CHAPTER THREE

RESULTS

3.1 PCR-SSP

In this study, the PCR – SSP technique was used to amplify the *GYP* hybrid gene from the genomic DNA samples and detect GP.Mur, GP.Bun, GP.Hop (148 bp), GP.HF and GP.Hut (151 bp). Presence of the *GYP* hybrid was indicated by an amplicon size between 100 bp and 200 bp; as the primer can amplify both a 148 bp DNA fragment (GP.Mur, GP.Bun, GP.Hop) and a 151 bp DNA fragment (GP.HF and GP.Hut). However, the two amplicons could not be separated by using 1.7% agarose gel electrophoresis. The band patterns from the PCR-SSP products of amplified *GYP (B-A-B)* genes were detected. For the positive control samples (HGH) the size of the product is 434 bp. In addition, when non-specific amplicons were detected, the test was repeated. The negative control (NTC) should not contain an amplified product; therefore, PCR-SPP was repeated also when product was detected in the NTC.

In the PCR-SSP, for the total of 77 samples which included in the study, the *GYP* hybrid gene was amplified in 15 out of 34 samples in Malays, 24 out of 34 in Chinese, and none was detected in 6 samples of Indians and the 3 samples of foreigners (Table 3.1).

Table 3.1: Result of PCR-SSP of Malays, Chinese and Indians.

Race	Number of samples	PCR-SSP (+ve)
Malays	34	15
Chinese	34	24
Indians	6	0
Foreigners	3	0

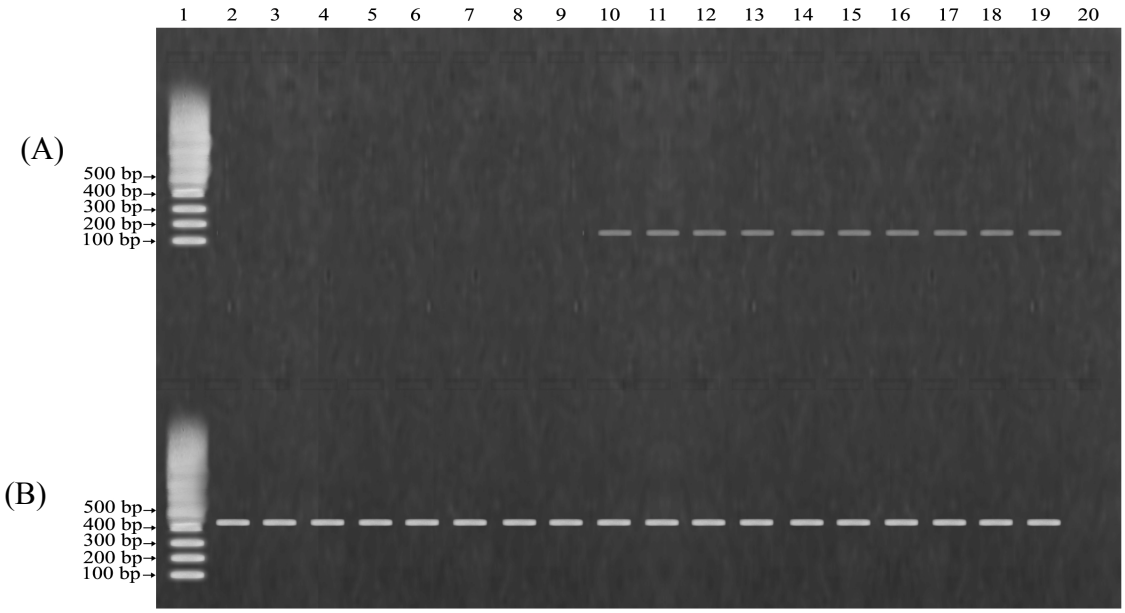

Fig. 3.1 Gel electrophoresis result of PCR-SSP products from different samples: (A) represents positive and negative results for *GYP* hybrid gene, the expected fragment sizes are 148 bp and 151 bp. Lanes 10 - 19: positive samples, Lanes 2 - 9: negative samples, Lane 20: negative control and Lane 1: 100-bp DNA Ladder Plus. (B) represents the control sample (HGH) which is 434 bp.

