Resource Summary Report

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CUT&Tag-direct with CUTAC

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Protocol Information

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Group: Human Cell Atlas Method Development Community

Summary: CUT&Tag@direct uses a modification of Bench-top CUT&Tag which includes incubation in 0.1% SDS post-tagmentation for quantitative release of targeted fragments, followed directly by PCR with Triton-X100 to neutralize the SDS. This protocol is performed in single PCR tubes from nuclei to sequencing-ready libraries and should be suitable for high throughput. The protocol has been enhanced by the addition of hyperaccessibility mapping by Cleavage Under Targeted Accessible Chromatin (CUTAC), where H3K4me2 CUT&Tag samples are tagmented in low salt for mapping of the hyperaccessible site close to the H3K4me2-labeled nucleosomes.In situ tethering for CUT&Tag chromatin profiling.a) The steps in CUT&Tag. Added antibody (green) binds to the target chromatin protein (blue) between nucleosomes (gray ovals) in the genome, and the excess is washed away. A second antibody (orange) is added and enhances tethering of pA-Tn5 transposome (gray boxes) at antibody-bound sites. After washing away excess transposome, addition of Mg++ activates the transposome and integrates adapters (red) at chromatin protein binding sites. After DNA purification genomic fragments with adapters at both ends are enriched by PCR. b) CUT&Tag is performed on a solid support. Unfixed cells or nuclei (blue) are permeabilized and mixed with antibody to a target chromatin protein. After addition and binding of cells to Concanavalin A-coated magnetic beads (M), all further steps are performed in the same reaction tube with magnetic capture between washes and incubations, including pA-Tn5 tethering, integration, and DNA purification.

Associated Publications: Henikoff S, Henikoff JG, Kaya-Okur HS, Ahmad K, Efficient chromatin accessibility mapping in situ by nucleosome-tethered tagmentation. eLife doi: 10.7554/eLife.63274

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