EzWay™ Human STR ID-23 System

1. Catalog No. KMHID-23

2. No. of Applications 100 Tests

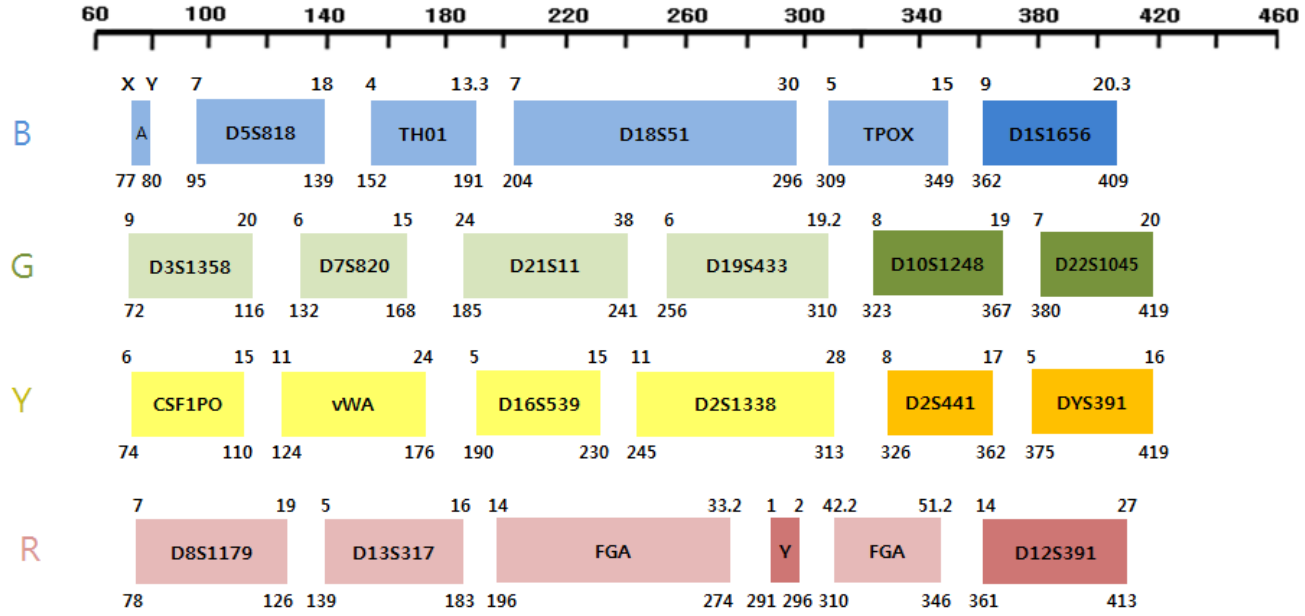
3. Storage EzWay Human STR ID-23 System is shipped on gel ice. Store the kit at -20°C on arrival. Repeated freezing and thawing of the components will affect the performance of the kit and must be avoided. Avoid prolonged exposure to the light
When stored under these conditions and handled correctly, the products can be kept at least until the expiration date without any reduction in performance. **When using control DNA, store it at 2-8 °C and avoid freezing and thawing repeatedly.**

4. Contents

Component	Volume
10x Hot STARplus PCR Buffer	0.5 ml
10x Hot Taq-fx DNA polymerase (5U/uL)	150 uL
3.3x STR ID-23 Primer Mix	0.75 ml
STR ID-23 Allelic ladder (Ver.1.1.8)	25 uL
9948 Control DNA (1ng/uL)	25 uL

5. Description EzWay Human STR ID-23 System is a multiplex PCR assay system that amplify the sex-determining marker (amelogenin), Y-InDel (M175, rs2032678), Y-chromosomal STR (DYS391) and 20 autosomal STR loci (D5S818, TH01, D18S51, TPOX, D1S1656, D3S1358, D7S820, D21S11, D19S433, D10S1248, D22S1045, CSF1PO, vWA, D16S539, D2S1338, D2S441, D8S1179, D13S317, FGA and D12S391).

EzWay Human STR ID-23 System contains all FBI recommended CODIS 12 core loci, 20 extended loci and ENFSI recommended 12 European Standard Set.



