Chromatrap

ChIP from 1000 cells

Background

Chromatrap®'s unique solid state patented technology provides unrivalled sensitivity allowing users to perform ChIP assays using as few as 1000 cells per immunoprecipitation.

Traditionally, ChIP techniques have required large cell numbers which can make it challenging when starting material is limited. Small samples, rare cell types or difficult cell types which provide low yield of chromatin have driven the development of a number of techniques to enable use of these chromatin sources. Many of the techniques available are long and complex and involve amplification of DNA before downstream analysis or spiking of samples with chromatin from another source to enable efficient ChIP from lower cell numbers. MicroChIP (μChIP) from 10,000 cells has been performed without amplification or spiking, using ChIP-on-chip (Acevedo et al., 2007). A modified version of the μChIP technique has been successfully employed using qPCR as the downstream process (Collas, 2011) but this remains a long and difficult ChIP process. Other methods which have been successful with ChIP using only a few cells rely on variations of the technique and are specifically for high abundant targets and take in excess of three days (Dahl and Collas, 2007; Sachs et al., 2013).

Here, we demonstrate Chromatrap®'s excellent sensitivity and wide dynamic range to perform ChIP from as little as 1000 cells with no difference in enrichment, independent of starting cell number.

Methods

Chromatin preparation

The human endometrial carcinoma cell line Hec50 (Holinka et al., 1996) was used for all chromatin extracted during the study. Cells were cultured according to standard protocols and allowed to reach appropriate cell numbers. For 1000 cell chromatin, cells were cultured in 96-well plates, for 10,000 cells in 12-well plates and for 100,000 cells in 6-well plates. Cell counts were performed on representative wells of all culture vessels using a TC-10 Automated cell counter (BioRad). Cells were fixed in an appropriate volume of 1% formaldehyde (100 µl for 1000 cells, 1 ml for 10,000 cells, 3 ml for 100,000 cells). Each monolayer of cells was quenched by decanting off the formaldehyde and adding an equal volume of 0.65 M glycine. Cells were subsequently scraped in to 1 ml, 400 µl or 100 µl ice cold PBS from 6-, 12- and 96-well plates respectively. Cell pellets were collected by centrifugation and cell membranes lysed in 100 µl Chromatrap® Hypotonic Buffer followed by nuclear lysis in 100 µl Chromatrap® Lysis Buffer. All chromatin stocks were sheared by sonication for 15min; 30s on, 30s off, using a Qsonica sonicator on the high setting.

Chromatin Immunoprecipitation

The Chromatrap® ChIP-seq Protein A kit (Cat no 500189) was used for ChIP according to the kit-supplied protocol. Slurries were prepared for immunoprecipitation using 100 µl chromatin stock from each extraction (1000 cells, 10,000 cells, 100,000 cells) with 2 µg H3K4me3 antibody (Chromatrap® Cat. No. 700010) or 2 µg rabbit IgG (Chromatrap® Cat. No. 700014) to determine non-specific background binding. An equivalent amount of chromatin for each IP was set aside as an input for use in downstream processes. Following reverse cross-linking and Proteinase K digestion, ChIP samples and inputs were purified using the Chromatrap® DNA purification columns supplied in the Chromatrap® ChIP-seq kit.

qPCR was carried out to determine the efficiency of precipitation of two gene loci using Chromatrap® primer sets for a positive gene target GAPDH (Chromatrap® Cat. No. 800000) and a negative gene target ß-globin (Chromatrap® Cat. No. 800010).

Results

Excellent signal was obtained for occupancy of the histone mark H3K4me3 onto the target gene GAPDH for all cell numbers tested (Figure 1).

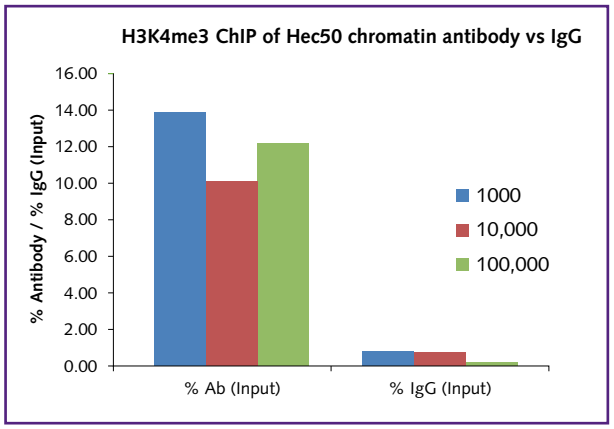

Figure 1 *GAPDH positive gene target only, H3K4me3 vs IgG*

The real signal was comparable, within 10-14%,regardless of whether the starting cell number was 1000, 10,000 or 100,000 cells for this target, with low background IgG in all cases. The selectivity of the assay was further demonstrated by the low binding of H3K4me3 to the negative gene target ß-globin (Figure 2).

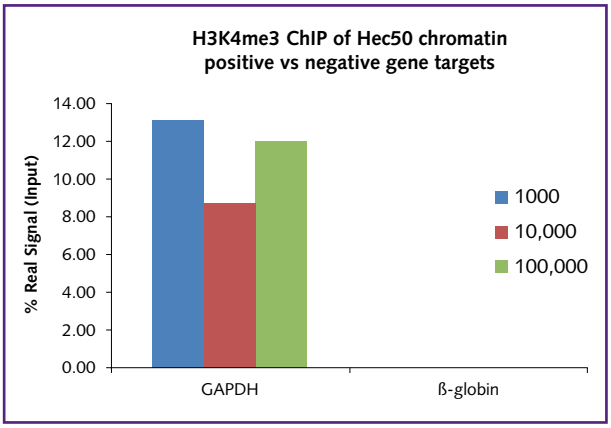

Figure 2 *GAPDH positive and ß-globin negative*

Conclusion

The flexibility, specificity and sensitivity of the Chromatrap® ChIP-seq Protein A kit allows robust and reproducible target enrichment from as little as 1000 cells per IP. Excellent signal strength is achieved and background noise is reproducibly low regardless of the number of cells used per IP.

References

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