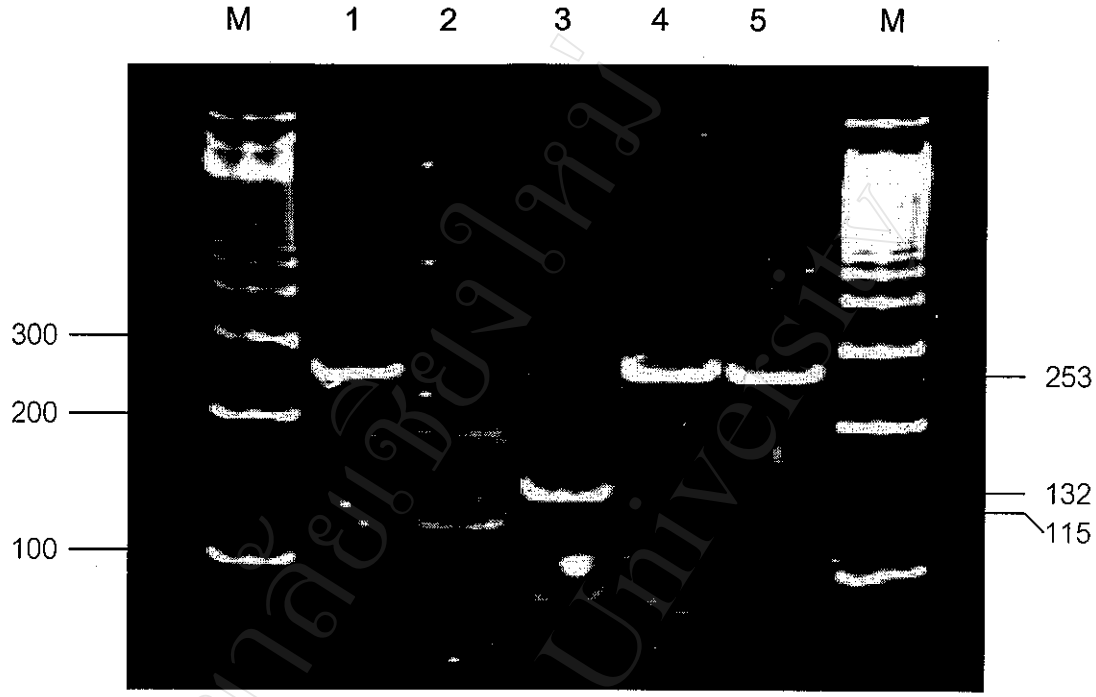

Figure 27. The illustration of the PCR-RFLP pattern of HPV type 59 (sample No. 12)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane M = 1 Kb DNA marker

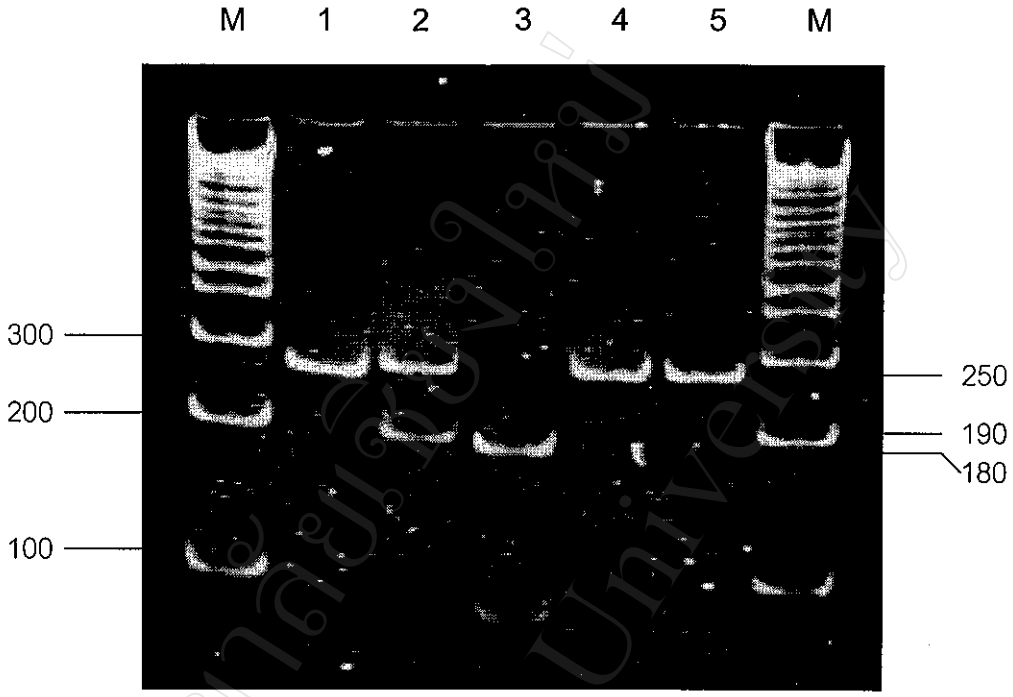

Figure 28. The illustration of the PCR-RFLP pattern of unidentified type (sample No. 40)

Lane M = 1 Kb DNA marker

Lane 1 = uncut fragment

Lane 2 = *Rsa* I digested products

Lane 3 = *Dde* I digested products

Lane 4 = *Hae* III digested products

Lane 5 = *Hinf* I digested products

Lane M = 1 Kb DNA marker

4. HPV E6 and E7 mRNA determination by RT-PCR

A total of 28 HPV-16 and HPV-18 positive samples were selected to determine E6 and E7 mRNA by using the RT-PCR technique. Two sets of primers, P16A-HPV16R(Hsu) and P16A-P16B were used to amplify the HPV-16 and HPV-18 transcripts, respectively. The amplified products were first screened by agarose gel electrophoresis, and then subjected to capillary electrophoresis to analyze further for the patterns and relative amount of the HPV transcripts by using an automatic genetic analyzer and genotyper computer software. The patterns of the transcripts were shown as an electropherotogram and the amount of each transcript was calculated in units of peak height or peak area. The SiHa cell line containing HPV16 genome and a Hela cell line containing HPV-18 genome were used as a control system for the HPV-16 and -18 expression, respectively. The HPV-16 and -18 plasmids were used as a negative control system.

