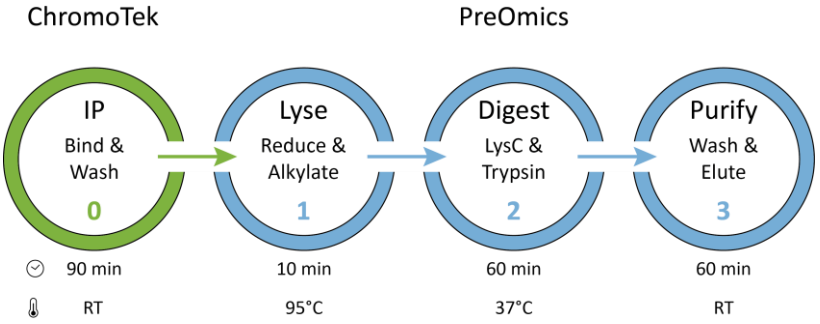
Application Note
Immunoprecipitation Sample Preparation Kit

OBJECTIVE

Sample preparation of immunoprecipitated EGFP-tagged PARP1 to identify protein-protein interactions.



BACKGROUND

Determination of protein-protein interactions provides valuable insights into protein function. Quantitative affinity purification followed by mass spectrometry (q-AP-MS) is a powerful approach to investigate physical interaction partners of a protein of interest. Recent advances in MS-based proteomics now allow the quantification of up to 96 interactomes per day (1). We now combine the GFP-Trap® (ChromoTek GmbH), which has been introduced in 2008 (2), and which since then has been cited in about 1,000 publications (3), for fast, reliable and efficient one-step immunoprecipitation of GFP-fusion proteins with the fast, sensitive and robust iST sample preparation kit (PreOmics GmbH). The combination of both workflows enables a streamlined sample preparation of immunoprecipitated GFP-fusion proteins for subsequent analysis by mass spectrometry.
In this application note, we analyze protein-protein interaction partners of an individual candidate protein in living cells. We express EGFP-tagged PARP1 and use the GFP-binding protein coupled magnetic beads (GFP-Trap®_M, gtm) to efficiently extract the EGFP-fusion proteins from the cellular extract and combine it with the reproducible iST method (4).

MATERIAL AND METHODS

EGFP-tag only and EGFP-tagged PARP1 proteins were transiently expressed in human embryonic kidney cells (HEK293T) in 10 cm dishes. Biological quadruplicates were used throughout the experiment. Each cell pellet of transfected and non-transfected control cells were lysed in 150 µL RIPA buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1% Triton X-100, 0.1% SDS, 1% deoxycholate, 1x Serva Protease Inhibitor Mix M, 1 mg/ml DNaseI, 2.5 mM $MgCl_2$, 1 mM PMSF). Cell extract was diluted with 150 µl wash buffer II (150 mM NaCl, 50 mM Tris pH 8.0, 1x Serva Protease Inhibitor Mix M, 1 mM PMSF) and used for immunoprecipitation. 30 µL of pre-equilibrated GFP-Trap®M suspension was added to the diluted cell extract and incubated at 4°C for 60 min tumbling end over end. The beads were separated using a magnetic stand. The supernatant was removed and the beads were washed twice with wash buffer I (150 mM NaCl, 50 mM Tris pH 8.0, 0.25% NP-40) followed by four times washing with wash buffer II (150 mM NaCl, 50 mM Tris pH 8.0). Beads were transferred to a fresh tube during the last washing step. Proteins bound to the GFP-Trap®_M beads were boiled for 10 min at 95°C in 50 µL LYSE buffer and transferred to the CARTRIDGES, before continuing with the protocol according to the iST Sample Preparation Kit instructions. MS analysis was performed basically as described (2) on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific). Data analysis was performed using the MaxQuant and Perseus software tools (5).

RESULTS

We determined interaction partners of PARP1 using EGFP-tagged PARP1 as bait protein. EGFP-only expressing cells or non-transfected HEK293T cells were used as control. PCA analysis revealed excellent reproducibility of both the immunoprecipitation and the sample preparation (Figure 1). We quantified EGFP-PARP1 as the most enriched protein in cells expressing the bait protein compared to non-transfected or EGFP-only expressing control cells (Figures 2A+B). Moreover, our approach retrieved many known protein interaction partners of PARP1 (Figures 2A+B, indicated by Gene Name). Pathway enrichment analysis of GeneOntology annotation terms revealed a significant enrichment of "non-homologous end joining repair" (GOCC, enrichment factor >268, p<1.1E-02, Benjamini-Hochberg controlled false discovery rate) and "nucleotide-excision repair" (GOBP, enrichment factor > 32, p<1.8E-02 BH-FDR).

