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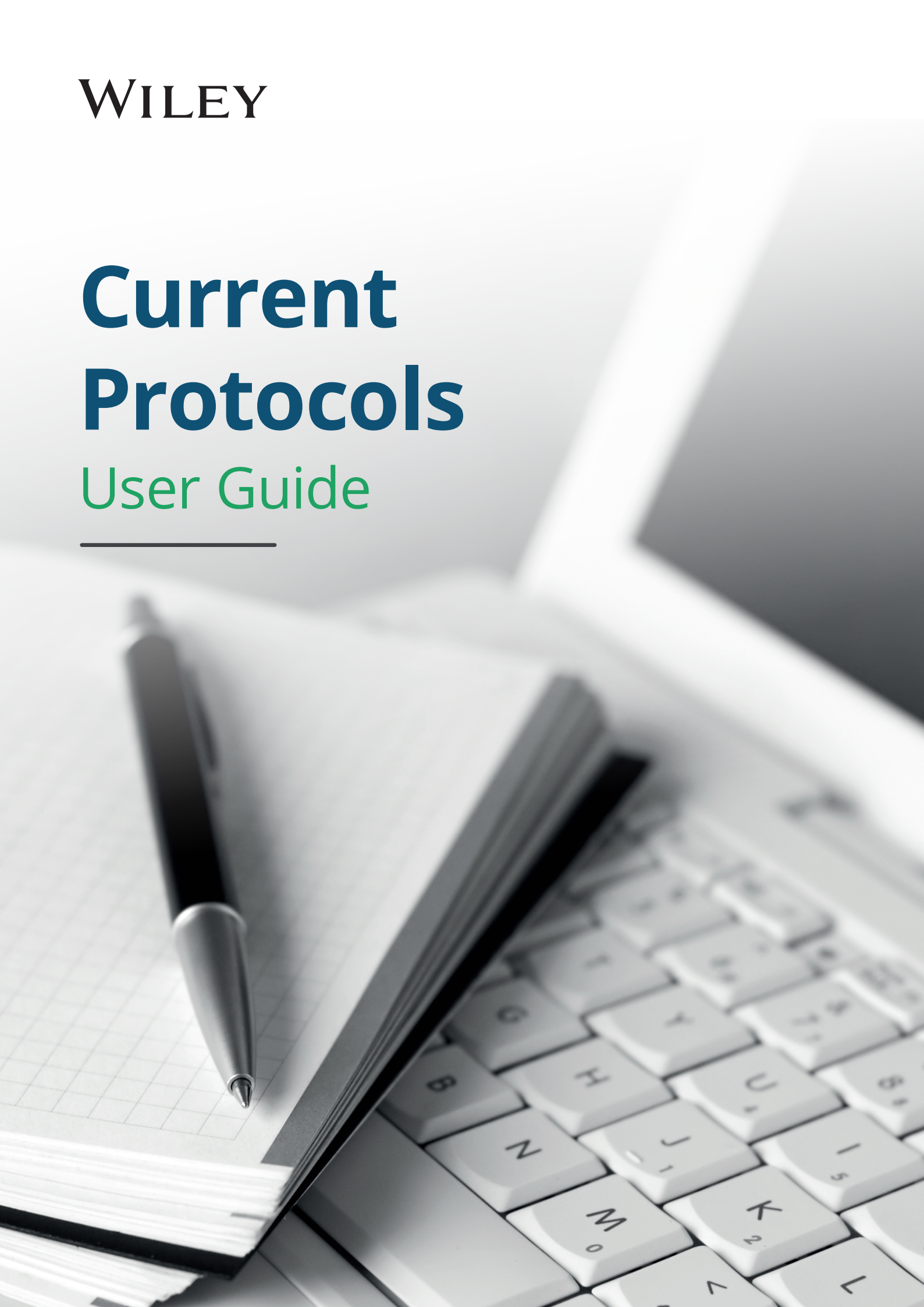
Current Protocols

User Guide



INDEX

What is Current Protocols?.....2

What are the benefits?.....3

Who should use Current Protocols?.....4

How do I access Current Protocols?.....4

How do I stay up-to-date?.....4

How do I use Current Protocols?.....5

- Homepage
- Logging in and registering
- Searching on the Current Protocols platform
- Searching within a Current Protocol title
- Exploring a Current Protocols Title
- Navigating a protocol
- Sections in a protocol

How do I publish a protocol?.....11

What is Current Protocols?