4.1 E6 and E7 mRNA determination in HPV 16 positive samples

Twenty-one HPV-16 positive samples were analyzed for E6 and E7 transcripts. The tumor histology of these samples is shown in Table 7. The samples comprised 3 HGSIL, 13 squamous cell carcinomas and 2 adenocarcinomas. After amplification by RT-PCR, the HPV-16 transcripts were detected in 18 out of 21 samples by using the genetic analyzer. However, when using the agarose gel electrophoresis, the HPV16 transcripts could be demonstrated in only some samples. The intensity of the DNA bands observed in the agarose gel was rather low, which indicated the low sensitivity of the agarose gel electrophoresis in the detection of the previously mentioned RT-PCR products (Fig. 29). At least two subsets of the transcripts were detected in all positive samples. They were fragments of approximately 400 and 279 bp (Fig. 29) and were supposed to represent the products of the spliced transcripts. These transcripts were thought to generate from the same splice donor at nucleotide position 226 and form different splice acceptors at nucleotide position 408 and 526, respectively. The 600-bp fragment that represented the full-length E6 transcripts was observed in some samples. The HPV-16 transcription patterns and the intensity of each transcript

reported in units from peak height and peak area were summarized in Table 7. The electrophoretograms of the HPV16 transcription patterns are shown in Figures 30-32.

Table 7. The summarized data of the HPV-16 transcription in cervical cancer.

Sample No.	Histology	Peak	Peak 1 (Full-length E6)		Peak 2 (E6*I)		Peak 3 (E6*II)	
			size (bp)	unit	size (bp)	unit	size (bp)	unit
SiHa cell line		Height	604.95	412	400.01	2741	279.62	1273
		Area		6647		41121		16177
3	HGSIL	Height	601.87	516	398.82	7513	279.08	3172
		Area		10176		114274		37992
5	HGSIL	Height	597.92	700	401.05	318	279.31	187
		Area		15388		4432		2118
14	HGSIL	Height			400.47	2868	279.31	2133
		Area				42437		23540
20	SCC, well	Height	602.48	1789	399.23	5059	279.12	2094
		Area		32663		74242		25263
51	SCC, well	Height	601.33	2047	398.7	4363	279.21	2508
		Area		25542		51366		25103
107	SCC, well	Height	590.78	460	400.22	858	279.51	940
		Area		6688		11632		10485
111	SCC, well	Height			399.46	1273	279.23	2091
		Area				16995		21190
24	SCC, mod	Height	603.09	2606	399.43	1803	279.54	2777
		Area		37391		24508		32818
34	SCC, mod	Height	603.09	3414	399.8	2439	279.23	2095
		Area		61435		37004		26689
41	SCC, mod	Height	602.96	220	399.11	6876	279.22	5410
		Area		2304		110952		66042

Table 7 The summarized data of the HPV-16 transcription in cervical cancer. (continue)

Sample No.	Histology	Peak	Peak 1 (Full-length E6)		Peak 2 (E6*I)		Peak 3 (E6*II)	
			size (bp)	unit	size (bp)	unit	size (bp)	unit
52	SCC, mod	Height	603.73	203	398.84	5304	279.22	4843
		Area		3823		87945		65547
63	SCC, mod	Height			406.26	1161	282.87	482
		Area				18841		7482
78	SCC, mod	Height	600.2	543	401.07	648	279.34	389
		Area		8124		9642		4514
26	SCC, poor	Height	601.4	4896	399.11	6968	279.12	6808
		Area		64059		61273		68890
32	SCC, poor	Height	604.32	67	399.37	2150	279.1	1214
		Area		925		30438		12406
60	SCC, poor	Height	600.44	2598	398.46	6421	279.04	3810
		Area		41377		82642		38379
39	AC, well	Height			399.39	7374	279.35	6918
		Area				115973		69371
43	AC, well	Height	601.65	3507	399.3	7369	279.31	7262
		Area		48726		113662		79728

