Introduction, Purification, Enzymatic Activity, and Mutagenesis of Dihydrofolate Reductase

Joanne M. Lau

Bellarmine University, 2001 Newburg Rd., Louisville KY 40205 USA
(jlau@bellarmine.edu)

Dihydrofolate reductase (DHFR) is a key enzyme in the metabolism of folate, catalyzing the reactions for purine synthesis, DNA synthesis, and certain amino acids. The objective of this project is to have students induce expression, purify, and enzymatically assay for DHFR activity. In addition, students conducted novel experiments by designing PCR primers for site-directed mutagenesis and examined specific activity of their mutant DHFR product and compared it to the wild-type DHFR gene. Furthermore, students also obtained protein concentrations of their purified DHFR protein product and performed Western Blot analysis to detect the glutathione S-transferase (GST) and Histidine (His) tags that were attached to the DHFR gene. In addition to standard biochemical and molecular techniques (e.g. protein purification, PCR amplification, transformation into competent expression cells, restriction enzyme digest, agarose gel electrophoresis, polyacrylamide gel electrophoresis, and Western blotting), students gained experience measuring specific enzyme activity. Also bioinformatics skills for primer design and sequence alignment were reinforced.

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit http://www.ableweb.org/.

Papers published in Tested Studies for Laboratory Teaching: Peer-Reviewed Proceedings of the Conference of the Association for Biology Laboratory Education are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

Citing This Article

http://www.ableweb.org/volumes/vol-34/?art=9

Compilation © 2013 by the Association for Biology Laboratory Education, ISBN 1-890444-16-2. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the copyright owner.

ABLE strongly encourages individuals to use the exercises in this proceedings volume in their teaching program. If this exercise is used solely at one’s own institution with no intent for profit, it is excluded from the preceding copyright restriction, unless otherwise noted on the copyright notice of the individual chapter in this volume. Proper credit to this publication must be included in your laboratory outline for each use; a sample citation is given above.