The Current Protocols collection includes nearly 25,000 step-by-step techniques and procedures that provide researchers with the reliable methods they need to reproducible results and pave the way for scientific discovery. Today we have 17 comprehensive collections ranging from *Essential Laboratory Techniques* for training undergraduate students to resources for developing as a scientist, like our flagship title, *Current Protocols in Molecular Biology.*

CURRENT PROTOCOLS
A Wiley Brand

- Microbiology
- Cytometry
- Essential Laboratory Techniques
- Molecular Biology
- Chemical Biology
- Immunology
- Plant Biology
- Human Genetics
- Cell Biology
- Stem Cell Biology
- Mouse Biology
- Bioinformatics
- Protein Science
- Pharmacology
- Neuroscience
- Nucleic Acid Chemistry
- Toxicology

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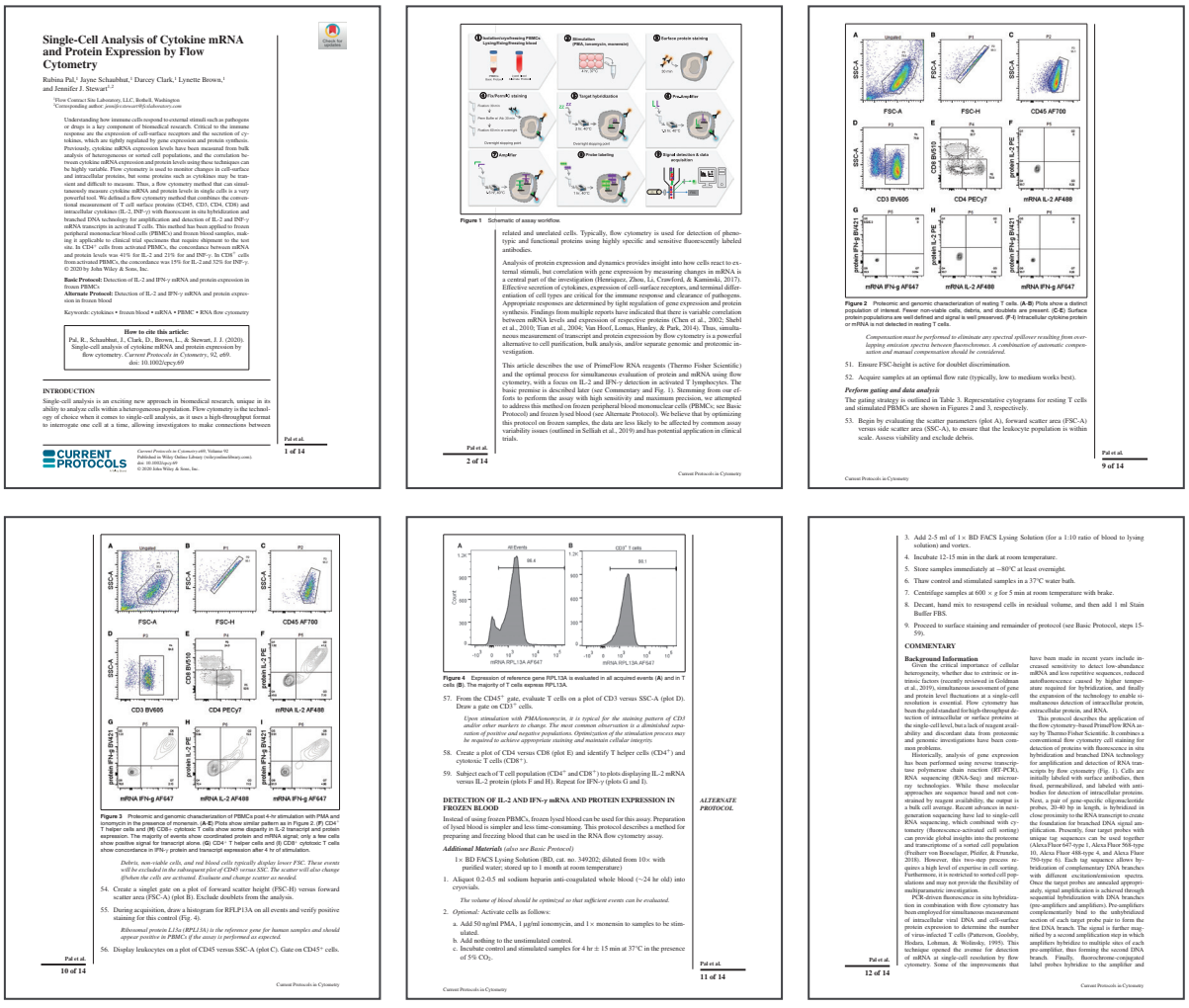
A single Current Protocols article is more than just a protocol: it often includes a collection of related protocols (basic, alternate, support). To make them easy to follow, Current Protocols features numbered steps, flow charts, illustrations, results, recipes, background, explanations, and advice.



