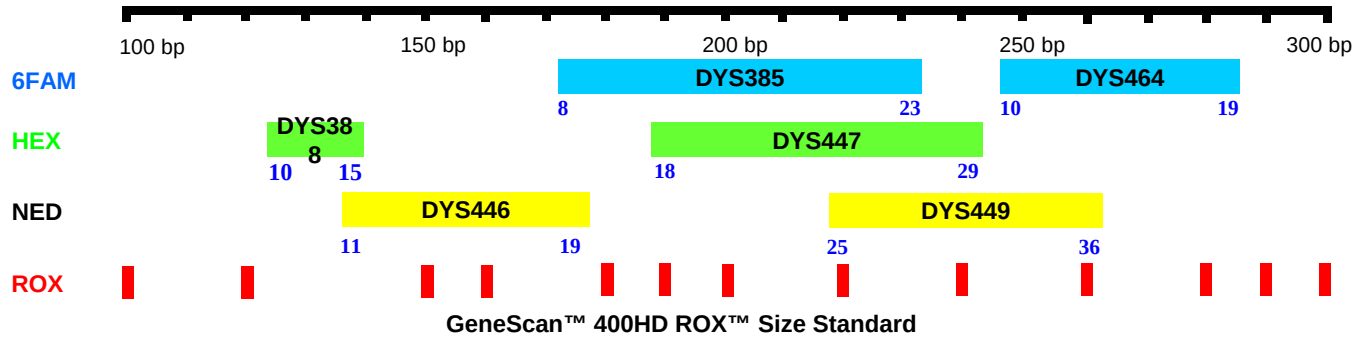
Y-STR Multiplex System III

DYS385, DYS388, DYS446, DYS447, DYS449, DYS464



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.
DYS385	F	GAA GGA AGG AAG GAA GGG AAA	0.5 µM
	R	6FAM-TAA GGG CTG CTG ACC AGA TT	0.5 µM
DYS388	F	HEX-GTG AGT TAG CCG TTT AGC GA	1.6 µM
	R	GCA GAT CGC ACC ACT GCG	1.6 µM
DYS446	F	GTA TTT TCA GTC TTG TCC TGT C	3.0 µM
	R	NED-GAG CTT GTA CCA CTG CAC TCA	3.0 µM
DYS447	F	HEX-GGT CAC AGC ATG GCT TGG TT	0.8 µM
	R	GGG CTT GCT TTG CGT TAT CTC T	0.8 µM
DYS449	F	NED-CTT GCT CTT TTT CTT TTC TCT CTT	4.2 µM
	R	GCA CTC TAG GTT GGA CAA CAA	4.2 µM
DYS464	F	6FAM-TTA CGA GCT TTG GGC TAT G	6.8 µM
	R	GCC TGG GTA ACA GAG AGA CTC TT	6.8 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 III

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™]

NED™ Matrix Standard (Applied Biosystems, Foster City, CA)

Run module GS STR POP4 (1 mLl) 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-STR3plus.gta