Poly(ADP-ribose) (PARylation) is a reversible post-translational modification found in higher eukaryotes, which is involved in many cellular processes, including DNA repair. During DNA damage, PARP1 binds to single-strand breaks and recruits proteins involved in base excision repair. These protein-protein interactions are PARylation-dependent (6). Our PARP1 pull-down experiments revealed several reported PARylation targets to be significantly enriched, such as RPA1, RPA2, XRCC1, XRCC5 and XRCC6.

Figure 1

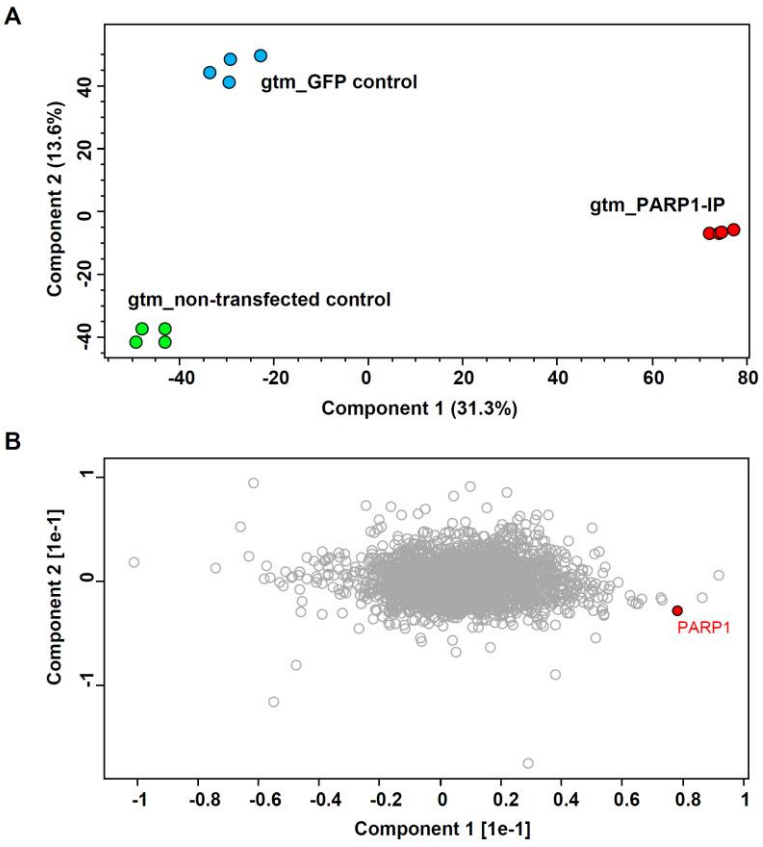


Figure 1

A. Principle component analysis, projections. Quadruplicate IPs and MS measurements show excellent reproducibility.

B. Principle component analysis, loadings. The proteins responsible for the separation of the three IP groups (Fig. 1A) are depicted in Fig. 1B. PARP1 as the bait protein in the gtm_PARP1-IPs is indicated in red.