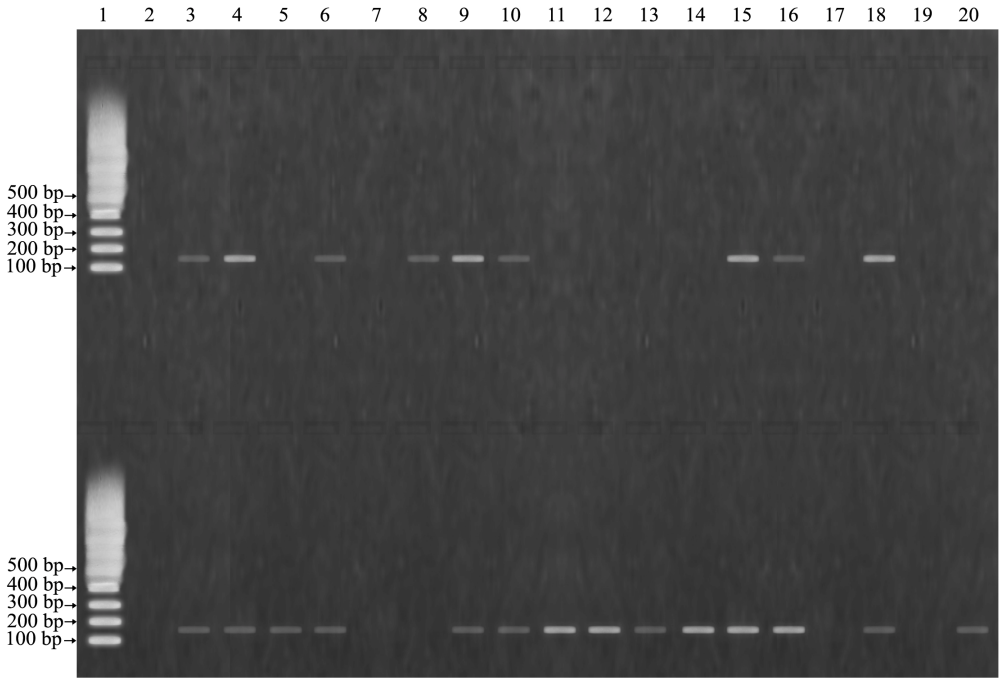

Fig. 3.2 Gel electrophoresis result of PCR-SSP products from different samples, the correct PCR product sizes are 148 bp and 151 bp, Ladder (L) is 100 bp and negative control is NTC (non template control).

Table 3.2: Amplification results of PCR products as in Fig. 3.2, including the different ethnic groups; M: Malays, C: Chinese, I: Indians and N: Non- Malaysian. From lane 1 – 20; lane 1: 100 bp DNA ladder, lanes (2 – 19): positive (+) and negative (-) sample and lane 20: negative control (NTC).

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**Lane No.**
L	50	49	48	46	43	44	42	41	40	27	26	25	24	23	22	5	4	3	NTC	**Sample No.**
	C	C	C	I	C	C	C	C	C	C	C	C	C	C	C	N	C	C		**Race**
	-	+	+	-	+	-	+	+	+	-	-	-	-	+	+	-	+	-	-	**Results**
L	78	77	76	75	70	69	68	64	63	62	61	60	59	58	57	56	55	54	53	**Sample No.**
	C	C	C	C	C	N	N	C	C	C	C	C	C	C	C	C	C	C	C	**Race**
	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	-	+	**Results**

