Protocol

Labeling Nuclear DNA with Hoechst 33342

Brad Chazotte

INTRODUCTION

A number of fluorescent stains are available that label DNA and allow easy visualization of the nucleus in interphase cells and chromosomes in mitotic cells. One advantage of Hoechst 33342 is that it is membrane permeant and, thus, can stain live cells. Hoechst 33342 binds to adenine-thymine-rich regions of DNA in the minor groove. On binding to DNA, the fluorescence greatly increases. This protocol describes the use of Hoechst 33342 to label nuclear DNA of cells grown in culture.

RELATED INFORMATION

Hoechst 33342 can also be used to stain fixed cells by substituting Hoechst 33342 for DAPI in the protocol described in **Labeling Nuclear DNA Using DAPI** (Chazotte 2011a). Autofluorescence from endogenous cellular molecules such as the reduced forms of nicotinamide adenine dinucleotide or flavin adenine dinucleotide can interfere with imaging by reducing the signal-to-noise ratio. This occurs when the excitation and/or emission wavelengths of the probe and the autofluorescing molecules are similar, e.g., frequently with excitation wavelengths <500 nm, and particularly at ultraviolet wavelengths. Autofluorescence can be reduced by careful selection of the excitation and the emission wavelengths used, by treating fixed cells with reducing agents (e.g., a 1% solution of sodium borohydride [$NaBH_4$] for 20 min), and by comparing the experimental images with unlabeled control slides. Avoid fixation with glutaraldehyde, because it can increase interference from cellular autofluorescence, most frequently at wavelengths <500 nm.

This protocol assumes that the cells of interest were grown on glass microscope coverslips immersed in small Petri dishes containing culture medium. Generally, labeling conditions vary by cell type, and it might be necessary to alter the protocol for a particular use. To mount cells labeled using the technique described here, see **Mounting Live Cells onto Microscope Slides** (Chazotte 2011b).

MATERIALS

CAUTIONS AND RECIPES: Please see the end of this protocol for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Cells of interest, grown on coverslips

<**!**>Hoechst No. 33342 (10 mg/mL in H_2O stock solution; Invitrogen H1399)

Store stock solution at 4°C, protected from light.

<**R**>Phosphate-buffered saline (PBS)

Prepare PBS with added $CaCl_2$ and $MgCl_2$ (PBS^+). This solution allows cells to adhere to each other and to the substrate. If cells are in medium containing no Ca^{2+} or Mg^{2+}, they will round up and detach from the substrate.

Adapted from *Imaging: A Laboratory Manual* (ed. Yuste). CSHL Press, Cold Spring Harbor, NY, USA, 2010.
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Equipment

Cell culture dishes, sterile
Microscope, fluorescence, equipped with an ultraviolet (UV) filter set
<!>*For confocal microscopy laser excitation, use a UV laser or, if sufficiently intense, the UV line of an argon-ion laser.*

METHOD

Do not allow the cells to dry out at any time during the protocol.

1. Dilute the Hoechst stock solution 1:100 in H_2O for use in labeling.
2. Aspirate the cell medium from cells grown on coverslips. Rinse the cells three times with PBS^+.
3. Incubate the cells in the Hoechst labeling solution (from Step 1) for 10-30 min at room temperature.
4. Aspirate the labeling solution. Rinse the cells three times in PBS^+.
5. Mount the coverslips as described in **Mounting Live Cells onto Microscope Slides** (Chazotte 2011b).
6. Image the cells (λ_{ex} ~353 nm, λ_{em} ~483 nm for Hoechst 33342).

ACKNOWLEDGMENTS

I thank my wife, Nancy, and my daughter, Bryanna, for their patience while I was writing this article. I dedicate this article in memory of my mother, Cozette Chazotte, 1919-2009.

REFERENCES

Chazotte B. 2011a. Labeling nuclear DNA using DAPI. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot5556.

Chazotte B. 2011b. Mounting live cells onto microscope slides. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot5554.

RECIPES

[**NOTE:** Recipes for reagents marked with the <R> symbol not listed below can be found online at http://www.cshprotocols.org/recipes.]

Phosphate-buffered saline (PBS)

Reagent	Amount to add (for 1X solution)	Final concentration (1X)	Amount to add (for 10X stock)	Final concentration (10X)
NaCl	8 g	137 mM	80 g	1.37 M
<!>KCl	0.2 g	2.7 mM	2 g	27 mM
Na_2HPO_4	1.44 g	10 mM	14.4 g	100 mM
KH_2PO_4	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
<!>$CaCl_2 \cdot 2H_2O$	0.133 g	1 mM	1.33 g	10 mM
<!>$MgCl_2 \cdot 6H_2O$	0.10 g	0.5 mM	1.0 g	5 mM

<!>PBS can be made as a 1X solution or as a 10X stock. To prepare 1 L of either 1X or 10X PBS, dissolve the reagents listed above in 800 mL of H_2O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H_2O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle or by filter sterilization. Store PBS at room temperature.

CAUTIONS

[**NOTE:** For reagents marked with the <**!**> symbol not listed below, please consult the manufacturer's Material Safety Data Sheet for further information.]

$CaCl_2$ (Calcium chloride) is hygroscopic and may cause cardiac disturbances. It may be harmful by inhalation, ingestion, or skin absorption. Do not breathe the dust. Wear appropriate gloves and safety goggles.

HCl (Hydrochloric acid, Hydrochloride) is volatile and may be fatal if inhaled, ingested, or absorbed through the skin. It is extremely destructive to mucous membranes, upper respiratory tract, eyes, and skin. Wear appropriate gloves and safety glasses. Use with great care in a chemical fume hood. Wear goggles when handling large quantities.

Hoechst 33342 (sometimes called bisbenzimide) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood. Do not breathe the dust.

KCl (Potassium chloride) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

$MgCl_2$ (Magnesium chloride) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood.

UV light and/or UV radiation is dangerous and can damage the retina of the eyes. Never look at an unshielded UV light source with naked eyes. Examples of UV light sources that are common in the laboratory include handheld lamps and transilluminators. View only through a filter or safety glasses that absorb harmful wavelengths. UV radiation is also mutagenic and carcinogenic. To minimize exposure, make sure that the UV light source is adequately shielded. Wear protective appropriate gloves when holding materials under the UV light source.

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