MCLAB
Molecular Cloning Laboratories

User Manual

Version 1.0

Product name: **NGS Library Distribution Kit**

Cat #: **NLDK-100, NLDK-200**

Description:

MCLAB's NGS Library Distribution Kit offers fast and reliable separation, sizing and quantification of DNA or RNA libraries utilizing Capillary Electrophoresis technology, which can separate molecules with a single base difference. The kit provides high resolution sizing and quality control of your DNA/RNA library.

Key Features:

- Easy and fast sample preparation: fluorescent labeled DNA oligos can be attached to your amplified NGS library within 5 minutes.
- High resolution: separate molecules with a single base difference.
- Ultra high sensitivity: one signal for each library molecule. Peak sizes are true to molecule quantity. No normalization is needed.
- Ultra high throughput: continuously analyze 960 samples* within a run.

  *(total sample numbers depends on the Genetic Analyzer model)
- High accuracy: DNA ladder added to each sample to eliminate equipment errors and increase sizing accuracy.

Component: Fluorescent Tag Mix

Recommended Storage Condition: -20 °C

Protocol

Fluorescent Tagging:

1. Set up the fluorescent tagging reaction in a PCR microtube:

Reagent	Volume (µl)
NGS library sample (PCR product)	1-2*
Fluorescent Tag Mix	4
Total volume	5-6

2. Place the PCR microtube in a thermo cycler with the lid heating to 100 °C and incubate using the following program:

5 min @ 37 °C
5 min @ 96°C
Keep @ 4 °C until next step (or place the microtube on ice in)

Prepare for Capillary Electrophoresis:

1. Set up the capillary electrophoresis mix in a 96 well plate:

Reagent	Volume (µl)
Fluorescent tagging rxn	1
Red Dye standard (premixed in nuclease-free water)	15.5
Total volume	16.5

2. Cover the plate with a 96-well cap mat, mix the reagent vigorously on a shaker, briefly centrifuge the plate to collect all liquid from the side of each well.
3. Place the covered 96 well plate in a thermo cycler with the lid heating to 100 °C and incubate using the following program:

5 min @ 96 °C (to denature the double helix and prepare for the CE)
Keep @ 20 °C

4. Load the prepared 96 well plate onto a genetic analyzer, your NGS library sample is ready to be validated.

Note:
* Prepared DNA library sample (after PCR) normally contents about 100ng/ul DNA fragments.
1. Keep both supplied reagents and the fluorescent tagging reaction in the dark at all time.
2. Store both supplied reagents at -20°C, thaw them on ice after removing from -20°C storage. Keep the reagents on ice after thawing.