GenePhile G-Plex

Human Autosomal STR Mapping Set

16 Powerful non-CODIS STR loci in a single PCR reaction

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The GenePhile G-Plex PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 15 non-CODIS autosomal STR loci and an amelogenin gender determining marker in a single PCR reaction. The G-Plex kit contains all the necessary reagents for the amplification of human DNA.
Each G-Plex kit contains materials sufficient to perform 50 reactions at a 25uL reaction volume.

GenePhile G-Plex PCR Amplification Kit

Kit Components	• 5X PCR buffer 400ul • G-Plex Primer mix 125ul • GenePhile Taq Polymerase(5U/ul) 13ul • Control DNA K01 (10ng/ul) 50ul • G-Plex Allelic Ladder 20ul
Number of Reactions	50
Reaction Volume	25 µL
Loci in Kit	Amelogenin, D21S1437, D22S683, D8S1110, D10S2325, D12S1090, D17S1294, PentaD, D3S1744, D14S608, D20S470, Penta E, D4S2366, D18S536, D13S765, D6S474
Amplicon Allele Size Range	100-460 base pairs
Dyes Used	6-FAM™, VIC®, NED™, PET®, LIZ® dyes
Storage Conditions	• 5X PCR buffer • G-Plex Primer mix • Control DNA (K01) Store at 2 to 8°C. • GenePhile Taq Polymerase Store at -15 to -25°C • G-Plex Allelic Ladder Store at -15 to -25°C upon receipt, 2 to 8°C after initial use
Matrix and Size Standards	Matrix Standards: ThermoFisher DS-33 Matrix Standard kit(Dye Set G5) (PN 4345833) Size standards: ThermoFisher GeneScan™ 500 LIZ® dye Size Standard (PN 4322682) ThermoFisher GeneScan™ 600 LIZ® dye Size Standard (PN 4366589)

IMPORTANT! The fluorescent dyes attached to the primers are light sensitive. Protect the primer mix from light when not in use. Amplified DNA, Allelic Ladder, and GeneScan 500/600 LIZ Size Standards should also be protected from light. Keep freeze-thaw cycles to a minimum.

1. G-Plex kit loci and alleles

The G-Plex kit uses a five-dye fluorescent system for automated DNA fragment analysis. The following table shows the loci amplified by the G-Plex kit and the corresponding dyes used. The GenePhile G-Plex Kit Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotypes of the Control DNA 9947A and K01 are listed in the table.

Locus	Designation Alleles Included in GPlex	Dye Label	9947A Genotype	Control DNA K01 Genotype
AMEL	X,Y	6-FAM™	X,X	X,Y
D21S1437	7-18		11,12	13,14
D22S683	9-22		12,16	12,20.2
D8S1110	23-31		28,28	27,27
D10S2325	6-34	VIC®	9,10	7,13
D12S1090	6-17		6,11	6,10
D17S1294	7-20		14,14	15,17
PentaD	5-18		12,12	11,14
D3S1744	11-22	NED™	17,17	18,20
D14S608	5-14		7,11	6,10
D20S470	6-21		15,16	14,17
PentaE	5.2-27		12,13	11,13
D4S2366	9-15	PET	11,13	9,11
D18S536	8-15		10,11	11,11
D13S765	7-13		8,9	9,10
D6S474	12-18		13,17	14,15

