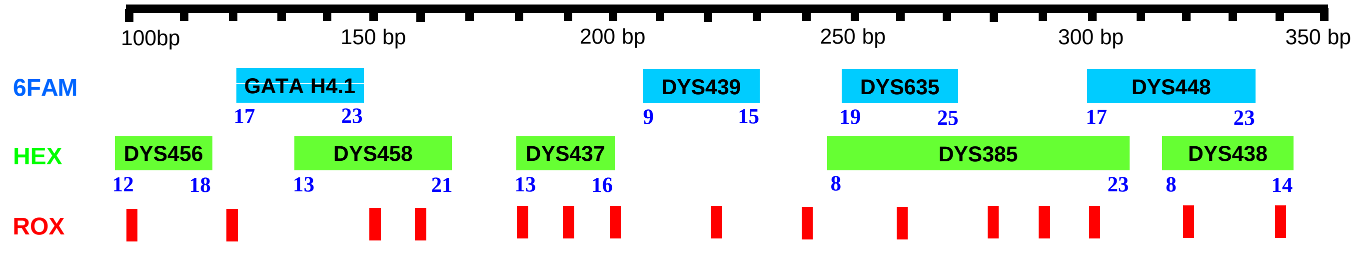
Y-STR Multiplex System II

DYS385, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA H4.1



GeneScan™ 400HD ROX™ Size Standard

10X Primer Mix:

STR Loci		Primer Sequence (5'-3')	Conc.
DYS385	F	HEX-AGC ATG GGT GAC AGA GCT A	3.2 µM
	R	GCC AAT TAC ATA GTC CTC CTT TC	3.0 µM
DYS437	F	HEX-GAC TAT GGG CGT GAG TGC AT	1.6 µM
	R	GAG ACC CTG TCA TTC ACA GAT GA	1.6 µM
DYS438	F	HEX-CCA AAA TTA GTG GGG AAT AGT TG	2.8 µM
	R	GAT CAC CCA GGG TCT GGA GTT	2.8 µM
DYS439	F	6FAM-TCG AGT TGT TAT GGT TTT AGG TCT	1.7 µM
	R	GTG GCT TGG AAT TCT TTT ACC C	1.7 µM
DYS448	F	6FAM-TGG GAG AGG CAA GGA TCC AA	7.5 µM
	R	GTC ATA TTT CTG GCC GGT CTG G	7.5 µM
DYS456	F	HEX-GGA CCT TGT GAT AAT GTA AGA TAG	1.0 µM
	R	GTA GAG GGA CAG AAC TAA TGG AA	1.0 µM
DYS458	F	HEX-GCA ACA GGA ATG AAA CTC CAA T	0.9 µM
	R	GTT CTG GCA TTA CAA GCA TGA G	0.9 µM
DYS635	F	6FAM-AGT GTC TCA CTT CAA GCA CCA AGC AC	4.3 µM
	R	GCA GCA AAA TTC ACA GTT GGA AAA ATG T	4.3 µM
GATAH4.1	F	6FAM-ATG CTG AGG AGA ATT TCC AA	2.0 µM
	R	GCT ATT CAT CCA TCT AAT CTA TCC ATT	2.0 µM

PCR Mixture:

PCR Component	Volume/Sample
dH$_2$O	6.0 µL
Gold ST*R 10X Buffer	1.6 µL
10X Primer Mix	1.0 µL
AmpliTaq Gold (5U/µL)	0.4 µL
DNA Template (1ng/µL)	1.0 µL
Total	10.0 µL

Thermal Cycling:

95ºC for 11 minutes, then:

94ºC for 1 minutes
59ºC for 1 minutes
72ºC for 1 minutes
for 30 cycles, then:

60ºC for 45 minutes
4ºC soak

Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

Y-STR Multiplex System II

Materials & Reagents Needed:

Dry heating block, water bath, or thermal cycler
310 capillaries, 47cm x 50 µm (Applied Biosystems, Foster City, CA)
Performance Optimized Polymer (POP4, Applied Biosystems, Foster City, CA)
Flourescent Amidite Matrix Standards [6FAM™, TET™, HEX™, TAMRA™, ROX™] (Applied Biosystems, Foster City, CA)
Run module GS STR POP4 (1 mL) D
GeneScan™ 400HD ROX™ Size Standard (Applied Biosystems, Foster City, CA)
Hi-Di™ Formamide (Applied Biosystems, Foster City, CA)

Creating Matrix:

According to the ABI PRISM® 310 Genetic Analyzer User's Manual

Preparing the Sample:

1. Prepare a loading cocktail by combining and mixing the 0.3 µL GeneScan™ 400HD ROX™ Size Standard and 20 µL Hi-Di Formamide per sample.
2. Vortex for 10 seconds.
3. Combine 20.3 µL of the prepared loading cocktail and 1.0 µL of the PCR product.
4. Preparing the allelic ladder, combine 20 µL of the prepared loading cocktail and 1.0 µl of the allelic ladder mix. Vortex the allelic ladder mix prior to pipetting.
5. Denature the samples and ladder by heating at 95ºC for 5 minutes and immediately chill on crushed ice. Denature the samples just prior to loading.
6. Assemble the tubes in the appropriate autosampler, and place the autosampler tray in the instrument.

310 Data Collection Software:

Prepared the samples are run using the Run module **GS STR POP4 (1 mL) D** and a described above **matrix**.

Samples are injected for 5 seconds at 15,000 V and separate at 15,000V for 24 minutes with run temperature of 60ºC.

Genotyper Software:

Y-STR2plus.gta