What are the benefits?

- **Support:** Using Current Protocols is like having an expert guiding your hand.
- **Confidence:** Our editors evaluate each protocol, so you know these techniques are the best available.
- **Efficiency:** Each protocol is carefully edited and rich in detail, so you get reliable results on the first try.

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Who should use Current Protocols?

Current Protocols is great for institutions with large research programs to keep discovery efficient and consistent. It is also essential for smaller institutions that may lack breadth of expertise in-house so they can build on a larger body of knowledge without wasting time. Students, technicians, and post-docs will find Current Protocols invaluable for their bench work. Lab heads, principal investigators, and department chairs will find Current Protocols useful for grant writing and budgeting, and for planning long-term research projects.

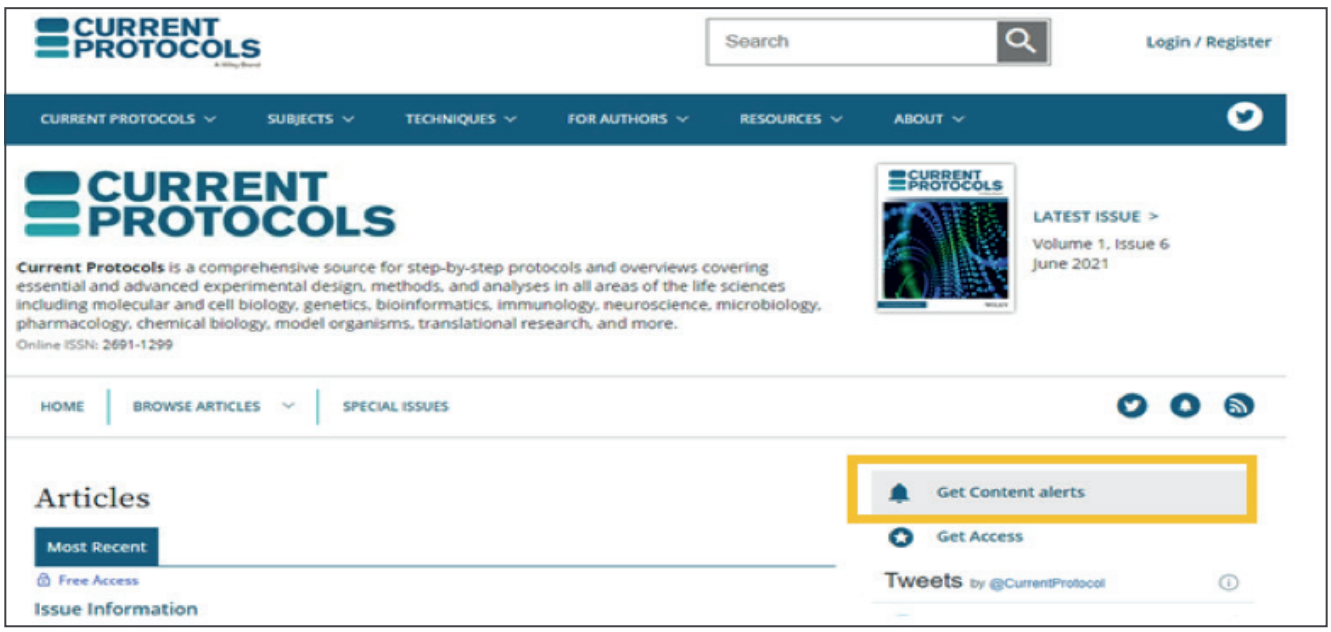
How do I access Current Protocols?

The URL to access Current Protocols is: **https://currentprotocols.onlinelibrary.wiley.com/**

How do I stay up-to-date?

Current Protocols are reviewed by the editorial board and updated on a regular basis. Running searches regularly and accessing the HTML versions will guarantee you are using the most-up-to-date information. We encourage the use of the HTML or online PDF versions so you will always find the latest version.

Be sure to sign up for alerts by clicking the alerts icon on the menu bar to the right of SPECIAL ISSUES within a specific Current Protocol page.