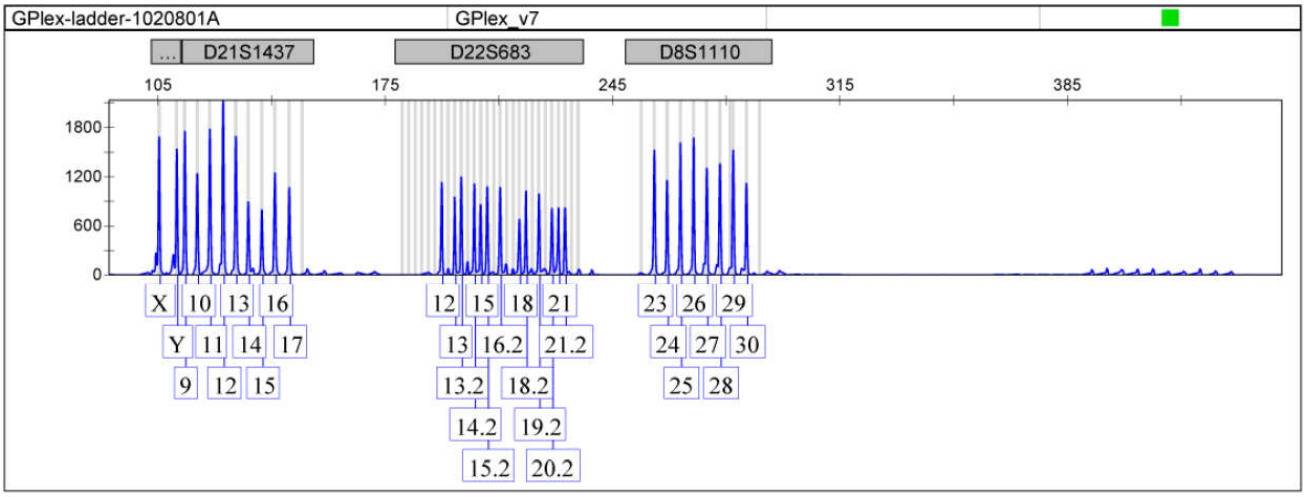
2. PCR reaction

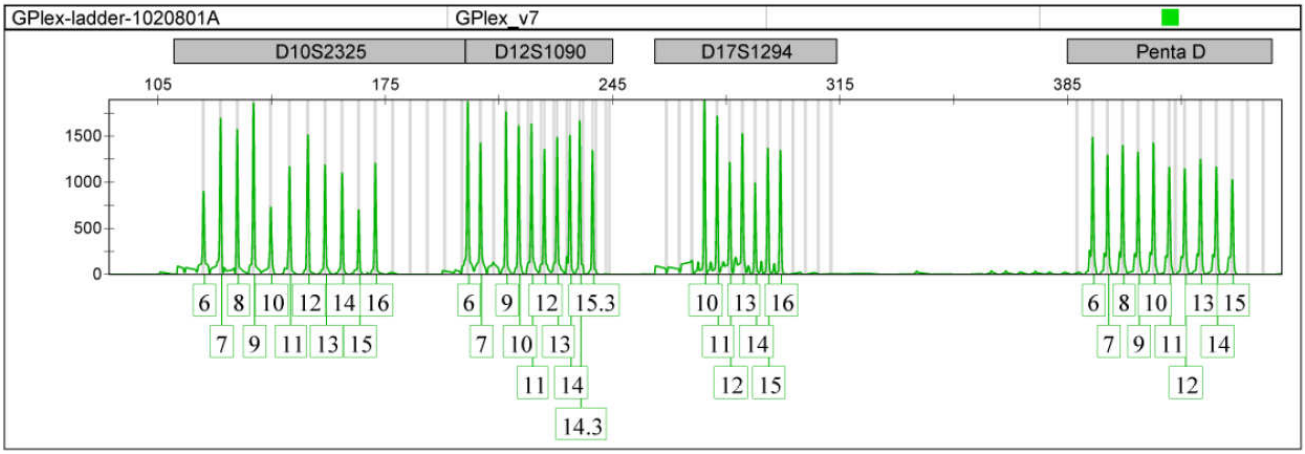
5X PCR Buffer	5.0ul
G-Plex Primer Mix	2.5ul
GenePhile Taq Polymerase(5U/ul)	0.15ul
dd H_2O	14.8ul
Genomic DNA(2~10 ng/ul)	2.5ul
Total	25.0ul