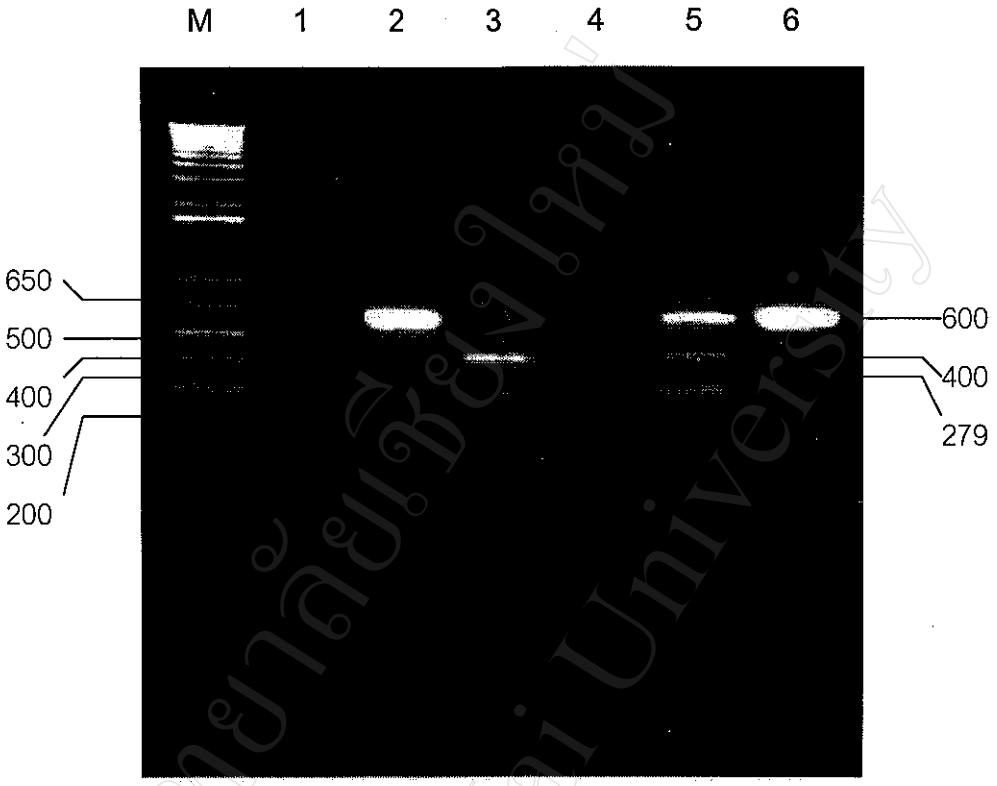

Figure 29. The illustration of the RT-PCR products of HPV 16 samples by gel electrophoresis

Lane M = 1 Kb DNA marker

Lane 1 = PCR negative control

Lane 2 = PCR positive control (HPV-16 plasmid DNA)

Lane 3 = Sample No.14 RT-PCR product

Lane 4 = Sample No.14 RT(-)-PCR* product

Lane 5 = Sample No.24 RT-PCR product

Lane 6 = Sample No.24 RT(-)-PCR* product

RT(-)-PCR* = The transcriptase enzyme was not added.

Figure 30. The electrophoretogram of the SiHa cell line RT-PCR product by using an automatic genetic analyzer. The blue line in the top frame is the analyzed signal of the SiHa cell line, which shows three peaks of RT-PCR product. The red line in the bottom frame is the analyzed signal of size standard fragments. The blue squares in the table, indicate the analyzed data of the three peaks, (2B, 1), (2B, 2) and (2B, 3), which refer to E6*II, E6*I and full-length transcription, respectively. The red squares with the black dot in the table, indicate the analyzed data of size standard fragments.

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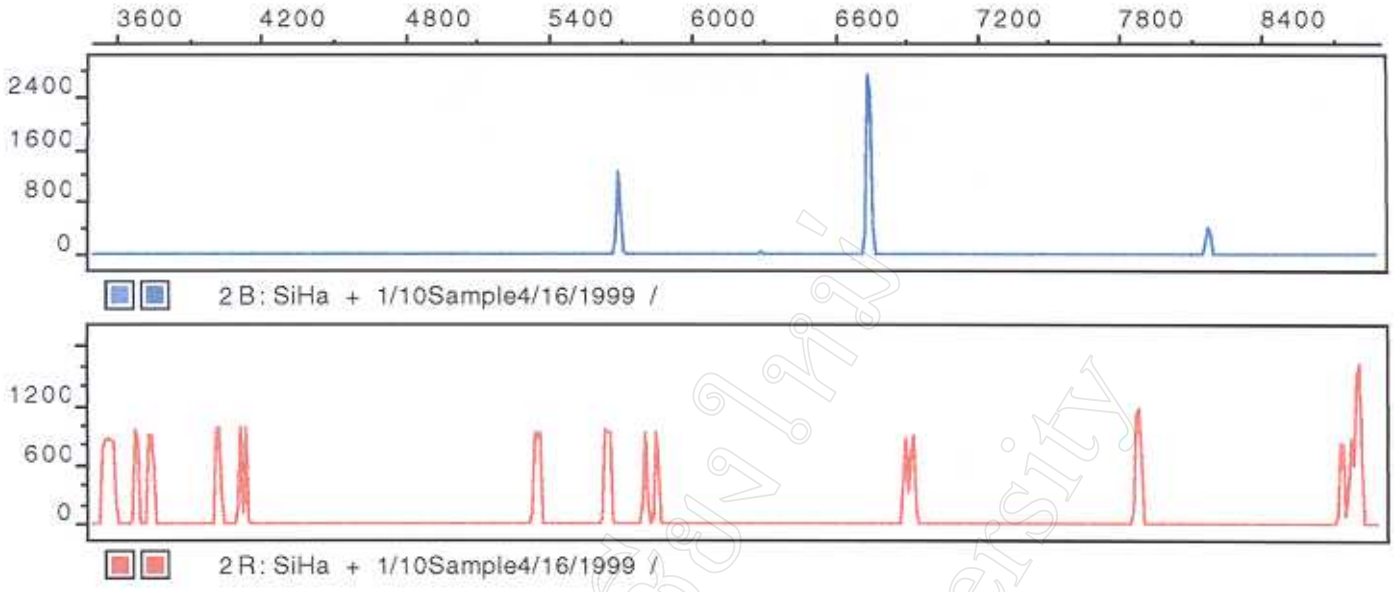

Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
2B,1	20.90	279.62	1273	16182	5699
2B,2	24.75	400.01	2742	41118	6749
2B,3	29.99	604.95	412	6645	8177
2R,1	13.05	60.22	853	5373	3557
2R,2	13.09	61.71	870	5538	3570
2R,3 •	13.17	64.00	857	5409	3590
2R,4	13.20	65.09	844	5531	3598
2R,5 •	13.52	75.00	961	6191	3686
2R,6	13.53	75.35	947	5204	3690
2R,7	13.74	80.19	903	5606	3747
2R,8 •	13.78	81.00	913	5487	3757
2R,9	14.77	107.10	972	5993	4028
2R,10 •	14.81	108.00	999	6150	4037
2R,11	15.12	115.80	993	6043	4122
2R,12 •	15.21	118.00	981	6195	4146
2R,13	19.63	242.24	937	8083	5352
2R,14 •	19.69	244.00	927	8066	5368
2R,15	20.71	273.46	954	8675	5646
2R,16 •	20.76	275.00	937	8817	5660
2R,17	21.31	293.23	936	8802	5812
2R,18 •	21.49	299.00	938	8891	5859
2R,19	25.31	417.37	891	10258	6902
2R,20 •	25.43	421.00	903	10603	6934
2R,21 •	28.90	539.00	1166	23549	7880
2R,22 •	32.02	674.00	838	11603	8731
2R,23	32.17	675.77	887	12257	8772
2R,24 •	32.28	677.00	1650	25340	8802

Figure 31. The electrophoretogram of the HPV-16 (sample No. 3) RT-PCR product by using an automatic genetic analyzer. The blue line in the top frame is the analyzed signal of the sample No. 3, which shows three peaks of RT-PCR product. The red line in the bottom frame is the analyzed signal of size standard fragments. The blue squares in the table, indicate the analyzed data of the three peaks, (2B, 6), (2B, 8) and (2B, 9), which refer to E6*II, E6*I and full-length transcription, respectively. The red squares with the black dot in the table, indicate the analyzed data of size standard fragments.