Loci	Chr.	Acc. No.	Repeats	Control DNA		STR ID-23		Global Filer	PowerPlex Fusion	CODIS 13	CODIS 20	ESS 12
				9948	2800M	Dye label						
Amelogenin	Xp, YP	NG_012040.1, NG008011.1	-	X, Y	X, Y	Blue	O	O	O		O	
D5S818	5q	AC008512.8	AGAT	11, 13	12, 12		O	O	O	O	O	
TH01	11p	D00269.2	TCAT	6, 9.3	6, 9.3		O	O	O	O	O	O
D18S51	18q	AP001534.2	AGAA	15, 18	16, 18		O	O	O	O	O	O
TPOX	2p	AC105450.1	AATG	8, 9	11, 11		O	O	O	O		
D1S1656	1q	G07820.1	TAGA	14, 17	12, 13		O	O	O		O	O
D3S1358	3p	AC099539.2	TCTR	15, 17	17, 18	Green	O	O	O	O	O	O
D7S820	7q	AC004848.1	GATA	11, 11	8, 11		O	O	O	O	O	
D21S11	21q	AP001675.1	TCTR	29, 30	29, 31.2		O	O	O	O	O	O
D19S433	19q	AC008507.11	WAGG	13, 14	13, 14		O	O	O		O	
D10S1248	10q	AL391869	GGAA	12, 15	13, 15		O	O	O		O	O
D22S1045	22q	AL022314	ATT	16, 18	16, 16		O	O	O			O
CSF1PO	5q	AC011382.4	AGAT	10, 11	12, 12	Yellow	O	O	O	O	O	
vWA	12p	M25858.1	TCTR	17, 17	16, 19		O	O	O	O	O	O
D16S539	16q	AC024591.3	GATA	11, 11	9, 13		O	O	O	O	O	
D2S1338	2q	AC010136.8	TKCC	23, 23	22, 25		O	O	O		O	
D2S441	2p	AC079112	TCWA	11, 12	10, 14		O	O	O		O	O
DYS391	Yq	AC011302	TCTA	10, 10	10, 10		O	O	O		O	
D8S1179	8q	AF216671.7	TCTR	12, 13	14, 15	Red	O	O	O	O	O	O
D13S317	13q	AL391354.12	TATC	11, 11	9, 11		O	O	O	O	O	
FGA	4q	AC107385.4	YTYY	24, 26	20, 23		O	O	O	O	O	O
Y-M175	Yq	rs2032678	TTCTC	2, 2	2, 2		O	O				
D12S391	12p	G08921	AGAY	18, 24	18, 23		O	O	O		O	O
SE33								O				
Penta D									O			
Penta E									O			

All Kits keep the same primer sequences to sustain kit concordance between evidence samples and reference samples when used in criminal DNA database.

The kits are designed for use with the following Applied Biosystems instruments* :
- Applied Biosystems 310 Genetic Analyzer filled with POP-4 & 36cm capillaries
- ABIPRISM® 3100/3100-Avant Genetic Analyzer filled with POP-4 & 36cm capillaries
- Applied Biosystems 3130/3130xl Genetic Analyzer filled with POP-4 & 36cm capillaries
- GeneAmp® PCR System 9600/9700/Veriti

* ABIPRISM® 3500/3700/3730(xl) Genetic Analyzer or Genetic Analyzers filled with POP-4™ polymer maybe used for the analysis of EzWay Human STR ID-23 System upon one's own risk.

Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes(B,G,Y and R dyes) are used in the EzWay Human STR ID-23 System to label samples and the fifth dye, LIZ®, is used to label the GeneScan™ 600 LIZ® (60-460) Size Standard. Either Filter Set G5 or its compatible BQ5 matrix standard may be used for multicomponent analysis of the kit.

6. Procedure

A. Materials & Reagents Needed*

- 36cm capillaries, 47 cm x 50 ㎛ (Applied Biosystems, Foster City, CA)
- Performance Optimized Polymer (POP-4™ Polymer)
- **DS-33** Matrix Standard for 3100/3130 [G5 Filter Set]
- Run module: **HID_FragmentAnalysis36_POP4**
- GeneScan™ 600 LIZ™(60-460) Size Standard
- Hi-Di™ Formamide
- Dry heating bl ck, water bath, or thermal cycler

* for ABI PRISM® 310/3100(Avant)/3130(XL) Genetic Analyzer.