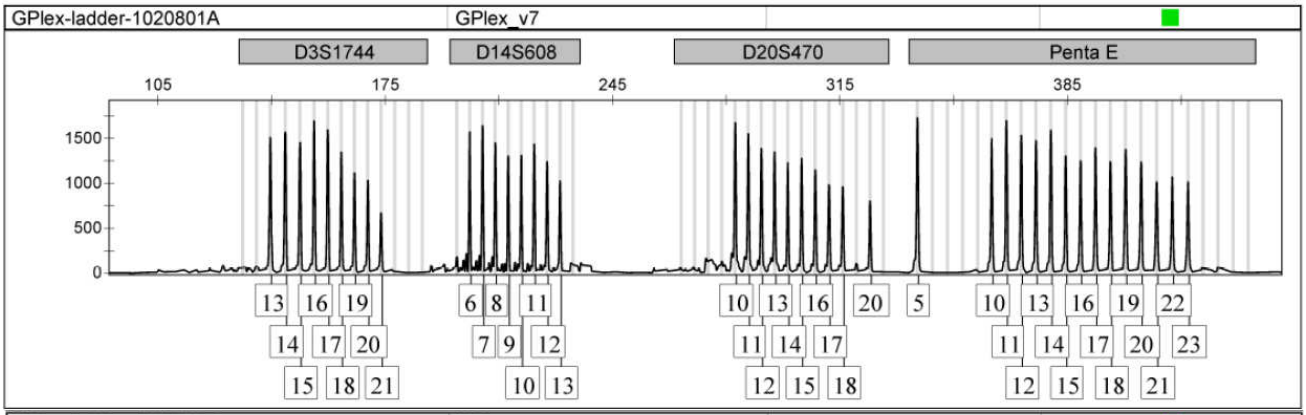
3.PCR cycle

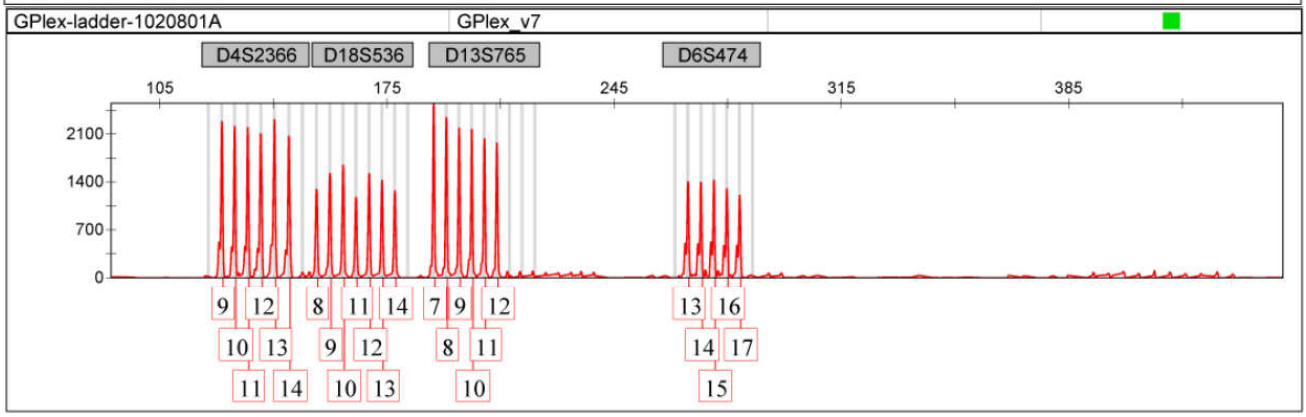
Initial Incubation Step	Cycle (32 cycles)			Final Extension	Final Hold
	Denature	Anneal	Extend		
95 ˚C	94 ˚C	59 ˚C	72 ˚C	60 ˚C	4 ˚C
10 min	1 min	1 min	1 min	45 min	Hold