Figure 2

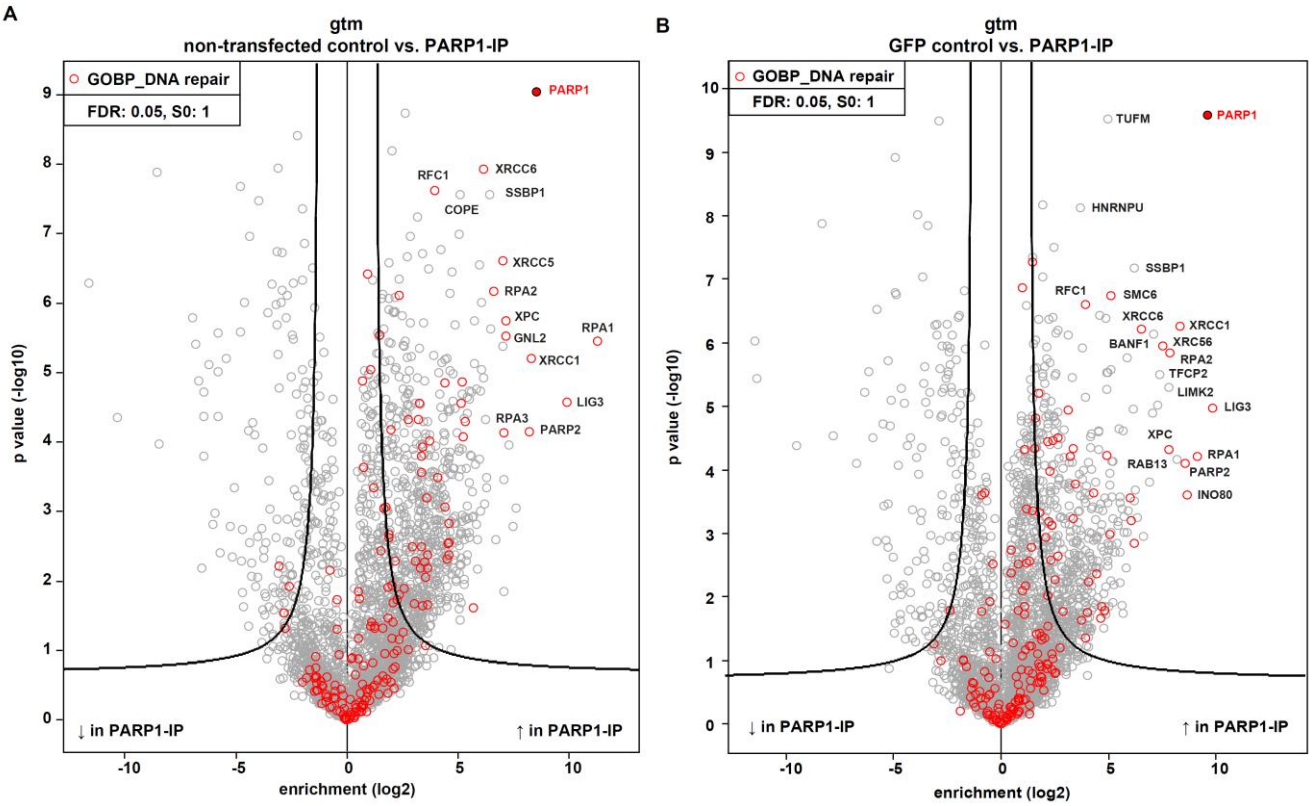


Figure 2

A. Non-transfected controls vs. PARP1-IP

B. EGFP-expressing controls vs. PARP1-IP

Volcano plot displays the enrichment of a given protein vs. the reproducibility of the enrichment in the four quadruplicate IPs. The position of the bait protein PARP1 is indicated in bold red. Proteins annotated with the GeneOntoloy term “DNA repair” are indicated as red circles. The most enriched proteins in the PARP1-IPs are displayed with their corresponding gene names. Many known protein-protein interactors of PARP1 are among the most enriched proteins, such as LIG3, PARP2, RPA1, RPA2, XRCC1, XRCC5 or XRCC6.

CONCLUSION

We show that GFP-Trap®_M can be combined in line with the iST Sample Preparation kit. This facilitates the isolation and immunopurification of an individual EGFP-fusion protein combined with the downstream sample preparation and mass spectrometry analysis.

As the interaction of PARP1 to several DNA repair proteins is mediated by PARylation but is not disrupted by either the IP washing buffers or the iST protocol used herein, the streamlined combination of the GFP-Trap®_M and the iST workflows proves to preserve PTM-mediated protein-protein interactions.

The strategy presented here can be applied to any EGFP-fusion protein that is amenable to GFP-Trap®_M immunoprecipitation. In addition, the iST Kit is also compatible with the GFP-Trap®_MA, and the workflow described in this application note can be expanded to magnetic agarose bead-based GFP-Traps®.

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ORDERING INFORMATION

Product	**Quantity**	**Manufacturer**	**Code**
iST Sample Preparation Kit	8 reactions	PreOmics GmbH	P.O.00001
	96 reactions		P.O.00027
GFP-Trap®_M	10 reactions, 250 µl	ChromoTek GmbH	gtm-10
	20 reactions, 500 µl		gtm-20
	100 reactions, 2.5 ml		gtm-100
	200 reactions, 5 ml		gtm-200
	400 reactions, 10 ml		gtm-400

For further information, please check our websites:
http://www.preomics.com
http://www.chromotek.com

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