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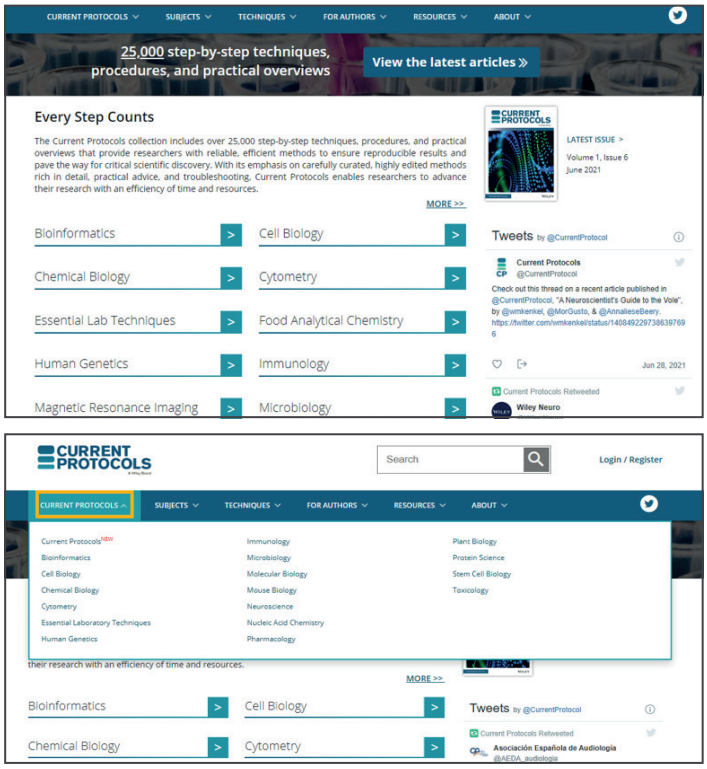
How do I use Current Protocols?

1 HOMEPAGE

First, go to:
currentprotocols.onlinelibrary.wiley.com

From the homepage, clicking on any topic will allow you to access a particular Current Protocols title. You can also use the dropdown Current Protocols menu to choose a specific title. There are also dropdown menus to explore by subject or by technique, described in more detail below.

Scroll down to see the latest protocols or to browse our "Best of" collection of protocols. You may want search across the entire collection using the search bar at the top of the page.

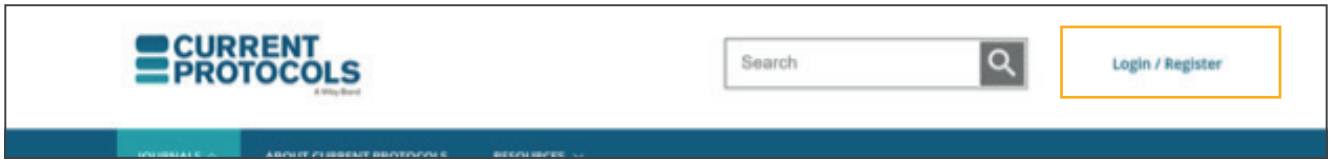


2 LOGGING IN AND REGISTERING

From the homepage, you can also click on **Login/Register** at the top right of the screen.

Registering or creating an account is easy and fast. You will be asked to use an institutional email and confirm that email during the registration process. Further instructions about how to log in and create your account can be found at the **Wiley Online Library training hub.**

Once you've logged in, you can save your favorite protocols for quick and easy access in the future. These will appear in the **Favorites** sections under **My Account** (once you have logged in, you can navigate to it from the top right corner by clicking on your name). You will need to log in to save searches, access saved searches, or set up alerts.



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3 SEARCHING ON THE CURRENT PROTOCOLS PLATFORM

The **search bar** at the top of the page will run keyword searches to find all relevant protocols. The results page features a number of **filters** on the left-hand menu.

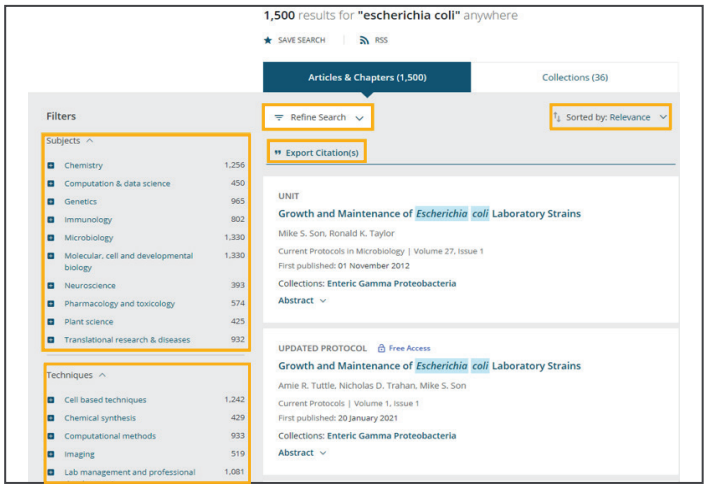
- Subjects: Use our custom Subject taxonomy to filter your searches. Each top level term can be expanded to narrow down your search results.
- Techniques: Use our custom Techniques taxonomy to filter your searches. Each top level term can be expanded to narrow down your search results. Subject and Techniques filters can be used together to rapidly focus on your research needs.
- Published in: Use this filter to narrow your search results to a particular Current Protocols title (e.g., Current Protocols in Molecular Biology). See 4. Searching Within a Current Protocols Title.
- Publication type: all protocols will appear as “Journals”.
- Publication date: you may use this filter to filter by year (last year, last two years, last five years or a custom range of your choice).