4. GPlex Allelic Ladder Profile: (Load 1ul Ladder DNA per well, in 10ul Hi-Di-LIZ500/600 mixture)



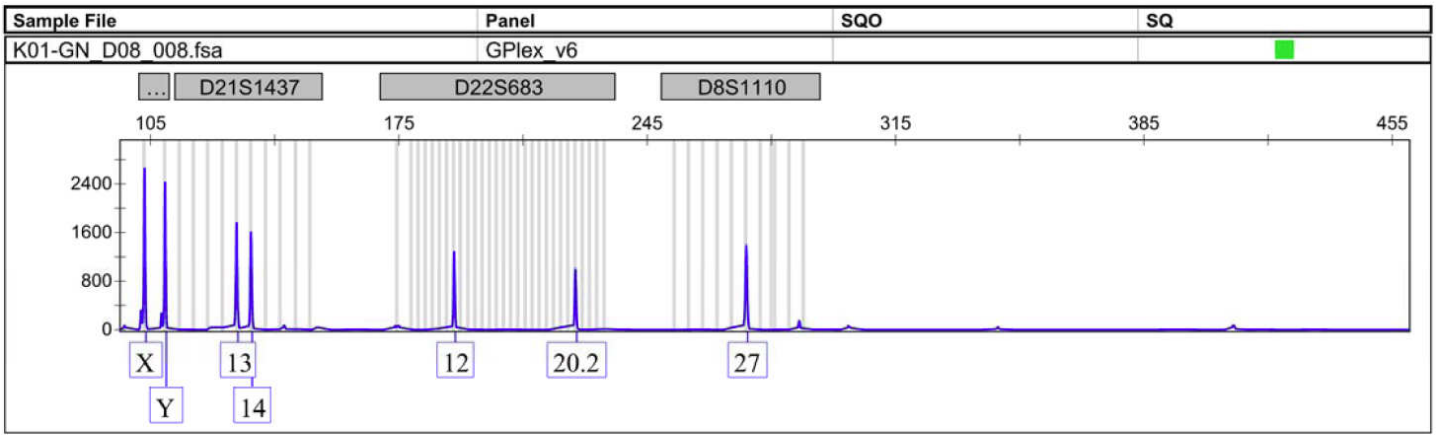


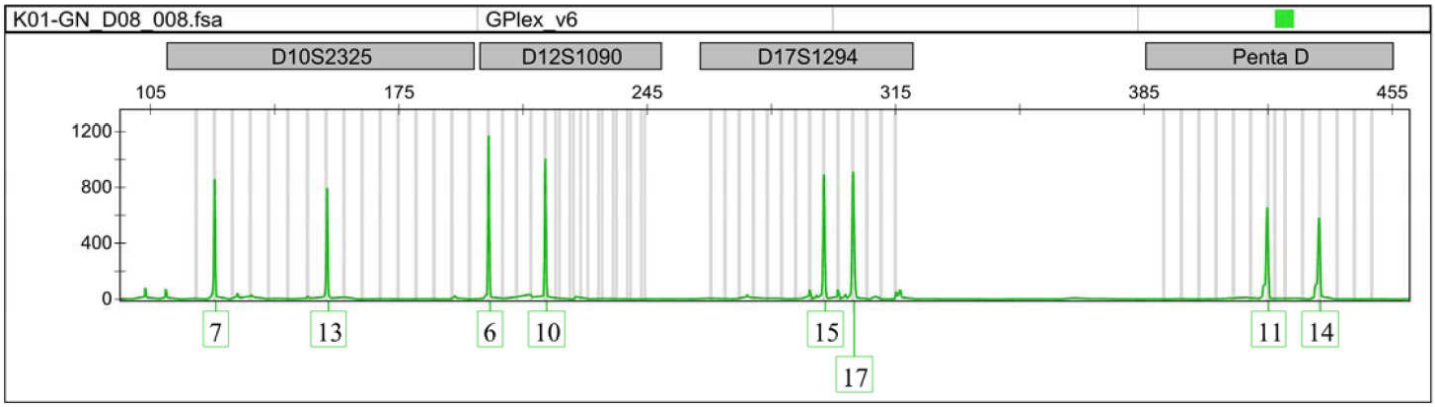


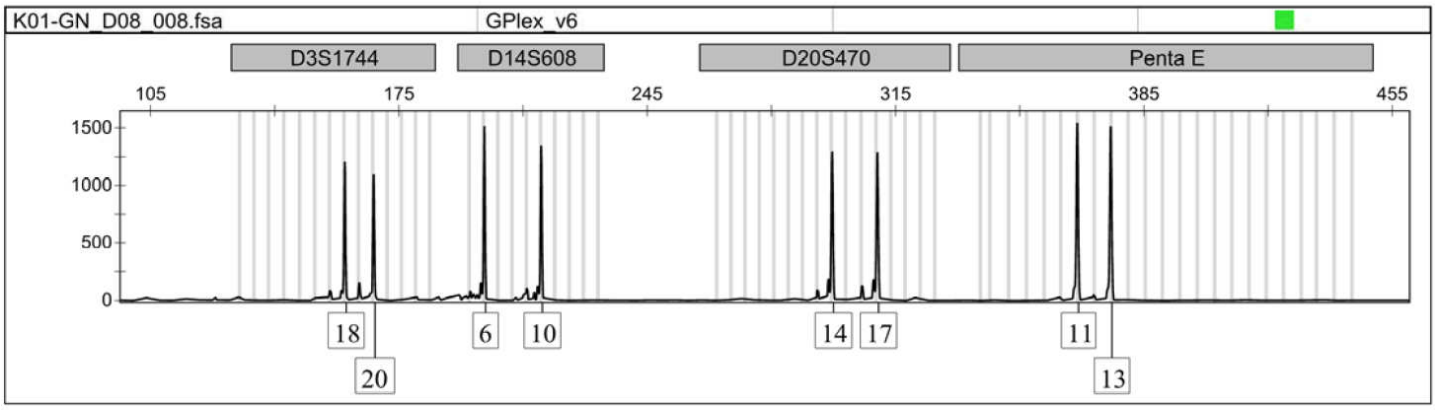


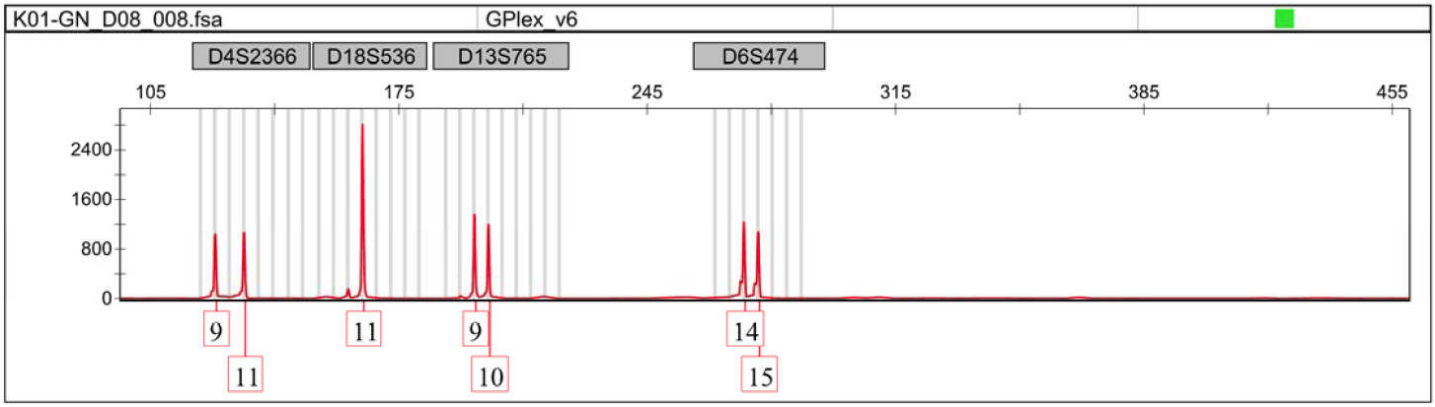
5. Control DNA K01 profile:

Control DNA K01 is a positive control for evaluating the efficiency of the amplification step and STR genotyping using the GenePhile G-Plex Kit Allelic Ladder.