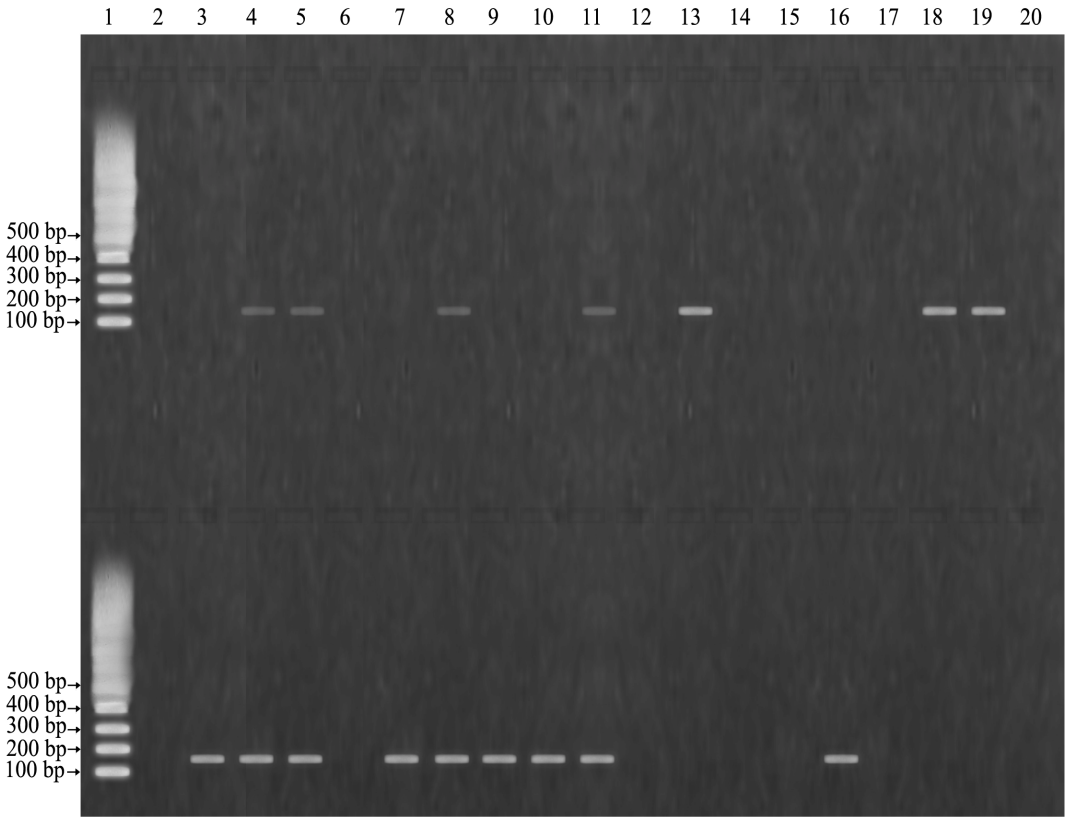

Fig. 3.3 Gel electrophoresis result of PCR-SSP products of different samples.

Table 3.3: Amplification results of PCR products as in Fig. 3.3, including the different ethnic groups; M: Malays, C: Chinese and I: Indians. . From lane 1 – 20; lane 1: 100 bp ladder, lanes (2 – 19): positive (+) and negative (-) sample and lane 20: negative control (NTC).

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**Lane No.**
L	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	2	1	NTC	**Sample No.**
	M	M	M	M	M	I	M	I	M	M	M	M	M	M	M	M	M	M		**Race**
	-	-	+	+	-	-	+	-	-	+	-	+	-	-	-	-	+	+	-	**Results**
L	66	65	52	47	45	43	42	39	38	37	36	35	34	33	32	31	30	29	28	**Sample No.**
	I	M	C	M	M	C	C	M	M	M	M	M	M	M	M	I	M	M	M	**Race**
	-	+	+	+	-	+	+	+	+	+	-	-	-	-	+	-	-	-	-	**Results**

3.2 Optimization of PCR-SSP

Although the PCR-SSP technique was performed in this study as monoplex PCR, it was initially started as a multiplex by using two sets of primers including the internal control (IC) primers. However, the amplified products were only for the IC (434 bp) even various dilutions from the stock or concentrated solution of the IC primers were done; for instance, 1:10 and 1:20. Fig.3.4 is an example for 1:10 dilution of IC primers.