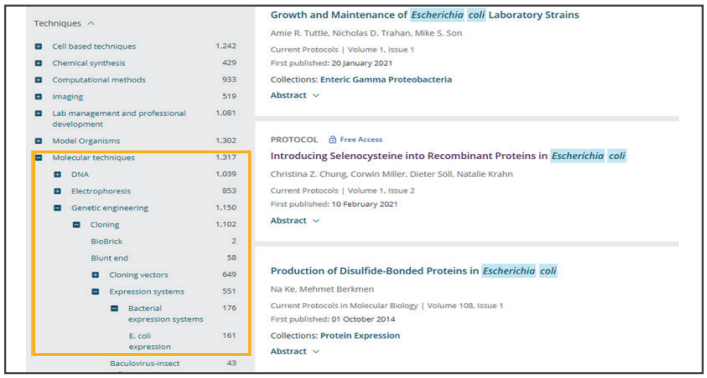
Below each protocol listed in your search, you can click **Abstract** to read the abstract while staying on the same page.

There are several different ways to sort your search: either by **relevance** or by **date** (from new to old or old to new).

You can also download citations for all results or selected results, by clicking on **Export citations** and selecting those you want to download.

If you wish to refine your search and be more specific, click on **Refine Search** on the top of the results page. Similar to an advanced search, this will allow you to add limits and additional keywords.





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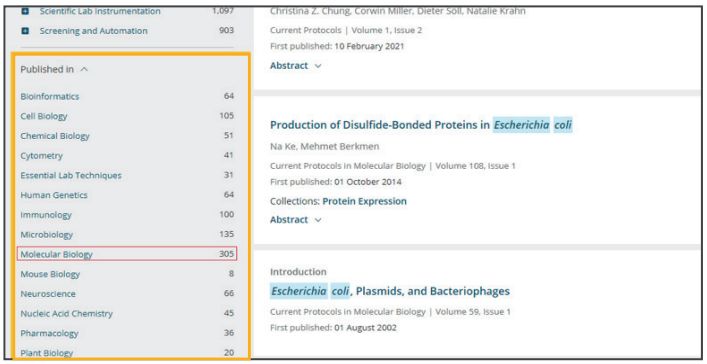
4 SEARCHING WITHIN A CURRENT PROTOCOL TITLE

After you use the search bar at the top of the page to search across all Current Protocols content, you can choose to restrict your results to a particular Current Protocols, as shown in the screenshot at the right.

Advanced Search (available from the search bar) will let you limit the search to author, title, keywords, etc., or to specify a publication date range. You can also click on **Citation Search** to search by citation.



You may find more information about these searching modalities in the **Wiley Online Library User Guide.**

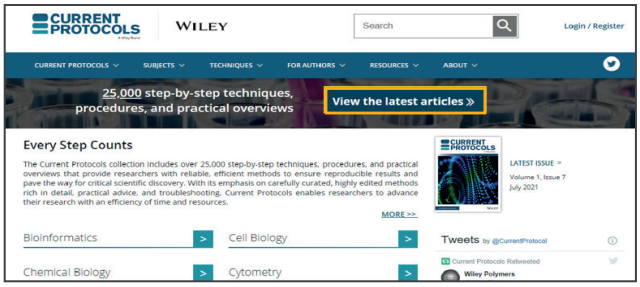
5 EXPLORING THE CURRENT PROTOCOLS COLLECTION

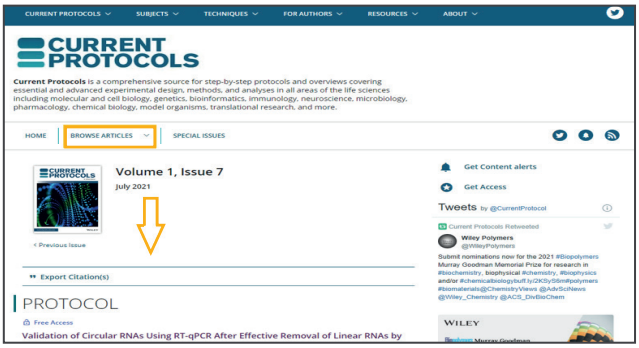
As of 2021, Current Protocols publishes monthly, interdisciplinary issues, while also sorting those articles into their traditional legacy Current Protocols titles. Here, we will illustrate the various ways to browse Current Protocols content.

