

LongSAGE Protocol

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LongSAGE ([Nature Biotechnology \(2002\) 20: 508 - 512](#)) is an adaptation of the SAGE approach that allows 21bp tags to be obtained from individual transcripts and matched directly to the human genome. LongSAGE can be used for gene expression analyses, experimental confirmation of gene predictions, and identification of novel genes and exons.

The LongSAGE method can be performed using either the standard SAGE protocol or the microSAGE protocol (available from http://www.sagenet.org/sage_protocol.htm) with the following modifications:

1. Linkers containing the Mmel recognition site are ligated to 3' cDNA ends after NlaIII digestion. Linker 1A and Linker 1B are annealed together and ligated to half the cDNA population and Linker 2A and Linker 2B are annealed together and ligated to the remaining half of the cDNA
 - a. Linker 1A 5'-TTTGGATTTGCTGGTGCAGTACAACCTAGGCTTAATATCCGACATG-3'
 - b. Linker 1B 5'-TCGGATATTAAGCCTAGTTGTAAGTGCACCAGCAAATCC AminoModified C7-3'
 - c. Linker 2A 5'-TTTCTGCTCGAATTCAAGCTTCTAACGATGTACGTCCGACATG-3'
 - d. Linker 2B 5'-TCGGACGTACATCGTTAGAAGCTTGAATTCGAGCAG AminoModified C7-3'
2. Linker tag molecules are released from the cDNA using the Mmel type IIS restriction endonuclease (University of Gdansk Center for Technology Transfer, Gdansk, Poland). Digestion is performed at 37C for 2.5 hrs using 40U Mmel in 300 uL of 10 mM HEPES, pH 8.0, 2.5 mM KOAc, 5 mM MgOAc, 2 mM DTT, and 40 uM S-adenosylmethionine.
3. To maximize the information content of the LongSAGE tags the 2 bp 3' overhang created by digestion with Mmel is not polished, and the Linker 1- tag and Linker 2-tag molecules are ligated together in a 6 ul reaction containing 4 U T4 DNA ligase (GIBCO BRL) in the supplied buffer for 2.5 hours at 16C.
4. The ditag molecules are amplified with Primer 1 and Primer 2 which are biotinylated with 2 sequential biotins on the 5' ends
 - a. Primer 1 Biotin - 5'-GTG CTC GTG GGA TTT GCT GGT GCA GTA CA-3'
 - b. Primer 2 Biotin - 5'-GAG CTC GTG CTG CTC GAA TTC AAG CTT CT-3'

Contact information for obtaining Mmel enzyme:

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