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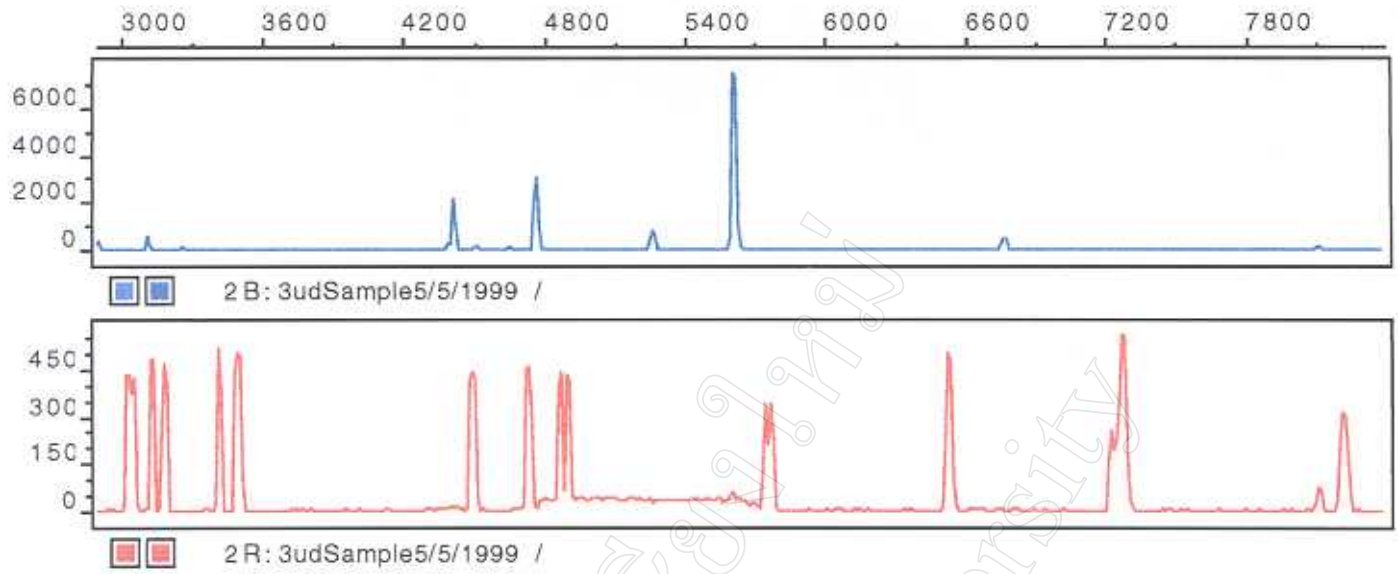
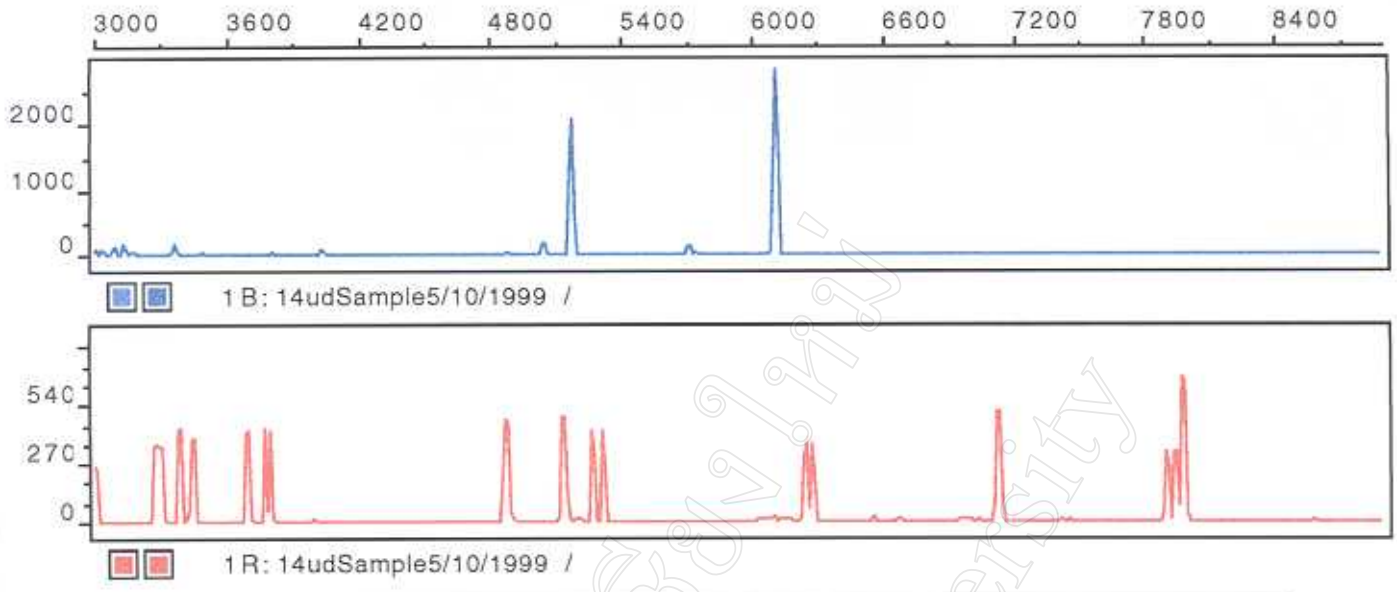


Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
2B,1	10.68	43.39	425	2438	2913
2B,2	11.46	73.06	584	3301	3124
2B,3	11.49	74.17	212	1323	3133
2B,4	16.12	228.83	272	2846	4396
2B,5	16.20	231.79	2159	23108	4418
2B,6	17.49	279.08	3172	37992	4770
2B,7	19.32	349.96	837	13584	5267
2B,8	20.59	398.82	7513	114293	5614
2B,9	24.82	601.87	516	10176	6769
2R,1	11.13	60.24	434	2323	3034
2R,2	11.16	61.63	433	2512	3044
2R,3 •	11.23	64.00	424	2377	3061
2R,4	11.25	64.98	425	2369	3067
2R,5 •	11.52	75.00	481	4615	3140
2R,6	11.69	80.09	465	2610	3188
2R,7 •	11.73	81.00	458	2513	3197
2R,8	12.54	107.27	478	2635	3420
2R,9 •	12.57	108.00	517	3109	3426
2R,10	12.82	115.87	507	2771	3496
2R,11 •	12.89	118.00	494	2732	3515
2R,12	16.48	241.95	444	3721	4493
2R,13 •	16.53	244.00	431	3864	4508
2R,14	17.35	273.63	455	4171	4732
2R,15 •	17.39	275.00	459	4178	4742
2R,16	17.87	294.18	442	5200	4872
2R,17 •	17.98	299.00	434	5248	4904
2R,18	21.08	417.75	344	4684	5748
2R,19 •	21.16	421.00	344	4794	5771
2R,20 •	23.95	539.00	501	10094	6530
2R,21 •	26.50	674.00	257	4887	7226
2R,22 •	26.68	677.00	560	16604	7275
2R,23	30.13		315	9611	8215

Figure 32. The electrophoretogram of the HPV-16 (sample No. 14) RT-PCR product by using an automatic genetic analyzer. The blue line in the top frame is the analyzed signal of the sample No. 14, which show two peaks of RT-PCR product. The red line in the bottom frame is the analyzed signal of size standard fragments. The blue squares in the table, indicate the analyzed data of the two peaks, (1B, 2) and (1B, 3), which refer to E6*II and E6*I transcription, respectively. The red squares with the black dot in the table, indicate the analyzed data of size standard fragments.



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
1B,1	18.53	263.60	204	2087	5053
1B,2	18.99	279.31	2133	23540	5178
1B,3	22.43	400.47	2868	42433	6115
1R,1	11.08	37.40	244	1273	3020
1R,2	12.02	63.10	356	2082	3277
1R,3	12.07	64.40	370	2225	3290
1R,4	12.13	66.00	351	2098	3306
1R,5	12.15	66.80	350	2191	3314
1R,6 •	12.46	75.00	441	4249	3396
1R,7	12.64	80.09	384	2306	3447
1R,8 •	12.68	81.00	387	2367	3456
1R,9	13.56	106.80	409	2327	3696
1R,10 •	13.60	108.00	430	2630	3707
1R,11	13.87	115.71	437	2557	3781
1R,12 •	13.95	118.00	426	2541	3803
1R,13	17.86	242.15	480	3345	4871
1R,14 •	17.92	244.00	463	3438	4886
1R,15	18.82	273.38	483	3743	5133
1R,16 •	18.87	275.00	490	3728	5146
1R,17	19.33	292.03	429	3605	5271
1R,18 •	19.51	299.00	423	3620	5321
1R,19	22.91	417.07	362	4076	6246
1R,20 •	23.02	421.00	366	4187	6277
1R,21 •	26.15	539.00	510	9431	7130
1R,22	28.98		325	4580	7902
1R,23	29.12		330	4646	7942
1R,24	29.25		672	10075	7976

The 400 bp fragment, which corresponded to the E6*I transcripts and putatively encoded the E7 protein, was found to be a major transcript that was consistently presented in all E6/E7 transcriptional active tumor cells. Whereas, the 280 bp fragment, which corresponded to an E6*II that encoded one of the two potential E6 proteins, was synthesized to a lesser degree than the E6*I, except in 3 cases of squamous cell carcinomas (Table 7). In order to see whether the relative proportions of different E6/E7 transcripts varied in tumor histology, the ratio of E6*I to E6*II from each sample was determined from the units of both peak height and area (Table 7). The ratio determined from either peak unit corresponded and varied from 0.61 to 2.42 (mean = 1.44) and 0.747 to 3.00 (mean = 1.79), respectively (Tables 8, 9). When related to tumor histology, the E6*I/E6*II ratio was shown to be independently varied histological types. The mean ratio of E6*I/E6*II in the HGSIL (mean = 1.81) was higher than those observed in the invasive squamous cell carcinomas (mean = 1.42). However, there was no statistically significant difference ($p>0.2$). Concerning the different histological forms of squamous cell carcinomas with well, moderate and poor differentiated patterns, the mean ratios of E6*I and E6*II were 1.42, 1.38 and 1.49, respectively (Table 8). By statistical analysis, those mean ratios were not significantly different ($P>0.2$).

Table 8. The ratio between E6*I and E6*II determined from the peak height unit of HPV-16 samples and the statistical analysis.