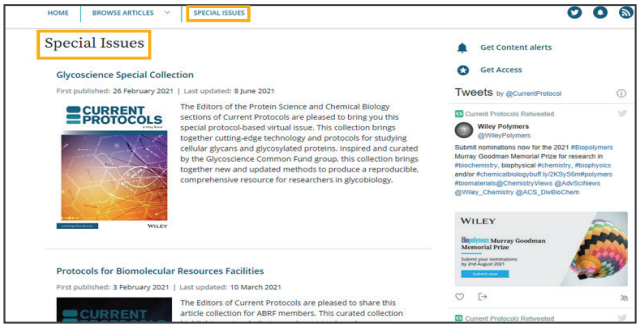
To see newly published protocols, click the **View the latest articles** button on the homepage. This brings you to the contents of the latest (in-progress issue).

From here, you can scroll to browse the issue. You can view previous issues from the **Browse Articles** dropdown menu.

Click on the **Special Issues** tab to view special collections of protocols. Here you will find protocols in new subject areas and interdisciplinary collections. Current Protocols editors carefully curate the content for each title – inviting the best researchers to share their detailed protocols – with an eye toward creating the comprehensive collection of the most important protocols in the field. Exploring the topics collections provides valuable context – understanding the various methods that can be employed to perform a task or answer a particular research question.







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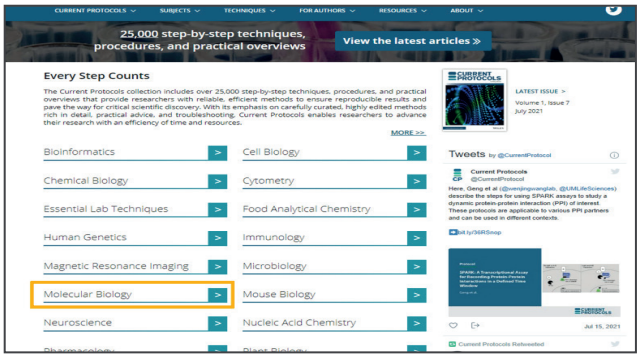
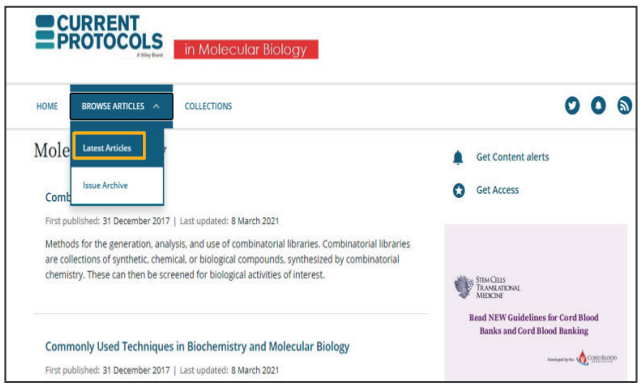
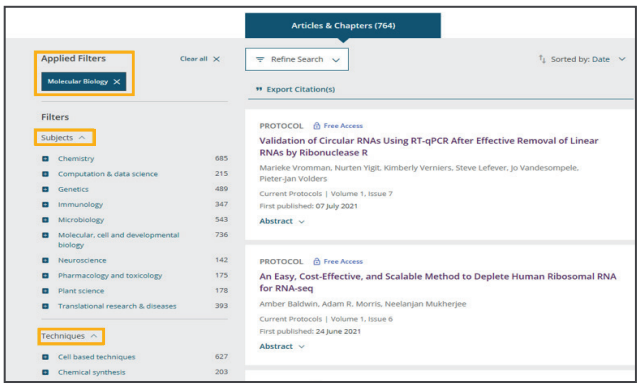
6 EXPLORING A CURRENT PROTOCOLS TITLE

Many readers will want to focus their browsing to a particular Current Protocols legacy title; this is accomplished by clicking on the desired CP title on the homepage – either from the center list or from the Current Protocols dropdown menu.

