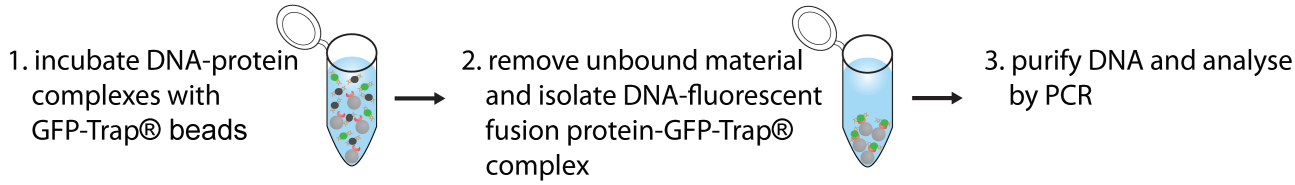
GFP-Trap® is a versatile tool for chromatin immunoprecipitation



Background

The chromatin immunoprecipitation (ChiP) assay is a versatile technique to study protein-DNA interactions. Immunoprecipitation of a specific, DNA-associated protein results in co-precipitation of the DNA binding region, which can be subsequently identified by different methods.
The high mobility group protein A1a (HMGA1a) is known to bind to the promotor regions of the Interleukin 6 (IL6) and Igf-binding protein 1 (IgfBP1) genes. To confirm that the GFP-Trap® is suitable for chromatin immunoprecipitations, ChIP experiments were performed using a mouse myoblast cell line stably expressing a GFP fusion of the high mobility group protein A1a (HMGA1a-GFP). After pull down of HMGA1a-GFP with the GFP-Trap®, promotor regions of the Interleukin 6 and Igf-binding protein 1 genes were analyzed by Real time PCR. Results were compared to ChIP experiments performed with commercially available GFP antibodies (1).

Materials and Methods

For chromatin immunoprecipitation experiments, formaldehyde was diluted to 1% in serum free media, and 15 ml were added to monolayers of C2C12 and C2C12/HMGA1a-GFP cells for 10 min at room temperature. The cross-link was stopped by adding 1,5 ml of 1,25 M glycine. After removal of the medium, cells were washed two times on plates with cold 1x PBS, scraped off, and washed again with cold 1x PBS and once with hypotonic LSB buffer (10 mM Hepes pH 7.9, 10 mM KCl, 1,5 mM $MgCl_2$). Cells were resuspened in 2,7 ml of LSB buffer and lysed by adding 300 ml of 20% Sarkosyl. The chromatin was carefully layered onto a 40 ml sucrose cushion (LSB buffer plus 100 mM sucrose) and centrifuged (10 min at 4°C at 4000xg) Supernatant was removed, and the chromatin was resuspended in 2 ml of TE (10 mM Tris, 0,5 mM EDTA, pH 8.0) and sonicated (Branson sonifier 250-D, 35% amplitude, 2 min in 1-s intervals). For each immunoprecipitation 500 µg of the nucleoprotein were adjusted with 1/10 volume of 1x NET (50 mM Tris, 150 mM NaCl, 0,5 M EDTA, 0,5% Nonidet P-40). 10 µg of two anti-GFP monoclonal antibodies (clones 7.1 and 13.1; Roche diagnostics), a mouse IgG1 isotype control, and 5 µg of GFP-Trap® were incubated overnight at 4°C. The purification of co-precipitated DNA was performed as described above. Real time PCR was performed with the Light Cycler instrument (Roche Diagnostics) using a ready to use reaction mixture (FastStart DNA Master SYBR Green I; Roche Diagnostics). Real time PCR analysis was performed according to manufacturer´s instructions using the same parameters as described previously (2).

Results

In ChIP experiments performed with the GFP-Trap® promotor fragments of IgfBP1 and IL6 were enriched 3 fold over distal sites, confirming known interactions of HMGA1a with IgfBP1 and IL6 promotor regions. This preferential binding of HMGA1a-GFP at the specific promotor sites was not detectable with conventional GFP antibodies (Fig. 1).

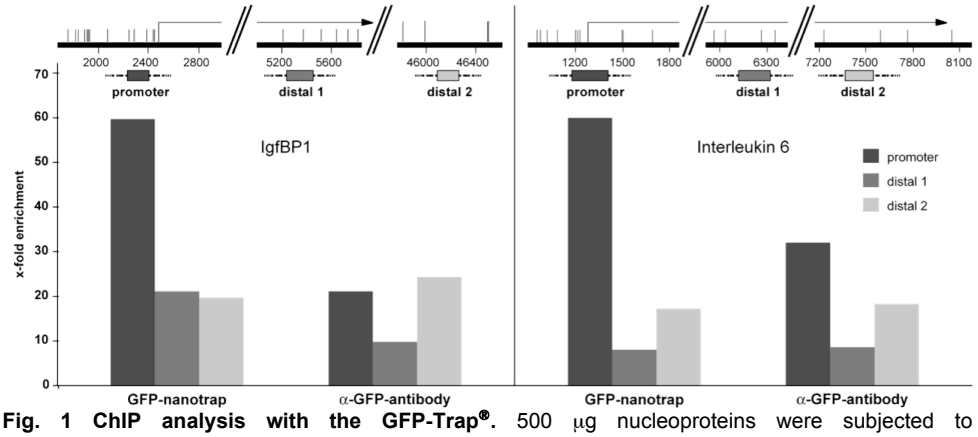


Fig. 1 ChIP analysis with the GFP-Trap®. 500 µg nucleoproteins were subjected to immunoprecipitation using 5 µg GFP-Trap®, 10 µg GFP-antibody, and 10 µg of mouse IgG1 isotype control, respectively. Co-precipitated DNA was quantified by real time PCR analysis using primer pairs of the IgfBP1 and Il6 promotor regions and indicated downstream regions (distal region 1/2). Results obtained for the Il6 and IgfBP1 promotor are shown in dark grey. Column heights indicate the relative enrichment obtained in two independent experiments as the difference between PCR values obtained with the GFP-Trap® and anti GFP-antibodies over mouse IgG1 control antibodies.

Conclusion

We showed that the GFP-Trap® is best suited for chromatin immunoprecipitations. In the experiment described above the GFP-Trap® demonstrated superior performance to commercial GFP antibodies, an observation we also have for conventional IP and Co-IP, since the known interactions between HMGA1a and the promotor regions of IgfBP1 and Il6 could only be reproduced in ChIP experiments using the GFP-Trap®.

References/relevant literature

(1) Rothbauer, U. et al. A versatile nanotrap for biochemical and functional studies with fluorescent fusion proteins. Mol Cell Proteomics 7, 282-289 (2008)
(2) Schepers, A. et al. Human origin recognition complex binds to the region of the latent origin of DNA replication of Epstein-Barr virus. EMBO J. 20, 4588-4602 (2001)

Bergbauer, M. et al., CpG-methylation regulates a class of Epstein –Barr virus promotor. Plos phathol.6 23;6(9):1001114 (2010)
Dubin, M., et al., Dynamics of a novel centromeric histone variant CenH3 reveals the evolutionary ancestral timing of centromere biogenesis Nucleic Acids Res. 38 (21): 7526-37 (2010)
Munoz I.M., et al. Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. Mol Cell 35:116-127(2009)

Ordering Information

Product	Quantity	Code
GFP-Trap® A	20 reactions	gta-20
GFP-Trap® A kit	20 reactions	gtak-20

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