Sample No.	Histology	Peak 1 (full-length E6)		Peak 2 (E6*I)		Peak 3 (E6*II)		P2/P3 Ratio*	
		size (bp)	unit	size (bp)	unit	size (bp)	unit		
SiHa cell line		604.95	412	400.01	2741	279.62	1273	2.15	
3	HGSIL	601.87	516	398.82	7513	279.08	3172	2.37	$\bar{X}$=1.81
5	HGSIL	597.92	700	401.05	318	279.31	187	1.70	SD=0.52
14	HGSIL			400.47	2868	279.31	2133	1.35	
20	SCC, well	602.48	1789	399.23	5059	279.12	2094	2.42	$\bar{X}$=1.42
51	SCC, well	601.33	2047	398.70	4363	279.21	2508	1.74	SD=0.82
107	SCC, well	590.78	460	400.22	858	279.51	940	0.91	
111	SCC, well			399.46	1273	279.23	2091	0.61	
24	SCC, mod	603.09	2606	399.43	1803	279.54	2777	0.65	$\bar{X}$=1.38
34	SCC, mod	603.09	3414	399.80	2439	279.23	2095	1.16	SD=0.60
41	SCC, mod	602.96	220	399.11	6876	279.22	5410	1.27	
52	SCC, mod	603.73	203	398.84	5304	279.22	4843	1.10	
63	SCC, mod			406.26	1161	282.87	482	2.41	
78	SCC, mod	600.2	543	401.07	648	279.34	389	1.67	
26	SCC, poor	601.4	4896	399.11	6968	279.12	6808	1.02	$\bar{X}$=1.49
32	SCC, poor	604.32	67	399.37	2150	279.10	1214	1.77	SD=0.41
60	SCC, poor	600.44	2598	398.46	6421	279.04	3810	1.69	
39	AC, well			399.39	7374	279.35	6918	1.07	$\bar{X}$=1.05
43	AC, well	601.65	3507	399.3	7369	279.31	7262	1.02	SD=0.04

*P2/P3 ratio = unit of peak 2/ unit of peak 3

Table 9. The ratio between E6*I and E6*II determined from the peak area unit of HPV-16 samples and the statistical analysis.

Sample No.	Histology	Peak 1 (full-length E6)		Peak 2 (E6*I)		Peak 3 (E6*II)		P2/P3 Ratio*	
		size (bp)	unit	size (bp)	unit	size (bp)	unit		
SiHa cell line		604.95	6647	400.01	41121	279.62	16177	2.54	
3	HGSIL	601.87	10176	398.82	114274	279.08	37992	3.01	$\bar{X}$=2.3
5	HGSIL	597.92	15388	401.05	4432	279.31	2118	2.09	SD=0.63
14	HGSIL			400.47	42437	279.31	23540	1.80	
20	SCC, well	602.48	32663	399.23	74242	279.12	25263	2.94	$\bar{X}$=1.73
51	SCC, well	601.33	25542	398.70	51366	279.21	25103	2.05	SD=0.97
107	SCC, well	590.78	6688	400.22	11632	279.51	10485	1.11	
111	SCC, well			399.46	16995	279.23	21190	0.80	
24	SCC, mod	603.09	37391	399.43	24508	279.54	32818	0.75	$\bar{X}$=1.64
34	SCC, mod	603.09	61435	399.80	37004	279.23	26689	1.39	SD=0.63
41	SCC, mod	602.96	2304	399.11	110952	279.22	66042	1.68	
52	SCC, mod	603.73	3823	398.84	87945	279.22	65547	1.34	
63	SCC, mod			406.26	18841	282.87	7482	2.52	
78	SCC, mod	600.2	8124	401.07	9642	279.34	4514	2.14	
26	SCC, poor	601.4	64059	399.11	61273	279.12	68890	0.89	$\bar{X}$=1.83
32	SCC, poor	604.32	925	399.37	30438	279.10	12406	2.45	SD=0.83
60	SCC, poor	600.44	41377	398.46	82642	279.04	38379	2.15	
39	AC, well			399.39	115973	279.35	69371	1.67	$\bar{X}$=1.55
43	AC, well	601.65	48726	399.3	113662	279.31	79728	1.43	SD=0.17

*P2/P3 = unit of peak 2/ unit of peak 3

4.2 E6 and E7 mRNA in HPV 18 positive samples

Seven HPV-18 positive tumor samples were analyzed for E6 and E7 mRNA by using the RT-PCR technique. The tumor histology of these samples is shown in Table 10. After analysis, E6/E7 transcripts were detected in 4 out of 7 samples. However, only one DNA peak was consistently observed in both the agarose gel electrophoresis and eletropherotogram (Figs. 33 - 35). Its molecular size was approximately 256 bp and corresponded to the E6*I transcript. The 440 bp fragments, which corresponded to the full-length E6 transcripts, was observed in some samples (Table 10).

Table 10. The summarized data of the HPV-18 transcription in cervical cancer.

Sample No	Histology	Peak	Peak 1 (Full-length E6)		Peak 2 (E6*I)	
			size (bp)	unit	size (bp)	unit
HeLa cell line		Height	445.39	534	256.26	4776
		Area		5992		39385
86	SCC, mod	Height	445.57	341	256.25	1203
		Area		3879		10087
42	AC, well	Height	440.29	560	255.57	4641
		Area		7248		40150
50	AC, poor	Height	442.22	169	257.34	651
		Area		3733		6571
33	Small cell CA	Height	440.87	6960	255.89	6507
		Area		73486		76006