B. PCR

PCR Component	Volume/Sample(uL)
10x HotSTARplus buffer	2.5
Hot Taq-fx DNA pol.(5U/uL)	1.5
3.3x STR ID-23 Primer Mix	7.5
Purified Genomic DNA (1ng/uL)	1.0
DW	Add to make final 25uL

C. Thermal Cycling

95°C for **15** minutes, then:

94°C for 20 seconds
59°C for 90 seconds
72°C for 60 seconds
for 29 cycles, then:

60°C for 60 minutes
4°C soak

D. Creating Matrix

According to the ABI PRISM® 3100/3130(XL) Genetic Analyzer User's Manual

E. Preparing the Sample

EzWay Human STR ID-23 System has been optimized for electrophoresis with the ABI PRISM 3100-Avant Genetic Analyzer, ABI PRISM 3100/3130(XL) Genetic Analyzer filled with POP-4™ polymer.
In addition to the instructions below, please refer to the instrument User Guides for electrophoresis details.
The quantity of the microsatellite PCR products varies depending on the amount and quality of the template used for the PCR reactions. When you first start to use EzWay Human STR ID-23 System, we recommend preparing a dilution series of the PCR products and running electrophoresis in order to optimize the allele fluorescence intensities.
For this experiment, use undiluted PCR products and 1:5, 1:10, 1:20 and 1:40 PCR product dilutions in H2O.
We recommend optimizing both the DNA template amount for PCR and the amount of PCR product used for electrophoresis so that the allele fluorescence intensities fall between 1000 ~ 4000 RFU. Peaks lower than ~300 RFU and higher than ~6000 RFU should be interpreted with caution.

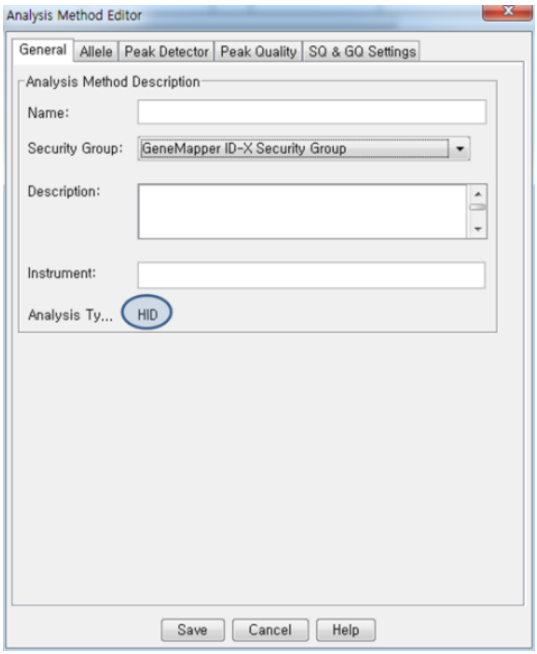
1. Prepare a loading cocktail by combining and mixing the 0.1μL GeneScan™ 600 LIZ™ (60-460) Size Standard and 10.0μL Hi-Di™ formamide per sample.
2. Vortex for 10 seconds.
3. Combine 10μL of the prepared loading cocktail and 1μL of the PCR product.
4. Note: It is recommended that at least 1 ladder should be run in a batch.
5. Preparing the allelic ladder, combine 10μL of the prepared loading cocktail and 1.0 μL of the allelic ladder mix. Vortex the allelic ladder mix prior to pipetting.
6. Seal the plate. Denature the samples and ladder by heating at 95°C for 2 minutes and immediately chill on crushed ice. Denature the samples just prior to loading
7. Place the plate in an auto-sampler tray and close the instrument doors.
8. Select the HID_FragmentAnalysis36_POP4 run module and dye set G5.
9. Begin electrophoresis according to ABI PRISM User Guide instructions.