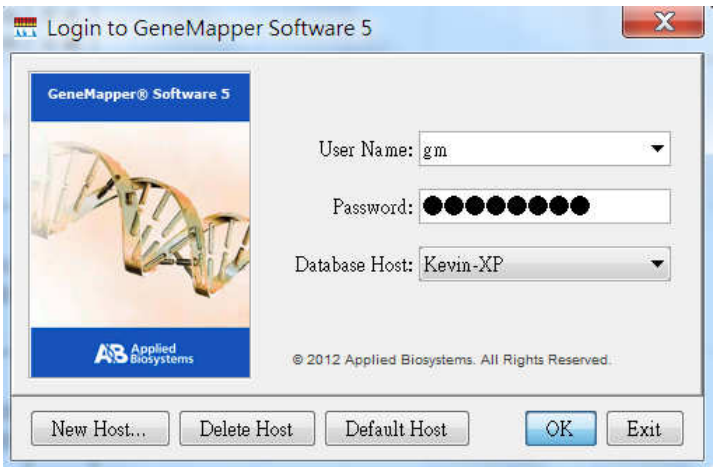
6. First-time installization of GPlex Panel & Binset: (For ABI GeneMapper users)

Download panel and bin set from website: http://www.genephile.com.tw/products/GPlex.rar

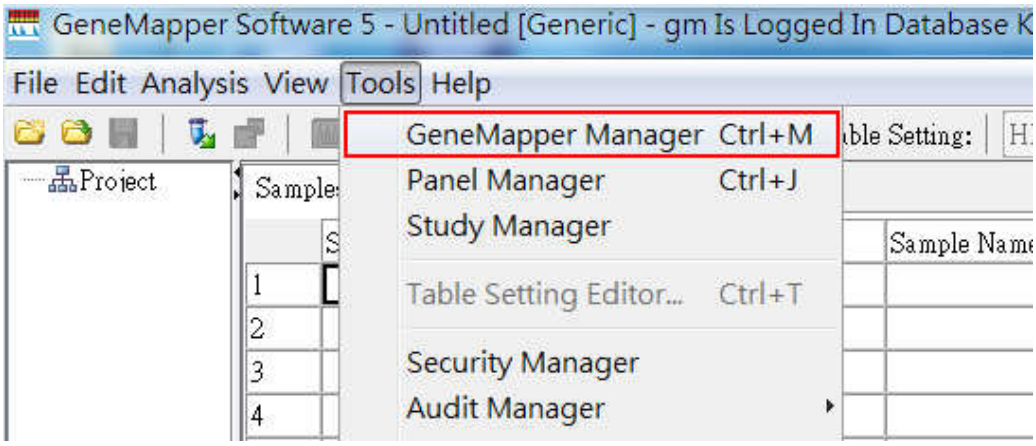
a. Save and Unzip the **GPlex.rar** to the desktop on your PC.

b. Run '**GeneMapper**' software

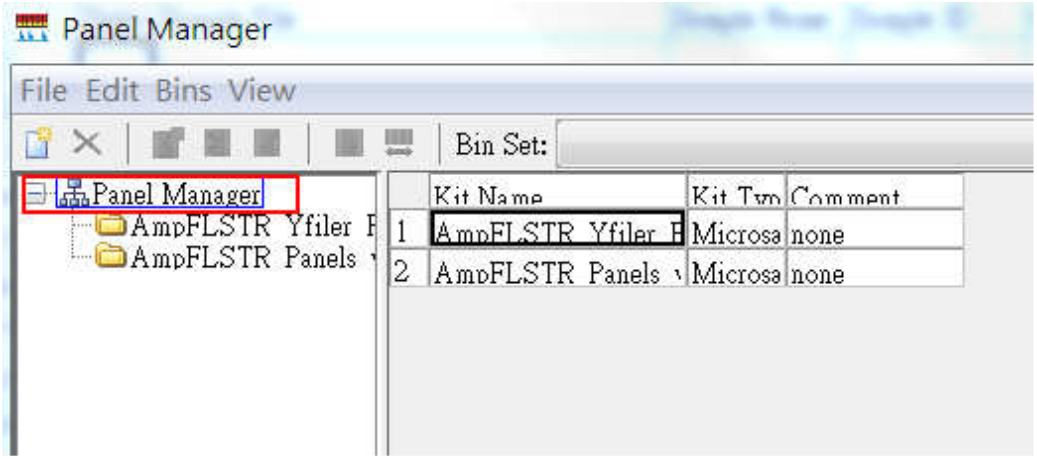
c. Login with registered user Name and Password.