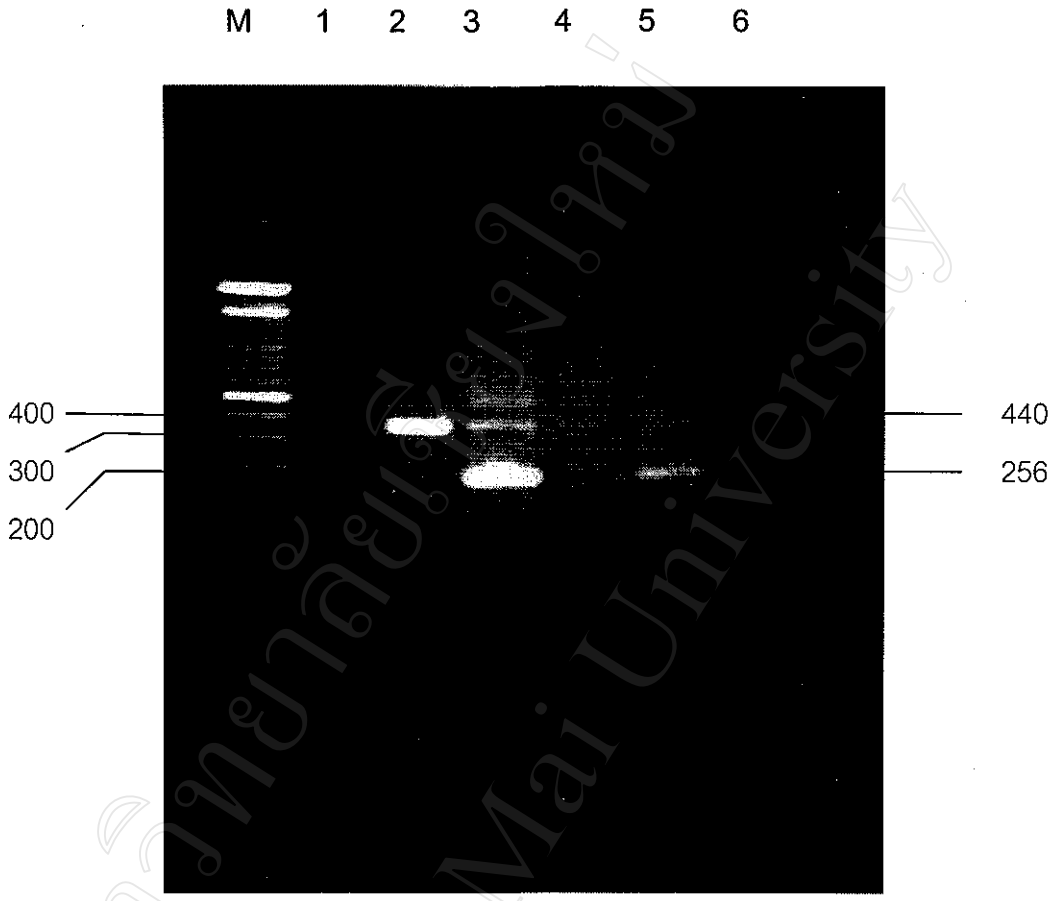


Figure 33. The illustration of the RT-PCR products of HPV 18 samples by gel electrophoresis

Lane M = 1 Kb DNA marker

Lane 1 = PCR negative control

Lane 2 = PCR positive control (HPV-18 plasmid DNA)

Lane 3 = Hela cell line RT-PCR product

Lane 4 = Hela cell line 14 RT(-)-PCR* product

Lane 5 = Sample No.42 RT-PCR product

Lane 6 = Sample No.42 RT(-)-PCR* product

RT(-)-PCR* = The transcriptase enzyme was not added .

Figure 34. The electrophoretogram of the HeLa cell line RT-PCR product by using an automatic genetic analyzer. The blue line in the top frame is the analyzed signal of the HeLa cell line, which shows two peaks of RT-PCR product. The red line in the bottom frame is the analyzed signal of size standard fragments. The blue squares in the table, indicate the analyzed data of the two peaks, (1B, 3) and (1B, 4), which refer to E6*I and full-length transcription, respectively. The red squares with the black dot in the table, indicate the analyzed data of size standard fragments.

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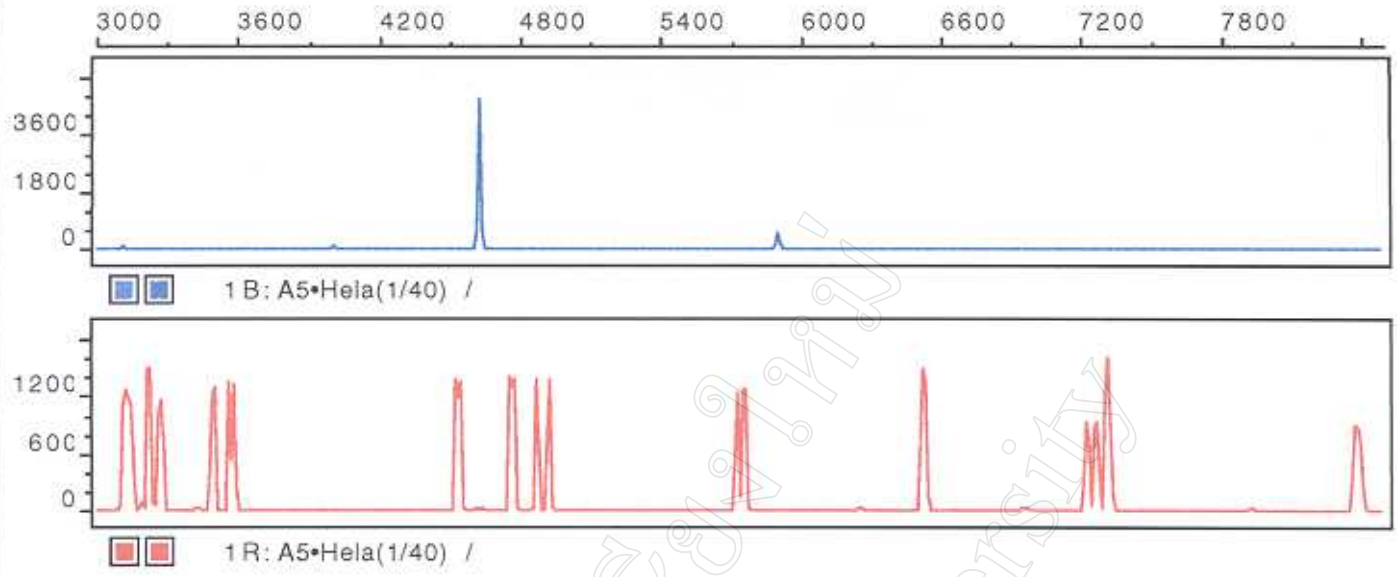

Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
1B,1	11.46	58.81	123	344	3124
1B,2	14.73	176.07	164	1235	4015
1B,3	16.99	256.26	4776	39385	4633
1B,4	21.66	445.39	534	5992	5905
1R,1	11.46	58.96	1136	7596	3125
1R,2	11.51	60.79	1274	8202	3137
1R,3	11.55	62.78	1121	6915	3150
1R,4 •	11.58	64.00	1083	6752	3158
1R,5	11.73	70.49	105	1073	3199
1R,6 •	11.85	75.00	1510	13340	3230
1R,7	11.88	76.37	100	622	3240
1R,8	11.92	77.71	104	933	3250
1R,9 •	12.01	81.00	1180	7130	3275
1R,10	12.05	82.23	1168	6893	3285
1R,11 •	12.82	108.00	1222	7327	3495
1R,12	12.87	109.72	1302	7835	3508
1R,13 •	13.09	118.00	1367	7733	3570
1R,14	13.17	120.87	1347	7728	3592
1R,15 •	16.63	244.00	1404	10737	4534
1R,16	16.68	245.78	1370	10770	4549
1R,17 •	17.47	275.00	1417	11167	4764
1R,18	17.53	278.06	1388	11166	4779
1R,19 •	17.88	299.00	1384	11157	4876
1R,20	18.07	307.77	1402	11698	4928
1R,21 •	21.02	421.00	1265	12978	5731
1R,22	21.13	425.29	1289	13340	5762
1R,23 •	23.93	539.00	1496	26881	6525
1R,24 •	26.49	674.00	953	14325	7224
1R,25 •	26.64	677.00	947	14758	7264
1R,26	26.81		1614	28329	7311
1R,27	30.69		911	12523	8370
1R,28	30.73		889	15947	8380

Figure 35 The electrophoretogram of the HPV-18 (sample No. 42) RT-PCR product by using an automatic genetic analyzer. The blue line in the top frame is the analyzed signal of the sample No. 42, which show the peak of RT-PCR product. The red line in the bottom frame is the analyzed signal of size standard fragments. The blue squares in the table, indicate the analyzed data of the peak, (3B, 3), which refer to E6*I transcription. The red squares with the black dot in the table, indicate the analyzed data of size standard fragments.

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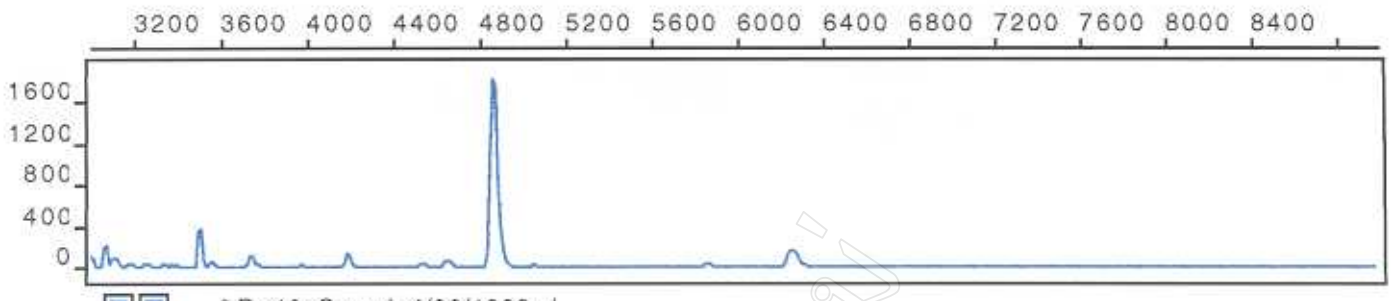
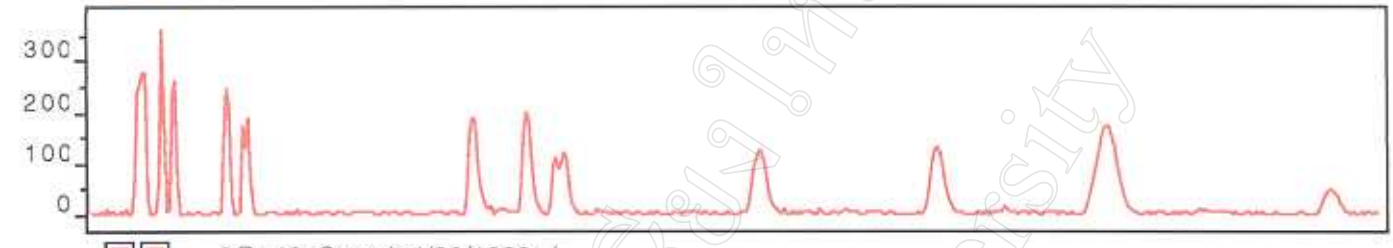


2B: 42+Sample4/20/1999 /



2R: 42+Sample4/20/1999 /

Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
2B,1	11.28	39.66	229	1765	3075
2B,2	12.88	94.39	366	5332	3513
2B,3	17.87	256.32	1833	56232	4872
2R,1	11.80	59.19	238	2024	3217
2R,2	11.84	60.84	258	2453	3229
2R,3	11.91	63.31	278	2152	3247
2R,4 •	11.93	64.00	279	2600	3252
2R,5 •	12.22	75.00	363	4401	3332
2R,6	12.41	80.22	235	2111	3383
2R,7 •	12.44	81.00	265	2848	3391
2R,8 •	13.33	108.00	246	4854	3635
2R,9	13.60	115.79	174	2153	3709
2R,10 •	13.68	118.00	190	2814	3730
2R,11 •	17.51	244.00	189	7063	4774
2R,12 •	18.41	275.00	202	7427	5019
2R,13 •	28.27	677.00	164	1252	7708
2R,14	28.29		172	1343	7715
2R,15	28.31		171	1179	7720
2R,16	28.32		166	659	7724
2R,17	28.34		172	1172	7729
2R,18	28.36		170	1441	7734
2R,19	28.39		154	3841	7742