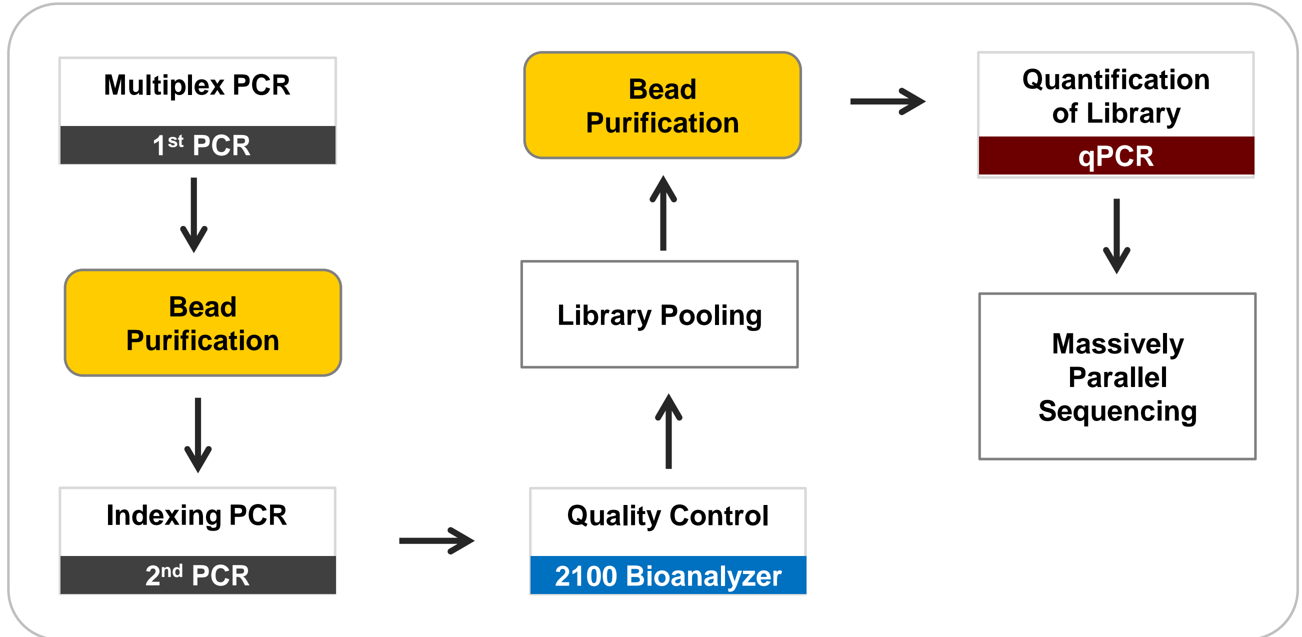
# Y-STR Analysis of Degraded DNA
## using In-house Massively Parallel Sequencing Panel

### Workflow




## Multiplex PCR

### Reagents Needed

- 5X KplexSeq-Y24 Primer Mix
- AmpliTaq Gold® DNA Polymerase (ThermoFisher Scientific, Waltham, MA)
- Gold ST*R 10X Buffer (Promega, Madison, WI)

### PCR Mixture

| PCR Component | Vol. (µl) |
| --- | --- |
| Nuclease-Free Water | 11.0 |
| 10X Gold ST*R Buffer | 2.0 |
| 5X Primer Mix¥ | 4.0 |
| AmpliTaq Gold (5U/µl) | 1.0 |
| Degraded DNA (< 100pg/µl) | 2.0 |
| Total | 20.0 |

¥5X primer mix can be divided into 2 ~ 3 sets

### Thermal Cycling

95℃ for 11 minutes, then:

94℃ for 20 seconds
60℃ for 60 seconds
72℃ for 45 seconds
for x 27 cycles, then:

72℃ for 5 minutes
4℃ soak

# Y-STR Analysis of Degraded DNA using In-house MPS Panel (continued)

## Bead-based Purification of the 1st PCR product

### Materials and Reagents Needed

**Agencourt® AMPure® XP beads (Beckman Coulter, Indianapolis, IN)**

**Freshly prepared 80% ethanol**

**DynaMag™-2 Magnet (ThermoFisher Scientific, Waltham, MA)**

**Elution buffer or Nuclease-Free Water**

**Dry heating block (for optional incubation in 37°C)**

### Protocol

1. **Remove the AMPure® XP beads from storage and let it stand for at least 30 minutes for equilibration to room temperature**
2. **Vortex the AMPure® XP beads to homogenize the suspension before use**
3. **Add 30µl of low EDTA TE buffer or Nuclease-Free Water to each sample tube that include 20µl of the 1st PCR product, and mix by tapping**
4. **Add x1.5 well-mixed AMPure® XP beads to 50µl each sample, then carry out procedures according to manufacturer's instructions**
5. **Elute PCR product in 20µl of the elution buffer or Nuclease-Free Water**

# Y-STR Analysis of Degraded DNA
## using In-house MPS Panel (continued)

## Indexing PCR

### Reagents Needed

Nextera® XT v2 index kit (Illumina®, Inc., San Diego, CA)

AmpliTaq Gold® DNA Polymerase (ThermoFisher Scientific, Waltham, MA)

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

### PCR Mixture

| PCR Component | Vol. (µl) |
|---|---|
| Nuclease-Free Water | 0.4 |
| 10X Gold ST*R Buffer | 3.0 |
| Index 1 (i7) | 3.0 |
| Index 2 (i5) | 3.0 |
| AmpliTaq Gold (5U/µl) | 0.6 |
| Purified 1st PCR product | 20.0 |
| Total | 30.0 |

### Thermal Cycling

95℃ for 15 minutes, then:

94℃ for 20 seconds
61℃ for 30 seconds
72℃ for 45 seconds
for x 16 cycles, then:

72℃ for 5 minutes
4℃ soak

## Quality Control

### Materials and Reagents Needed

Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA)

Agilent DNA 1000 kit (Agilent Technologies, Inc., Santa Clara, CA)

### Protocol

According to the Agilent 2100 Bioanalyzer and DNA 1000 kit User's Manuals

Finally, normalize each library to 10ng/ul and pool them in equal volume

# Y-STR Analysis of Degraded DNA
## using In-house MPS Panel (continued)

## Bead-based Purification of the Pooled Library

### Materials and Reagents Needed

- Agencourt® AMPure® XP beads (Beckman Coulter, Indianapolis, IN)
- Freshly prepared 80% ethanol
- DynaMag™-2 Magnet (ThermoFisher Scientific, Waltham, MA)
- Elution buffer or Nuclease-Free Water
- Dry heating block (for optional incubation in 37°C)

### Protocol

1. Remove the AMPure® XP beads from storage and let it stand for at least 30 minutes for equilibration to room temperature
2. Vortex the AMPure® XP beads to homogenize the suspension before use
3. Add x1.1 well-mixed AMPure® XP beads to 50μl pooled library, then carry out procedures according to manufacturer's instructions
4. Elute library in 50μl of the elution buffer or Nuclease-Free Water

## Quantify Libraries

### Materials and Reagents Needed

- KAPA Library Quantification Kits (KAPA Biosystems, Wilmington, MA)
- AB 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA)

### Protocol

According to the KAPA Library Quantification Kits User's Manual