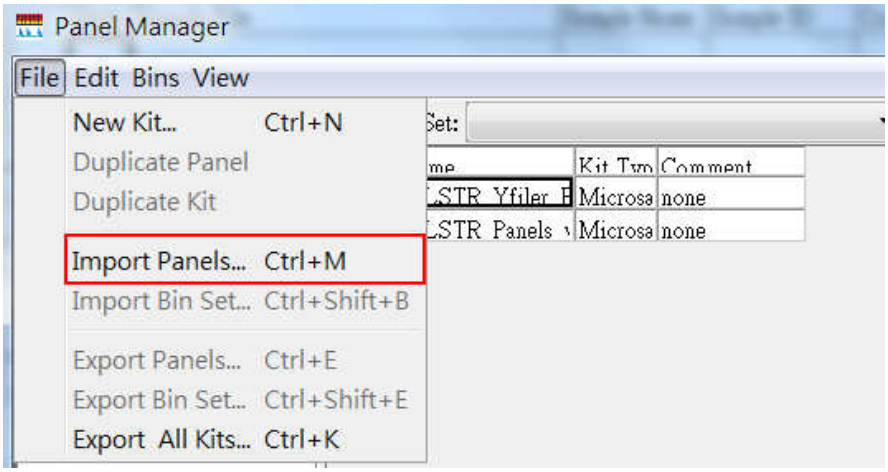
d. Select '**Tools**' → '**Panel Manager**'



e. Click on '**Panel Manager**'



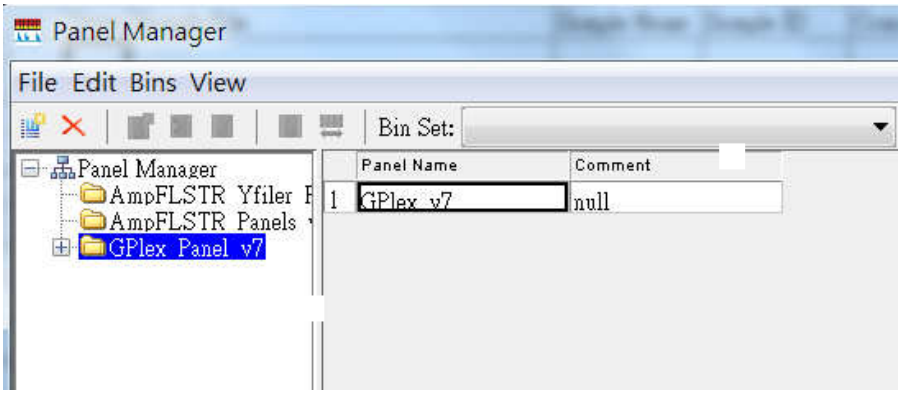
f. '**File**' → '**Import Panels**'



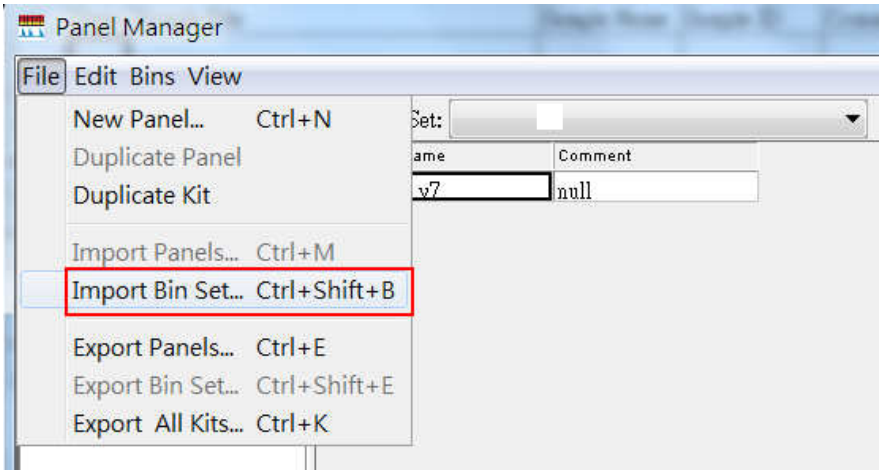
g. Direct to the file named '**GPlex_Panel_v7.txt**'

h. Click 

i. Click on '**GPlex_Panel_v7**'

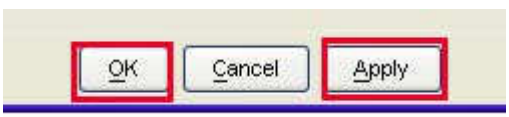


j. '**File**' → '**Import Bin Set**', direct to the file named '**GPlex_Binset_v7.txt**'



k. Click 

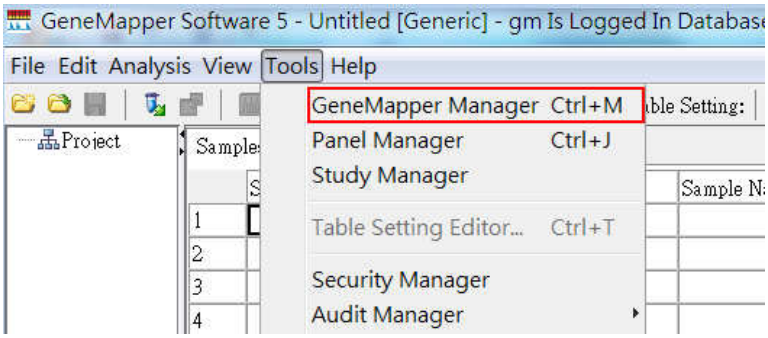
l. Click '**Apply**' or '**OK**' and exit.