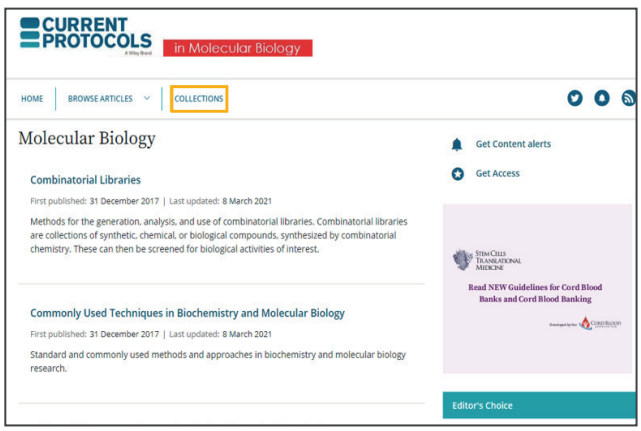


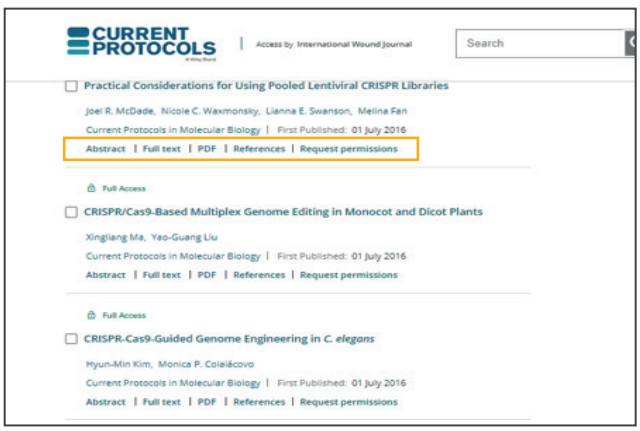
Clicking on Molecular Biology will take you to the Current Protocols in Molecular Biology page. From here, click on the **Browse Articles** dropdown menu to either view the latest molecular biology articles, or view the CPMB issue archive.





Clicking on the **Collections** tab will bring you to the familiar CPMB collections (formerly topics or chapters). This provides the curated view of all of the protocols published by Current Protocols in Molecular Biology, organized by topic.





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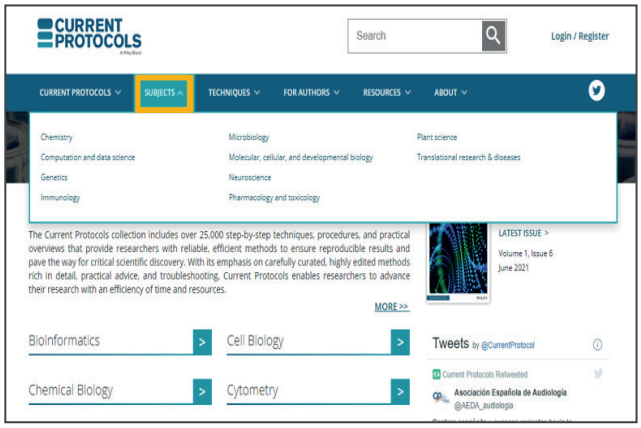
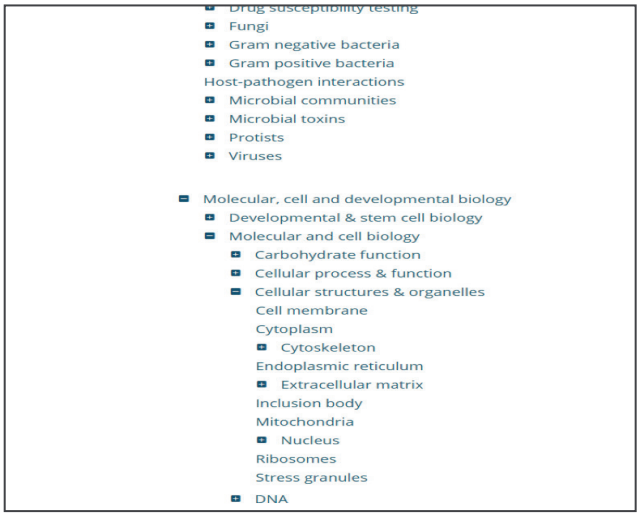
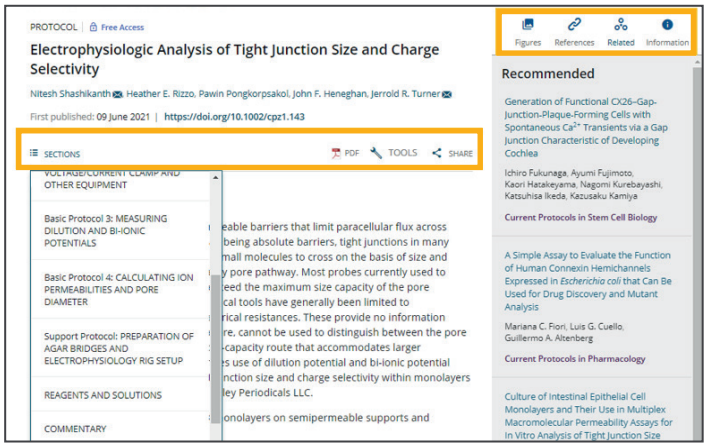
You can also take advantage of our custom taxonomies to browse the complete Current Protocols collection by either Subject or by Technique, which you can select from the dropdown menu on the Current Protocols homepage. Choosing your top level topic of interest will bring you to a page displaying the complete Subject or Technique taxonomy. This can be expanded to drill down to your particular area of interest. Clicking on your term of interest will return a complete set of articles across the entire CP collection on that topic. The list can be filtered as shown in the section describing Search, above.





