

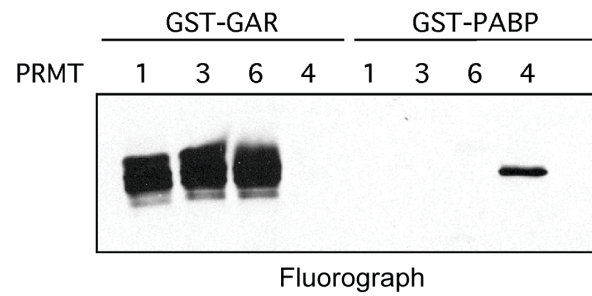
***In vitro* Arginine Methylation Assays**

PRMTs transfer a methyl group from S-adenosylmethionine (AdoMet) to the guanidino nitrogen of arginine, resulting in S-adenosylhomocysteine (AdoHcy) and methyl arginine. The analysis of protein methylation can be performed *in vitro* using either recombinant or endogenous enzymes, in the presence of selected substrates. The specificities of each of the PRMTs are largely unique. *In vitro* methylation assays are used to determine the specificity of PRMTs, to discover new PRMT substrates, or to establish the activity of newly identified PRMTs. For best results, freshly prepared enzymes and substrates should always be used.

1. In a 1.5-mL Eppendorf Safe-Lock tube, mix 0.5-1 µg of substrate, 1 µL of S-adenosyl-L-[methyl-³H] methionine (85 Ci/mmol from a 0.5 mCi/ml stock solution; Perkin-Elmer), 3 µL of 10X PBS, and add H₂O up to 30 µL. The methylation reaction is initiated either with the addition of 0.2-0.5 µg of recombinant enzyme (see **Purification of Recombinant Methyltransferase Enzymes**) or beads that carry an immunoprecipitated PRMT (see **Immunoprecipitated PRMTs**).
2. Mix the tube by tapping.
3. Incubate sample tubes at 30 °C for 1-1.5 h.
4. Stop the reaction by adding 6 µL of 6X SDS protein sample loading buffer (180 mM Tris-HCl, pH 6.8, 30% glycerol, 10% SDS, 0.6 M dithiothreitol (DTT), 0.012% bromophenol blue) and heat at 95 °C for 5 min.
5. Then run 18 µL of the reaction on a 10-15% SDS-PAGE gel at 100 volts for 1-2 h using Tris/Glycine running buffer.
6. The separated samples are then transferred from the gel to a PVDF membrane using a semidry electroblotter.
7. The PVDF membrane harboring the immobilized protein samples is then sprayed with EN³HANCE (Perkin Elmer) twice, waiting 10 min between each application.
8. Finally, the PVDF membrane is left to fully dry for 30 min and then exposed to X-ray film overnight (or longer).

Results of *in vitro* methylation assays using different recombinant PRMTs and histones, GST-GAR, and GST-PABP1 substrates are depicted in **Figure 1**.

A



B

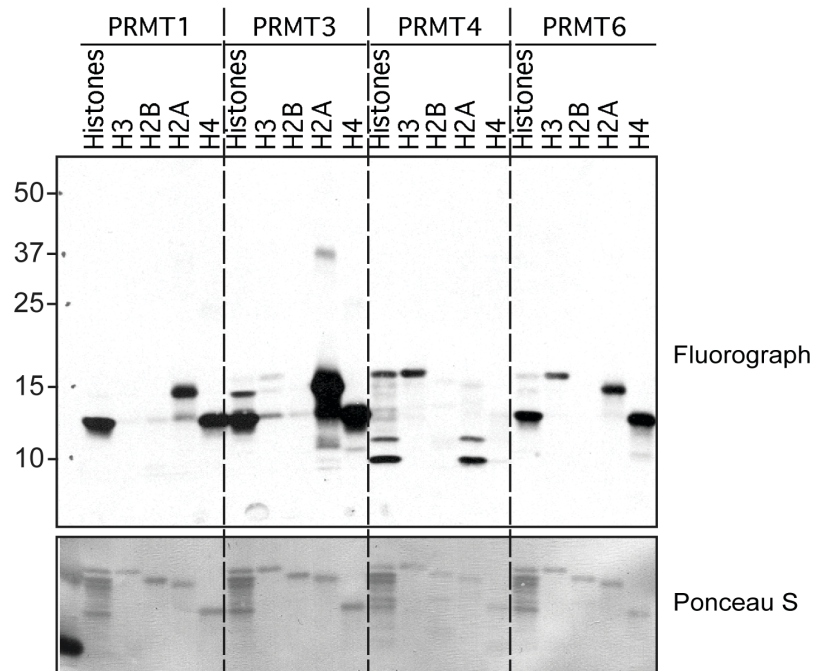


Figure 1. *In vitro* methyltransferase activity of different PRMTs using methylation assays. **(A)** The indicated PRMTs were used to methylate the non-histone substrates GST-GAR and GST-PABP1. Substrate specificity is clearly observed in this experiment. **(B)** The indicated PRMTs were used to methylate core histones. The methylated proteins were separated by SDS-PAGE, transferred to a PVDF membrane, sprayed with EN³HANCE. The membranes were exposed to X-ray film overnight.

References

Cheng, D., Vemulapalli, V., Bedford, M. T. 2012. Methods Applied to the Study of Protein Arginine Methylation. *Methods in Enzymology*, 512, 71-92.