F. Analysis and interpretation of the results

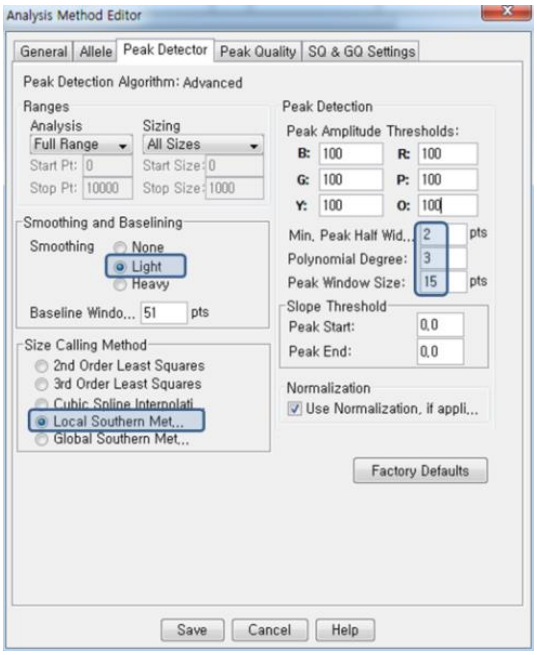
1. Prior to analysis, open the project of GeneMapper ID software and confirm that analysis type of Analysis Method Editor is HID module.
2. Import STR ID-23 Panel, Bin Set and Marker Stutter for GeneMapper ID-X software sequentially using Panel Manager. All files are supplied by email upon request after you purchase the kit.

3. Set analysis parameter as the followings.
   (The setup of the ABI PRISM 35XX series equipment is also performed according to this procedure.)

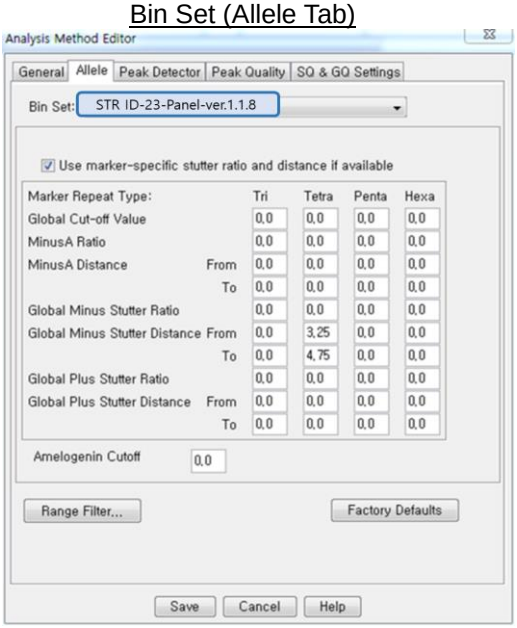
Analysis Type (General Tab)



Analysis Parameter (Peak Detector Tab)



Bin Set (Allele Tab)



4. Make New Project and Add Samples to the Project. Before adding samples to the project, confirm that ladder file is located in the same folder with the samples.

5. Designate ladder files as allelic ladder and then all parameters such as analysis method, panel, size standard and etc. have been designated properly, press start button.