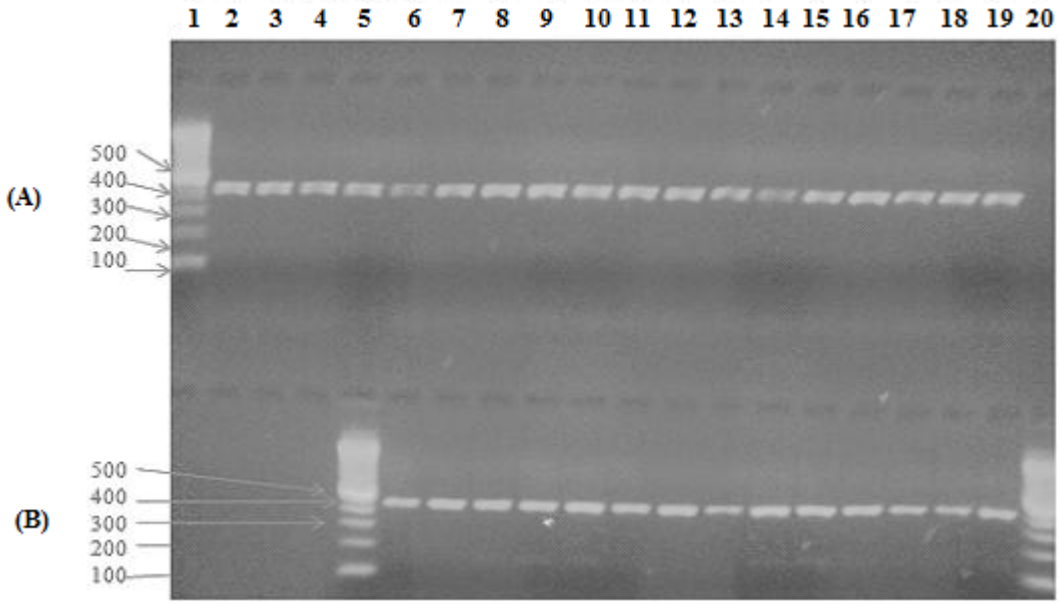

Fig. 3.4 The amplification results of the PCR-SSP products, gel electrophoresis image represents results of amplification products for different samples using PCR-SSP as a Muliplex. The bands explain that the ampilification results were for the IC (434 bp); lanes 2 – 19 (A) and 6 – 19 (B): different samples, lane 1(A), lane 5(B) and lane 20(B): 100 bp DNA ladder.

3.3 Comparison of PCR-SSP Results In Different Ethnic Populations

The PCR-SSP technique which was used in this study detected and confirmed the presence of one of the *GYP* hybrid gene (*GYP B-A-B*) in different ethnic group of Malaysian populations (Malays, Chinese and Indians) and showed difference in prevalence rates between them. It was detected more commonly in Chinese compared to Malays.Whereas not detected in Indian ethnic group (Table 3.4) and (Fig. 3.4).

Table 3.4: Result of PCR-SSP for Malays, Chinese and Indians as a percentage (%); compared to the total number of each ethnic group.

Race	Number of samples	PCR-SSP (+ve)	%
Malays	34	15	44.1 %
Chinese	34	24	70.6 %
Indians	6	0	0 %

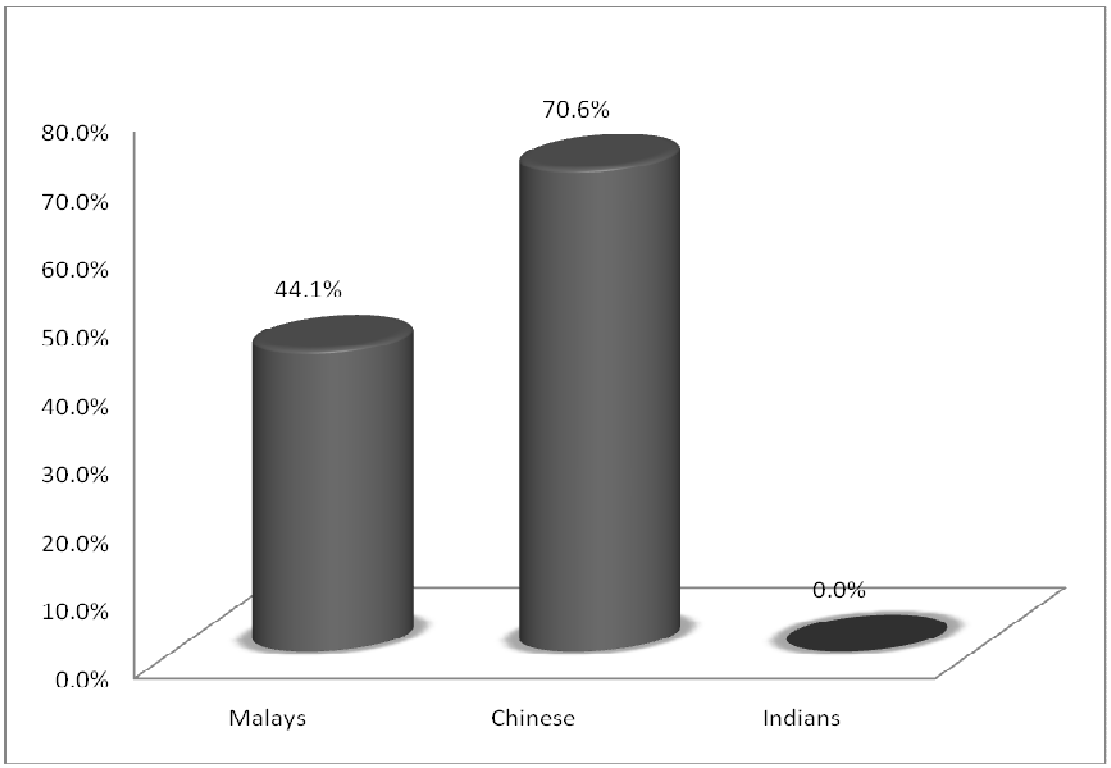

Fig. 3.5 Percentage of *GYP* hybrid genes for different ethnic groups; Malays, Chinese and Indians.

Overall, the results of this study identified the presence of the *GYP* hybrid gene in Chinese and Malays but not in Indians.