Using Total Cell Extracts as an Enzyme Source

Whole cell extract can also be used as a source of methyltransferase activity to transfer a tritium-labeled methyl group from AdoMet onto a protein substrate. Ideally, a recombinant substrate is immobilized on glutathione sepharose beads, and then mixed with a cell extract and the labeled methyl-donor for a *in vitro* methylation reaction. PRMT knockout and wild type MEF lysates have been used to perform this experiment, using GST-PABP1 (Yadav et al., 2003) and GST-CA150 (Cheng et al., 2007) as substrates. Using this approach, we can demonstrate that CARM1 is the only PRMT that can methylate these substrates. PRMT1 (Yu et al., 2009) and PRMT3 (Swiercz et al., 2007) knockout cells have been generated as well, and they can be used in similar assays. In addition, specific PRMTs can be targeted using shRNA knockdown techniques, and lysates from these cells can be used to evaluate the specificity of substrate methylation.

1. Purify the substrates as in **Purification of Recombinant Methyltransferase Enzymes**. Stop at step 14 and do not elute samples with elution buffer.
2. Quantify the protein amount on beads by SDS-PAGE separation and Coomassie staining, using 1 μg, 2 μg, and 5 μg of BSA as a reference.
3. Adjust the immobilized protein substrate to 1-2 μg/20 μL beads using ice-cold 1X PBS washed glutathione sepharose beads.
4. Aliquot 20 μL adjusted immobilized protein beads to a 1.5 mL Eppendorf Safe-Lock Tube.
5. Seed MEFs on a 10-cm culture plate until they reach 80-90% confluence.
6. Wash twice with cold 1X PBS.
7. Drain the PBS completely and add 400 μL of cold mild lysis buffer (150 mm NaCl, 5 mm EDTA, 1% Triton X-100, 10 mM Tris-HCl, pH 7.5) with protease inhibitor cocktail.
8. Collect the cells from the culture plate with a plastic scraper and gently transfer the cell suspension into a pre-cooled microcentrifuge tube.
9. Sonicate twice for 8 s with pulses of 0.5 s on and 0.5 s off (amplitude 30%) to disrupt the cells.
10. Centrifuge at 15,000 *g* for 15 min at 4 ºC.
11. Transfer the supernatant (300 μL) into the tube from step 4.
12. Add 3 μL of S-adenosyl-L-[methyl-^{3}H]methionine.
13. Incubate for 2 h at 30 ºC.
14. Wash the beads three times with cold mild buffer.
15. Carefully remove the supernatant after the last wash and add 25 μL of 2X protein sample loading buffer and heat at 95 ºC for 5 min.
16. Run SDS PAGE and transfer separated samples from the gel to a PVDF membrane using a semidry electroblotter.
17. Spray the PVDF membrane harboring the immobilized protein samples with EN^{3}HANCE two times, with a 10 min delay between each application.

18. Allow the PVDF membrane to fully dry for 30 min and then expose to X-ray film overnight.

Results of an *in vitro* methylation assay using CARM1 knockout and wild type MEF extracts as an enzyme source are depicted in **Figure 1**.

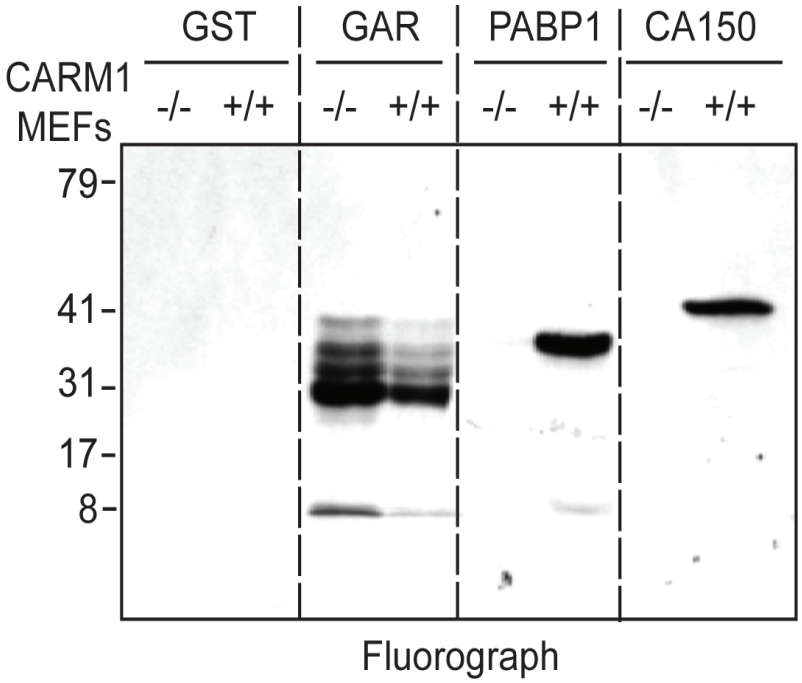

Figure 1. Cell extracts from CARM1 wild type (+/+) and knockout (-/-) MEFs were used in an *in vitro* methylation assay. GST and GST-fusion proteins of GAR, PABP, and CA150 PGM were purified and left bound to glutathione sepharose beads and methylated in the presence of tritiated AdoMet, using the indicated total cell lysate. After the methylation reaction, the beads harboring the recombinant substrate were washed and eluted by boiling with protein sample loading buffer. Proteins were run on a gel, transferred onto a membrane, sprayed with EN^{3}HANCE, and exposed to X-ray film overnight. The molecular-mass markers are shown on the left in kDa. PABP1 and CA150 are clearly CARM1 substrates, and they cannot be effectively methylated by any other PRMT.

References

Cheng, D., Cote, J., Shaaban, S., Bedford, M. T., 2007. The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. Mol Cell. 25**,** 71-83.

Swiercz, R., Cheng, D., Kim, D., Bedford, M. T., 2007. Ribosomal protein rpS2 is hypomethylated in PRMT3-deficient mice. J Biol Chem. 282**,** 16917-23.

Yadav, N., Lee, J., Kim, J., Shen, J., Hu, M. C., Aldaz, C. M., Bedford, M. T., 2003. Specific protein methylation defects and gene expression perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. Proc Natl Acad Sci U S A. 100**,** 6464-8.

Yu, Z., Chen, T., Hebert, J., Li, E., Richard, S., 2009. A mouse PRMT1 null allele defines an essential role for arginine methylation in genome maintenance and cell proliferation. Mol Cell Biol. 29**,** 2982-96.