6. After Analysis, save the project

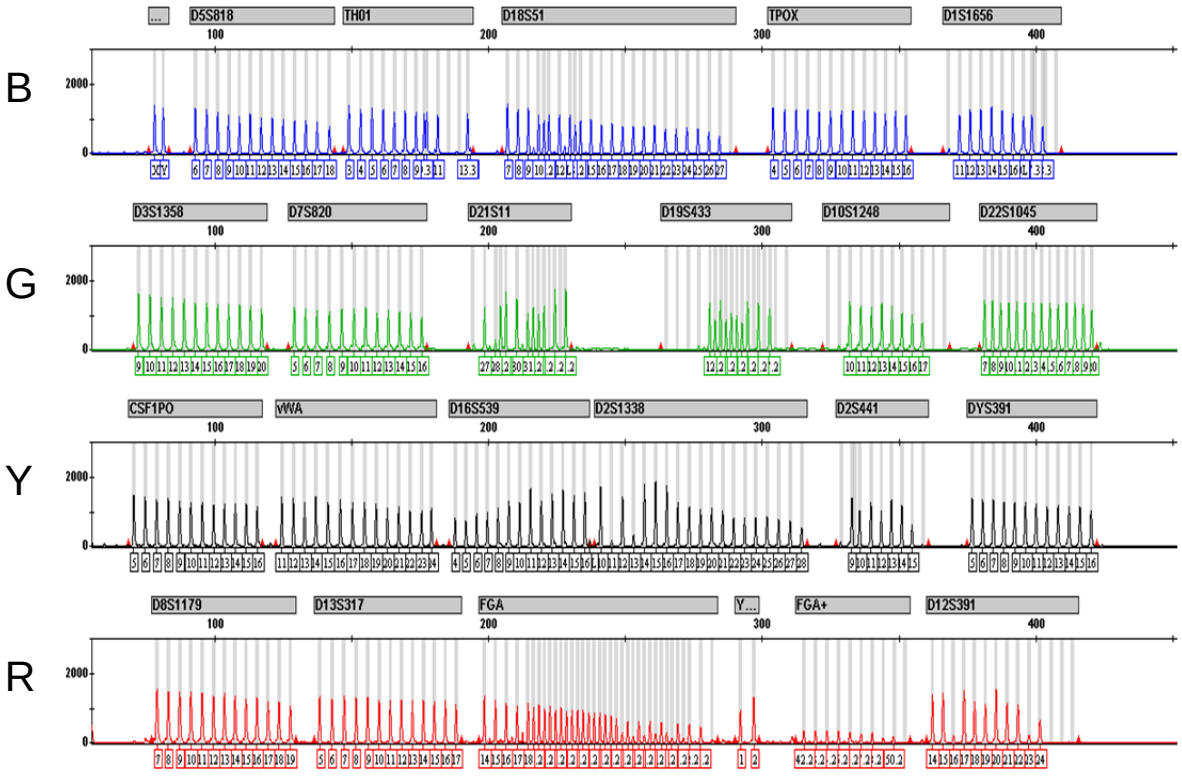


Fig.1. The allelic ladder for EzWay Human STR ID-23 System

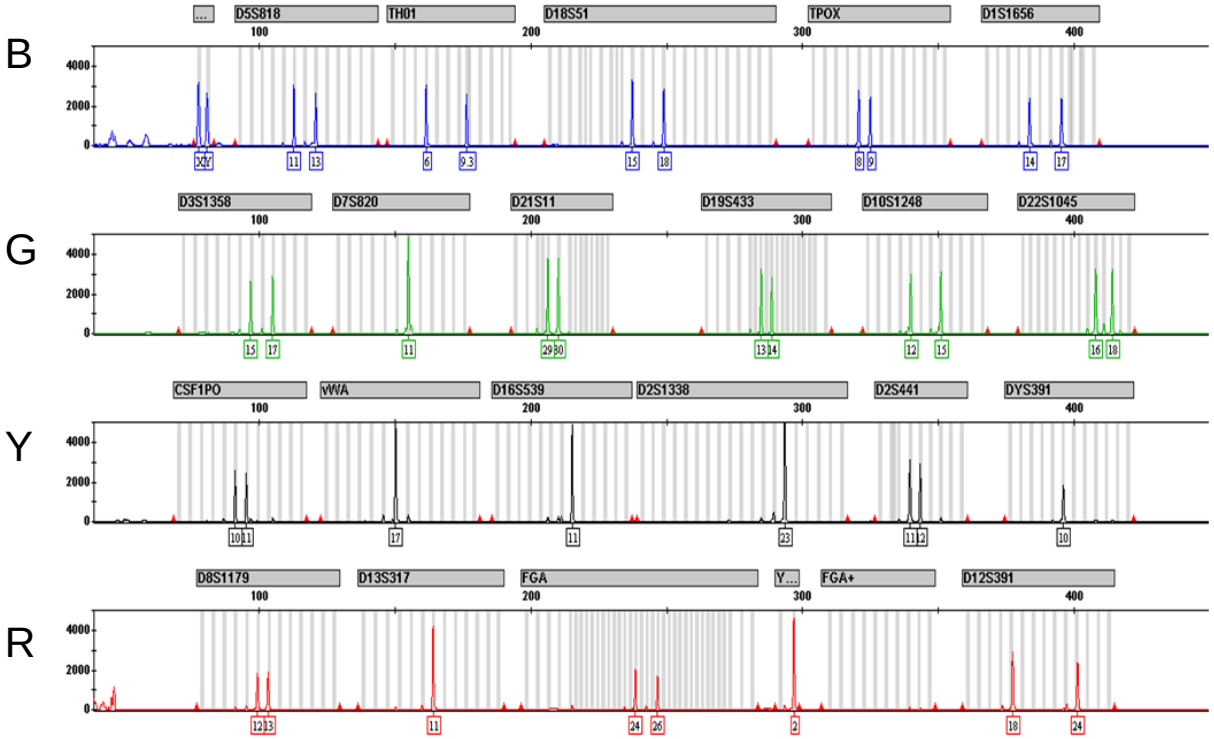


Fig.2. 9948 control DNA - 1ng as template