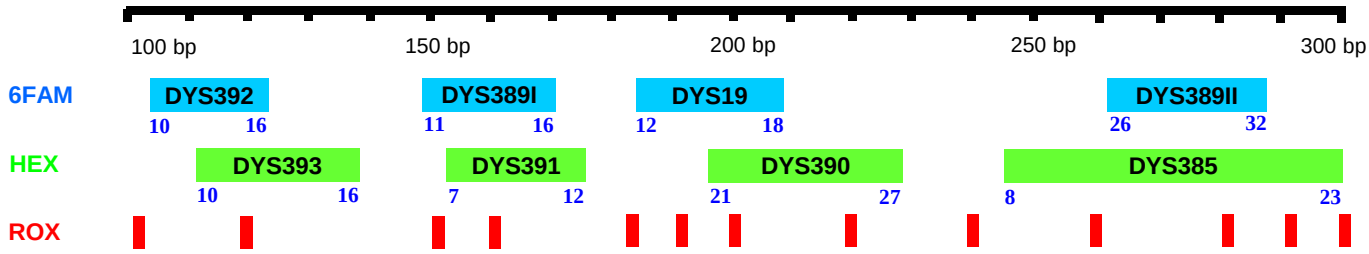
Y-STR Multiplex System I

Minimal Haplotype Loci



GeneScan™ 400HD ROX™ Size Standard

Reagents Needed:

10X Primer Mix

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)

Gold ST*R 10X Buffer (Promega, Madison, WI)

10X Primer Mix:

STR Loci		Primer Sequence (5'-3')	Conc.
DYS19	F	6FAM-CTA CTG AGT TTC TGT TAT AGT	4.6 µM
	R	ATG GCC ATG TAG TGA GGA CA	4.6 µM
DY389I/II	F	6FAM-CCA ACT CTC ATC TGT ATT ATC T	3.2 µM
	R	TTA TCC CTG AGT AGC AGA AGA AT	3.2 µM
DYS390	F	HEX-TAT ATT TTA CAC ATT TTT GGG CC	2.2 µM
	R	TGA CAG TAA AAT GAA CAC ATT GC	2.2 µM
DYS391	F	HEX-CTA TTC ATT CAA TCA TAC ACC CAT AT	0.7 µM
	R	ACA TAG CCA AAT ATC TCC TGG G	0.7 µM
DYS392	F	6FAM-AAA AGC CAA GAA GGA AAA CAA A	0.5 µM
	R	AAA CCT ACC AAT CCC ATT CCT T	0.5 µM
DYS393	F	HEX-GTG GTC TTC TAC TTG TGT CAA TAC	0.4 µM
	R	AAC TCA AGT CCA AAA AAT GAG G	0.4 µM
DYS385	F	HEX-AGC ATG GGT GAC AGA GCT A	0.8 µM
	R	CCA ATT ACA TAG TCC TCC TTT C	0.8 µM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	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 I

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 20.0 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-STR1.gta