

5'RACE Sequencing Protocol (UCSF Human Genetics Sequencing Core)

1. Sequencing reaction plate setup. (Applied Biosystems cat.#N801-0560, Costar Thermowell Sealers cat#.6570 [VWR 29445-080].

The volumes and order of the reagents are as follows:

Template (2 nd 5'RACE PCR product SAP/EXO treated)	2.2 μ l
dH ₂ O	3 μ l
Sequencing Primer (10ng/ μ l)	1 μ l
Big Dye (Applied Biosystems cat.#4314414) & Half BD (GenPak cat.# HBD200) 1:1 ratio	4 μ l
Total	10 μ l

2. Sequencing Reaction Cycling:

95 ⁰ C	10 seconds
50 ⁰ C	15 seconds
60 ⁰ C	4 minutes
25 cycles	

3. Centrifuge Edge Filter Blocks (cat. #63875) rcf 850 \times g, 2 minutes to remove buffer.
4. Add sequencing reaction samples to respective wells. Centrifuge blocks rcf 850 \times g, 5 minutes.
5. Collect samples in ABI Plates (cat.#N801-0560). Seal plate.
6. Run on ABI Prism 3700 DNA Analyzer.