Histone Methyltransferase Assay *in vitro*

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[Abstract] Histone methylation is an important epigenetic modification that plays important roles in plant development and growth. Histone methytransferases are the enzymes to establish histone methylation and here we describe a simple and effective protocol for detecting methyltransferase activity and specificity *in vitro*.

Materials and Reagents

1. ^{14}C labeled-methyl-adenosyl-L-methionine (SAMS) (Sigma-Aldrich)
2. Purified recombinant histones (from *E. coli*)
3. Histone H3 (Roche Diagnostics, catalog number: 1034758)
4. Core histones (Sigma-Aldrich, catalog number: H9250)
5. Mononuclosomes and Oligonucleosomes (see procedure)
6. Coomassie brilliant blue R-250
7. MiliQ water
8. SDS-PAGE
9. KCl
10. $MgCl_2$
11. Sucrose
12. $ZnCl_2$
13. β-mercaptoethanol
14. MAB buffer (see Recipes)

Equipment

1. Water bath

Procedure

A. Substrate:

Purified recombinant Histones (Ding *et al.*, 2007), histone H3, core histones, mononuclosome

and oligonucleosome (from Hela cells or recombinant ones from *E.coli*).

Note: The mononucleosomes and oligonucleosomes prepared from Hela cells were gifts from Yi Zhang (UNC Chapel Hill, USA). The recombinant oligonucleosomes from E. coli were gifts from Bing Zhu (NIBS, Beijing, China).

B. Reaction

A mixture containing 1-5 µl enzyme (such as SDG725), 5 µl 4x MAB buffer, 250 nCi ^{14}C labeled SAM and 1 µg substrates to 20 µl volume, is kept at 37 °C for 1-2 h. The reaction is stopped by adding 5 µl 5x SDS-PAGE loading buffer and boiling at 100 °C for 5 min, and 5 µl samples will be analyzed by 15% SDS-PAGE gel electrophoresis and visualized by Coomassie brilliant blue R-250. The SDS-PAGE gel is dried and exposed to X-ray films or scanned by Typhoon (GE) (15% refers to the concentration of Acr/Bis. Different histones can be separated more clearly by 15% SDS-PAGE).

Recipes

1. MAB buffer

   1x MAB buffer

   50 mM Tris-Cl (pH 8.5)

   20 mM KCl

   10 mM $MgCl_2$

   250 mM Sucrose

   100 µM $ZnCl_2$

   10 mM β-mercaptoethanol

Acknowledgments

The protocol was adapted from previously published papers (Rea *et al.,* 2000; Ding *et al.,* 2007).

References

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