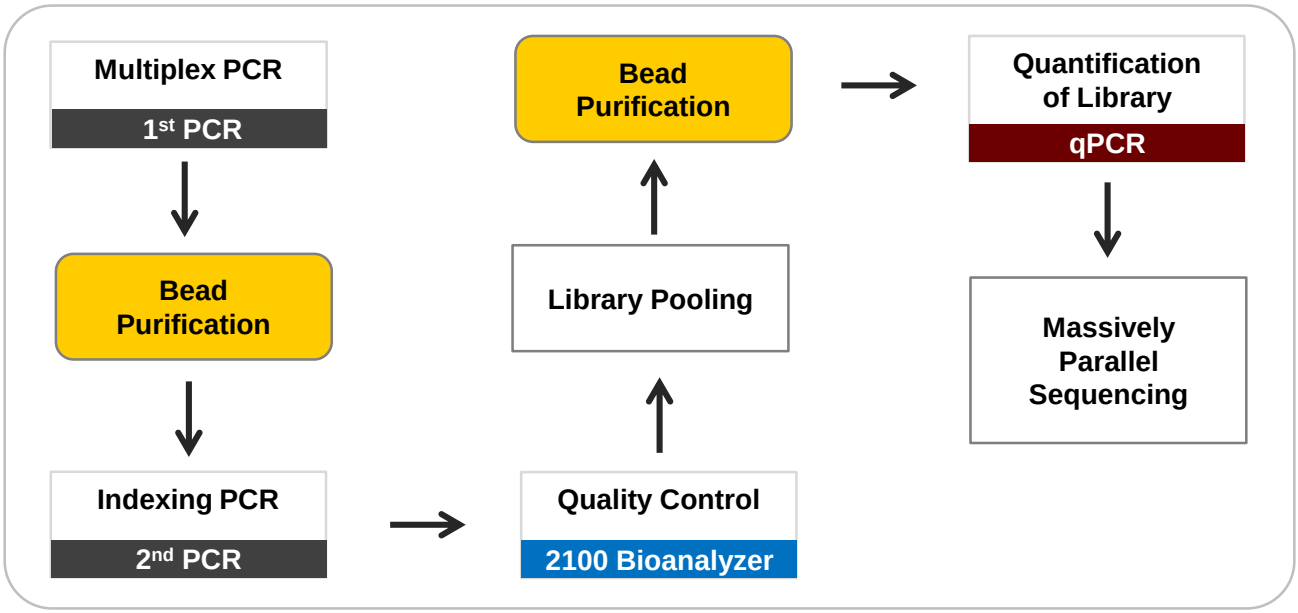
Autosomal STR Analysis of Degraded DNA
using In-house Massively Parallel Sequencing Panel

Workflow



Multiplex PCR

Reagents Needed

5X KplexSeq-A25 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
59℃ for 60 seconds
72℃ for 45 seconds
for x 26 cycles, then:

72℃ for 5 minutes
4℃ soak

Autosomal 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

Autosomal 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 15 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

Autosomal 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 (ThermoFisher Scientific, Waltham, MA)

Protocol

According to the KAPA Library Quantification Kits User's Manual