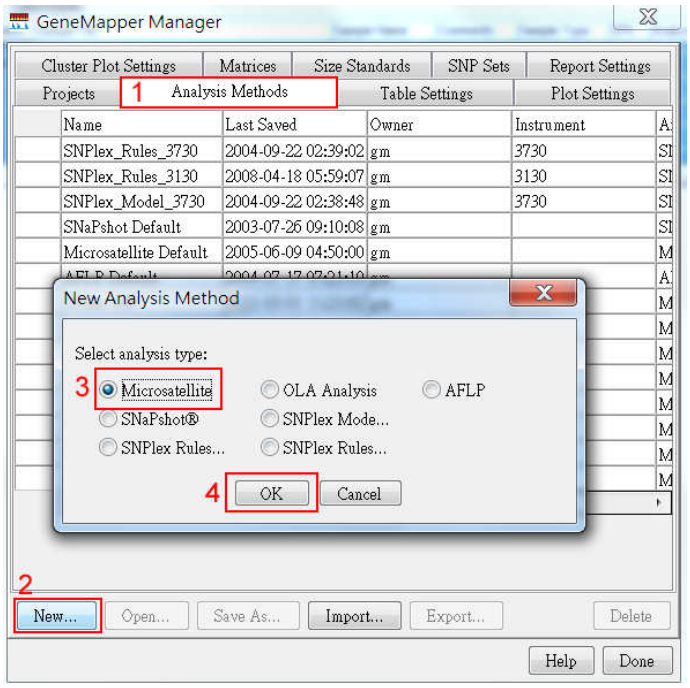
7. First-time installation of GPlex Analysis method: (For ABI GeneMapper ID users)

a. Start **GeneMapper** software, login with **User Name** and **Password**.

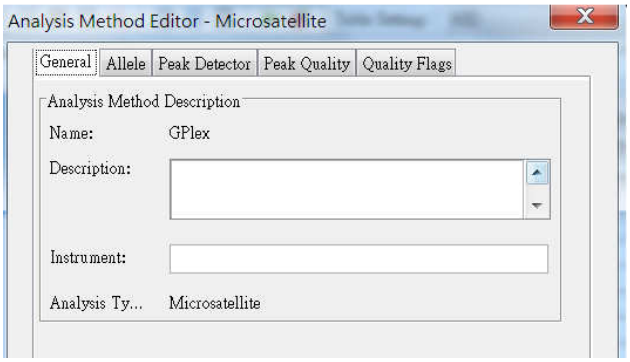
b. Click '**Tools**' → '**GeneMapper Manager**'

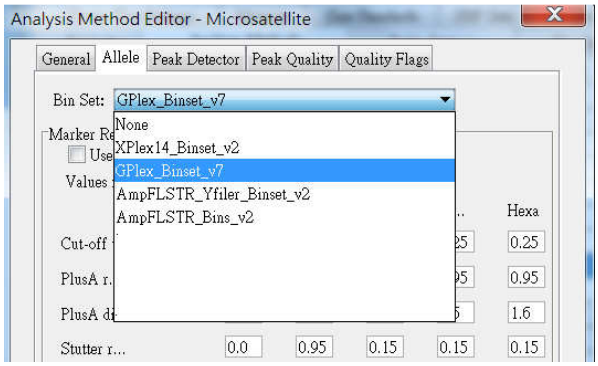


c. Select '**Analysis Methods**' 1 sheet → '**New**' 2 → '**HID**' 3 → '**OK**' 4

