Purification of Recombinant Methyltransferase Enzymes

To facilitate the use of recombinant enzymes to test the substrate specificity *in vitro*, PRMTs were cloned in frame into pGEX vectors using standard molecular-biology techniques. All nine PRMTs can be expressed in E. coli as glutathione S-transferase (GST) fusion proteins. Except for PRMT9, which has no reported activity, all other GST fusion PRMTs have been shown to have enzymatic activity toward certain substrates. Type I GST-PRMT1, 2, 3, 4, 6, and 8 can catalyze the production of aDMA, while type II GST-PRMT5 catalyzes the formation of sDMA. It should be noted that GST-PRMT5 purified from E. coli has been reported to have very weak *in vitro* activity (Rho et al., 2001). It is well documented that endogenous PRMT5 forms a complex with the cofactors MEP50 and pICln, which are required for full activity (Martin et al., 2010; Rho et al., 2001). It is thus necessary to co-express MEP50 and pICln with GST-PRMT5, using a tricistronic expression plasmid, to ensure the full activity of GST-PRMT5 activity *in vitro* (Martin et al., 2010). GST-PRMT7 was shown to catalyze the formation of MMA or SDMA by different groups (Lee et al., 2005; Miranda et al., 2004; Zurita-Lopez et al., 2012). To generate recombinant PRMT enzymes, the pGex vectors that express the GST-PRMTs are transformed into competent bacterial.

1. Pick a single colony of transformed cells and set up a 5 mL culture in LB broth containing 50 μg/mL Ampicillin. Inoculate at 37 ºC for 16–18 h or overnight with vigorous shaking (250 rpm).
2. Transfer 1-5 mL of the overnight culture to 50 mL of LB broth containing 50 μg/mL Ampicillin in a 250 mL flask. Multiple flasks can be used to scale-up expression.
3. Incubate at 37 °C for 1-2 h until density of A600 is 0.5.
4. Remove 10 μL of culture for analysis by SDS-PAGE (pre-induced sample).
5. Induce the remaining culture by adding 50 μL 0.1 M IPTG (final concentration can be 0.1 mM) and continue to incubate for an additional 4 h at 37 ºC or overnight at 30 ºC (may increase soluble target protein expression) with shaking.
6. Remove 10 μL of the culture for analysis by SDS-PAGE (induced sample).
7. Harvest the cells by centrifugation at 4000 *g* for 5 min at 4 ºC and discard the supernatant. The pellets can be stored at -80 ºC for a month.
8. Resuspend the pellet in 1 mL of cold 1X PBS.
9. Lyse cells by 3 sets of 20 s sonication with pulses of 0.5 s on and 0.5 s off (amplitude 30%).
10. Collect the cellular debris by centrifugation at 15,000 *g* for 15 min at 4 ºC and retain 10 μL of the supernatant and resuspend the pellet for analysis by SDS-PAGE.
11. Analyze the preinduced sample, induced sample, supernatant and pellet using standard SDS-PAGE methods and Coommassie Blue staining to determine expression levels and solubility.
12. Wash 50 μL packed Glutathione sepharose beads two times with ice-cold 1X PBS in a 1.5 mL microcentrifuge tube and add the supernatant from step 10.
13. Rock sample tubes for 3 to 5 hours or overnight at 4 ºC.

14. Wash the rocked beads at least 3 times with ice cold 1X PBS
15. Prepare fresh Elution buffer that contains 100 mM Tris pH 8.0, and 120 mM NaCl with 10 mM reduced L-Glutathione.
16. Add 100-300 μL of freshly made Elution buffer to the beads and rock it for 2 hours at room temperature or overnight at 4 ºC.
17. Spin down beads at 5000 *g* for 1 min and remove the supernatant carefully. The supernatant contains the active enzyme.
18. The purified enzyme can be kept at 4 ºC for 1-2 d or used directly.
19. An aliquot of the supernatant can be analyzed using standard SDS-PAGE methods and Coommassie Blue staining (**Figure 1**).

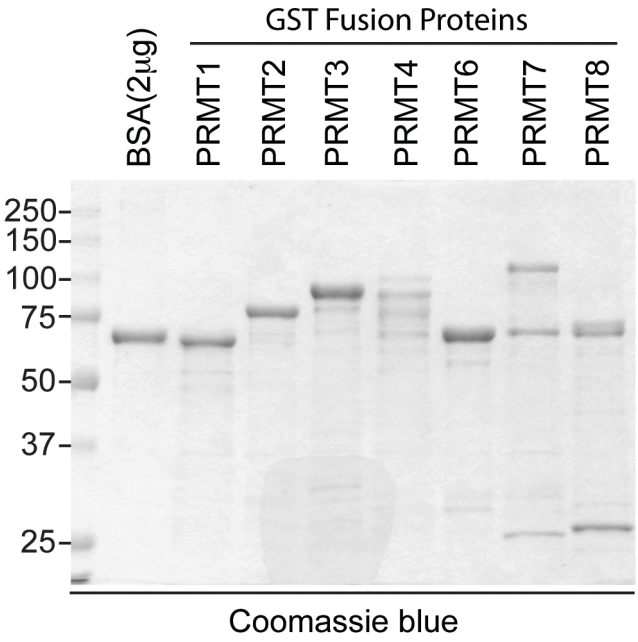

Figure 1. Recombinant arginine methyltransferase enzymes. PRMTs fused to glutathione S-transferase (GST) were expressed in *E.coli* and purified. The GST fusion proteins (1–2 μg) were separated by 10% SDS-PAGE and stained with Coomassie blue. The molecular-mass markers are shown on the left in kDa. BSA (2 μg) serves as a loading control.

References

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