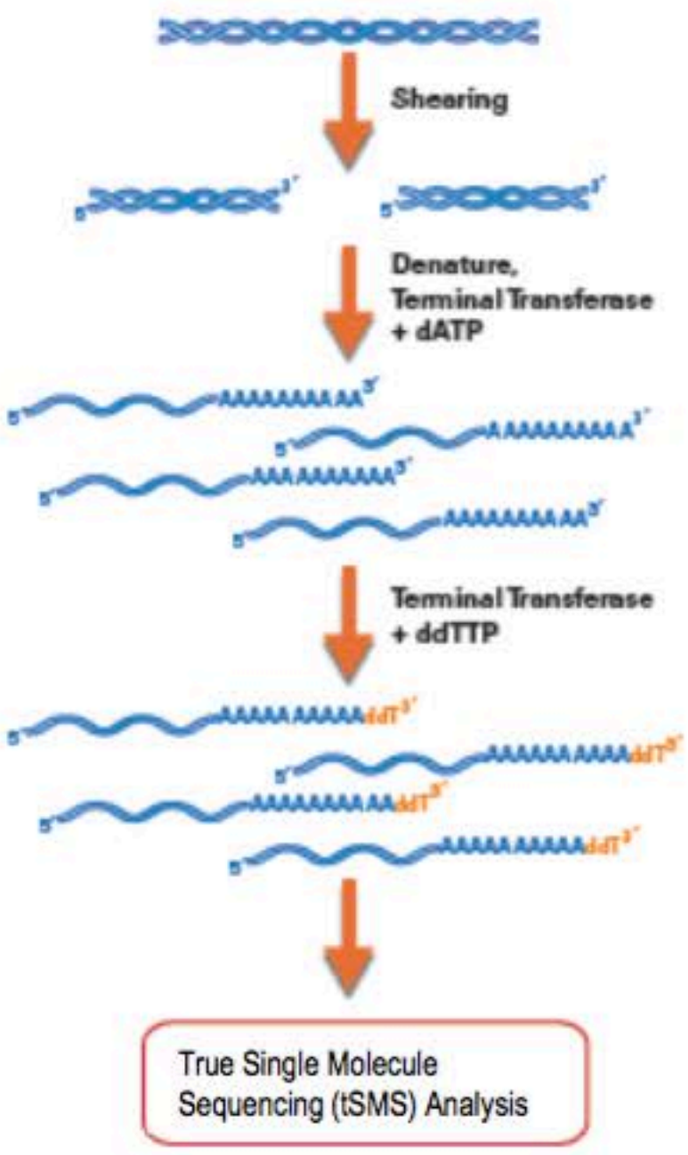
Reagent Kits for Targeted Resequencing Assay Protocol

Targeted Resequencing Sample Preparation Process



Step 1: DNA Fragmentation and Quantitation

Step 1a: Ultrasonic Shearing of DNA Samples

30 minutes per sample

Shear 1-4 µg of DNA to an average size of 200 bp using a Covaris S2 instrument.

Samples may be stored at -20°C.

Step 1b: Size Selection of DNA fragments

30 minutes

Load each sheared DNA sample onto a size exclusion spin column.

Wash sample twice with distilled water.

Elute DNA sample.

If needed, concentrate DNA sample to 50 µl using a vacuum concentrator.

Step 1c: Calculating the Approximate Concentration of 3' Ends

1 hour 30 minutes

Run an aliquot of each sheared DNA sample on a 1X TBE 4-20% • gradient polyacrylamide gel.

Image gel and determine the average size of the sheared DNA fragments by comparison to 25 bp ladder standard.

Determine double-stranded DNA concentration using a small volume spectrophotometer.

Calculate approximate concentration of DNA 3' ends.

Step 2: PolyA Tailing of the DNA

Step 2a: PolyA Tailing Reaction

1 hour 45 minutes

Prepare a control reaction in parallel with the sample reactions to • determine success of polyA tailing step.

Denature DNA samples by heating for 5 minutes at 95°C. Snap • cool.

Add Terminal Transferase enzyme, dATP nucleotides, and buffer. • Mix well and spin down.

Incubate for 1 hour at 37°C, then incubate for 10 minutes at 70°C.•

Step 2b: Determining the Success of the Tailing Reaction

1 hour

Run an aliquot of the control reaction on a 1X TBE 4-20% gradient • polyacrylamide gel.

Image gel and determine polyA tail length by comparison to 100 bp • ladder standard..

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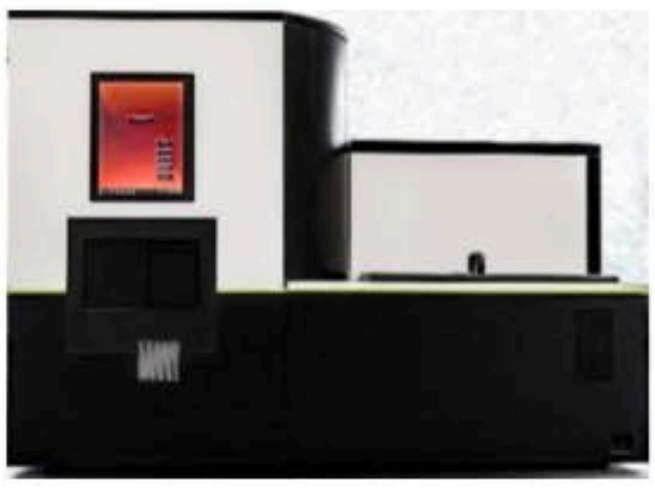
Protocol Summary

Day 1 Step 1: DNA Fragmentation and Quantitation
- 1a: Ultrasonic Shearing of DNA Samples
- 1b: Size Selection of DNA Fragments
- 1 c : Calculating the Approximate Concen. of 3' Ends

Step 2: PolyA Tailing of the DNA
- 2a: PolyA Tailing Reaction
- 2b: Determining the Success of the Tailing Reaction

Step 3: Blocking
- 3a: 3' Blocking Reaction
- 3b: Sample Clean-up and Quantification

Step 4: Flow Cell Loading
- 4a: Sample Loading
- 4b: Fill & Lock



Step 3: Blocking

Step 3a: 3' Blocking Reaction

1 hour 15 minutes

Denature DNA samples by heating for 5 minutes at 95°C.

Snap cool.

Add Terminal Transferase enzyme, ddTTP nucleotides, and buffer. Mix well.

Incubate for 30 minutes at 37°C, then incubate for 10 minutes at 70°C.

Step 3b: Sample Clean-up and Quantification

2 hours

Load each DNA sample onto a size exclusion spin column.

Wash sample twice with distilled water.

Elute DNA sample.

Concentrate DNA sample to 10-20 µl using a vacuum concentrator.

Determine single-stranded DNA concentration using a small volume spectrophotometer.

Calculate estimated concentration of DNA strands.

Step 4: Hybridize to Flow Cell and Sequence

Reagent Kit: includes fluorescent-labeled nucleotides, enzymes, and buffers need for sample loading and sequencing. Reagents either allow for 1) fluorescent nucleotide incorporation and rinsing or 2) for single molecule imaging of fluorescent labels.

Flow Cells: Glass surfaces that are fitted with a custom frame and gasket to allow for a vacuum seal on the HeliScope. These are the equivalent of a microscope slide and cover slip; immersion oil is added to the cover slip and a microscope objective is used to view the slide. These flow cells are specially prepared surfaces that allow for DNA/RNA sequencing and subsequent imaging.

		Part Number
Heliscope Reagent Kit	50 channels	10001