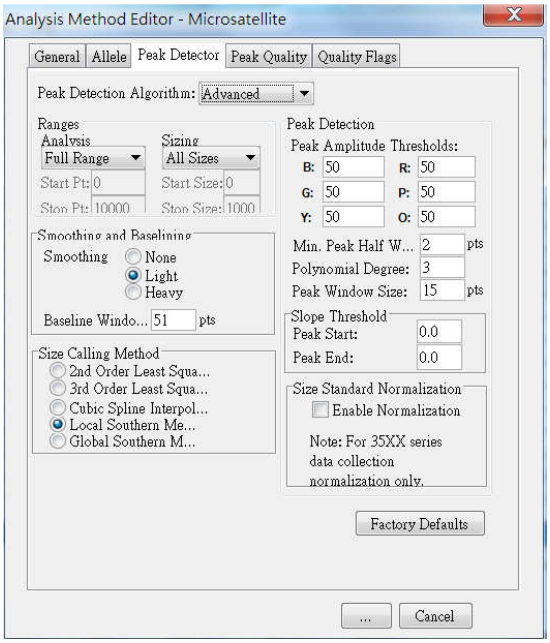


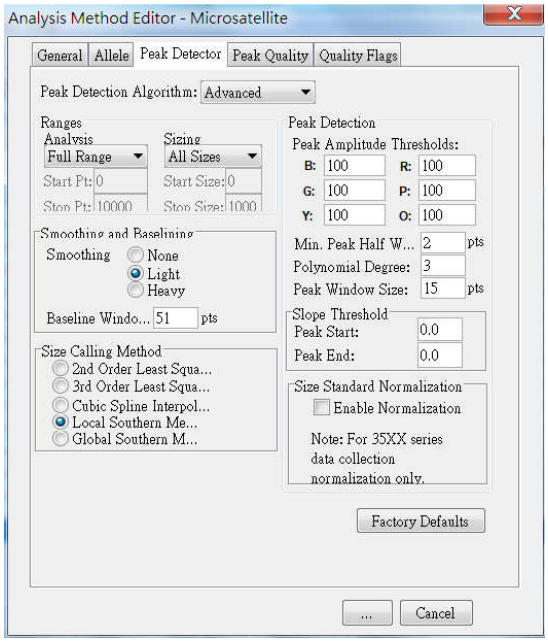
d. Assign a name for this method: '**GPlex**'. In the '**Allele**' sheet, select '**GPlex_Binset_v7**'.





e. In the '**Peak Detector**' sheet, users can modify the peak amplitude thresholds to meet analytic requirements. Press '**OK**' to finish a setting.

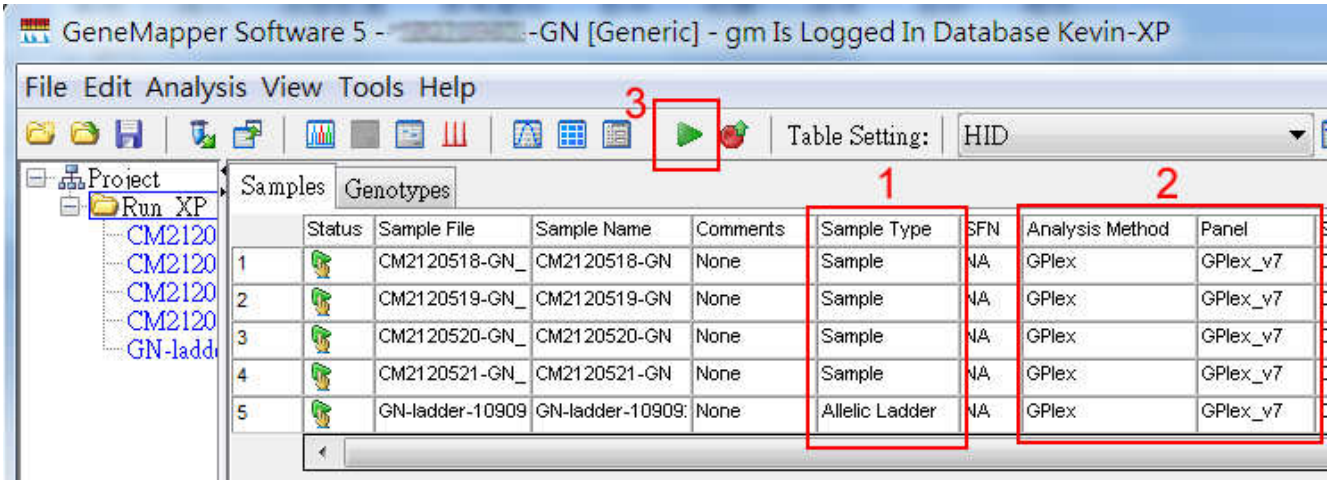




8. Perform a GPlex analysis:

a. In the sample list view, create a new project and import a "**GPlex ladder**" file and "**sample**" files. (Note: GPlex ladder file and sample files must be placed in a same folder.)

b. Assign sample types [1], set Analysis Method to '**GPlex**' and Panel to '**GPlex_v7**' [2]. Finally, press  button to start an analysis [3].



c. Select samples and perform genotypes.

9. Publication:

Hwa HL, Chang YY, Lee J CI, Yin HY, Tseng LH, Su YN, Ko TM. **Fourteen non-CODIS autosomal short tandem repeat loci multiplex data from Taiwanese.** *Int J Legal Med. 2011 Mar;125(2):219-26.*

Hwa HL, Chang YY, Lee JC, Lin CY, Yin HY, Tseng LH, Su YN, Ko TM. **Fifteen non-CODIS autosomal short tandem repeat loci multiplex data from nine population groups living in Taiwan.** Int J Legal Med. 2012 Jul;126(4):671-5.

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