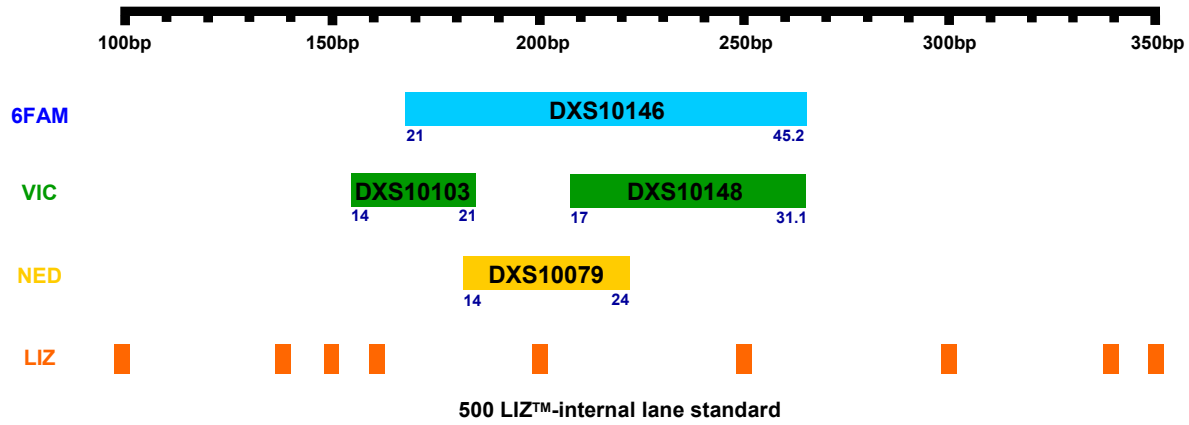
# X-STR Multiplex System III

## DXS10146, DXS10103, DXS10148, DXS10079




**500 LIZ™-internal lane standard**

## Reagents Needed:

10X Primer Mix

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

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

## 10X Primer Mix:

| STR Loci | | Primer Sequence (5'→3') | Conc. |
|---|---|---|---|
| DXS10146 | F | 6FAM-CTG CCT TGC CCT TCC TAC C | 8.0 µM |
| | R | GAA AAA GAA AGA AAG ACA GAG A | 8.0 µM |
| DXS10103 | F | VIC-TCA TAA TCA CAT ATC ACA TGA GC | 3.0 µM |
| | R | AAA CAG AAC CAG GGG AAT GAA | 3.0 µM |
| DXS10148 | F | AAA AAA GGG GGA AGG AAG GA | 10.0 µM |
| | R | VIC-GGC TAT TTC TCC TGC ATA AG | 10.0 µM |
| DXS10079 | F | NED-CTG GGT GAC CAA GTG AGA CC | 1.0 µM |
| | R | TGT GTT GTT GAG TTC AGT TTG C | 1.0 µM |

## PCR Mixture:

| PCR Component | Volume/Sample |
|---|---|
| $dH_2O$ | 6.7 µL |
| Gold ST*R 10X Buffer | 1.0 µL |
| 10X Primer Mix | 1.0 µL |
| AmpliTaq Gold (5 U/µL) | 0.3 µL |
| DNA Template (1 ng/µL) | 1.0 µL |
| Total | 10.0 µL |

## Thermal Cycling:

95°C for 11 minutes, then:

94°C for 1 minute
59°C for 1 minute
72°C for 1 minute
for 30 cycles, then:

60°C for 45 minutes
4°C soak

# Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

## X-STR Multiplex System III

### Materials & Reagents Needed:

Dry heating block, water bath, or thermal cycler
310 capillaries, 47 cm x 50 ㎛ (Applied Biosystems, Foster City, CA)
Performance Optimized Polymer (POP-4™ Polymer, Applied Biosystems, Foster City, CA)
DS-33 Matrix Standard Set [6FAM™, VIC®, NED™, PET®, and LIZ® dyes]
(Applied Biosystems, Foster City, CA)
Run module GS STR POP4 (1mL) G5v2
GeneScan™ 500 LIZ™ 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 0.2 μL GeneScan™ 500 LIZ™ Size Standard and 20.0 μL Hi-Di™ Formamide per sample.
2. Vortex for 10 seconds.
3. Combine 20.2 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 95°C 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 (1mL) G5v2** and a described above **matrix**.
Samples are injected for 5 seconds at 15,000 V and separate at 15,000 V for 24 minutes with run temperature of 60°C.

### Genotyper Macro:

X-STR-3.gta

# Electropherogram of X-STR Multiplex System III Allelic Ladder and Genotyping of Standard 9947A DNA

## X-STR Multiplex System III