7 NAVIGATING A PROTOCOL

In the HTML version, you will notice a number of sections and functionalities.



Top navigation

- **Sections:** open a drop-down menu and move to the section you want to read first.
- **PDF:** access the protocol in PDF format, which you can download, zoom in/out, and print as needed.
- **Tools:** request permissions, add to favorites or track citations.
- **Share:** share the content with others.

Left-hand menu

- **Figures:** see all the figures within the protocol and download the files as .png or .ppt files.
- **References:** find all the references of other primary and secondary sources used to write this protocol.
- **Related:** find related protocols and other articles related to this protocol.
- **Information:** find information such as metrics, related keywords (which are hyperlinked and allow you to run a new search with one click), publication history and copyright information.

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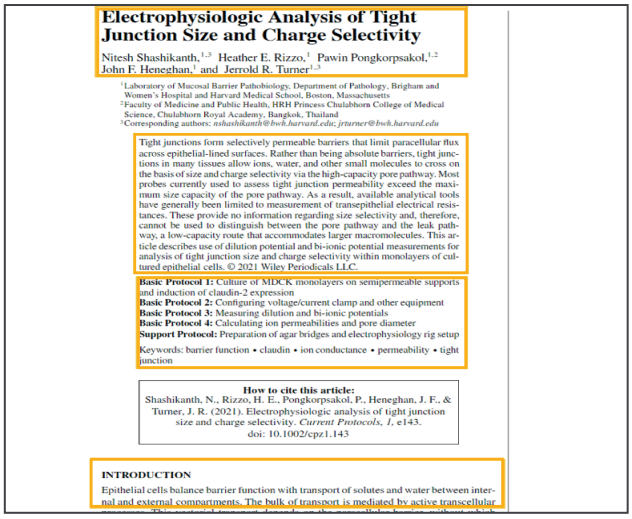
8 SECTIONS IN A PROTOCOL

- **Title**
- **Author(s)**
- **Abstract,** including list of protocols contained in the article
- **Introduction** to the article



Electrophysiologic Analysis of Tight Junction Size and Charge Selectivity

Nitesh Shashikanth,[1,3] Heather E. Rizzo,[1] Pawin Pongkorpsakol,[1,2] John F. Heneghan,[1] and Jerrold R. Turner[1,3]

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[2]Faculty of Medicine and Public Health, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy, Bangkok, Thailand
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Tight junctions form selectively permeable barriers that limit paracellular flux across epithelial-lined surfaces. Rather than being absolute barriers, tight junctions in many tissues allow ions, water, and other small molecules to cross on the basis of size and charge selectivity via the high-capacity pore pathway. Most probes currently used to assess tight junction permeability exceed the maximum size capacity of the pore pathway. As a result, available analytical tools have generally been limited to measurement of transepithelial electrical resistances. These provide no information regarding size selectivity and, therefore, cannot be used to distinguish between the pore pathway and the leak pathway, a low-capacity route that accommodates larger macromolecules. This article describes use of dilution potential and bi-ionic potential measurements for analysis of tight junction size and charge selectivity within monolayers of cultured epithelial cells. © 2021 Wiley Periodicals LLC.

Basic Protocol 1: Culture of MDCK monolayers on semipermeable supports and induction of claudin-2 expression
Basic Protocol 2: Configuring voltage/current clamp and other equipment
Basic Protocol 3: Measuring dilution and bi-ionic potentials
Basic Protocol 4: Calculating ion permeabilities and pore diameter
Support Protocol: Preparation of agar bridges and electrophysiology rig setup

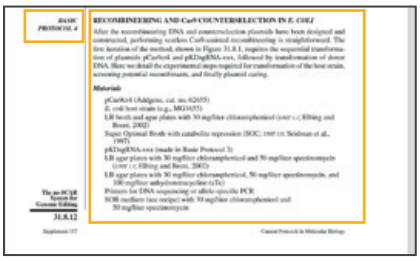
Keywords: barrier function • claudin • ion conductance • permeability • tight junction

How to cite this article:
Shashikanth, N., Rizzo, H. E., Pongkorpsakol, P., Heneghan, J. F., & Turner, J. R. (2021). Electrophysiologic analysis of tight junction size and charge selectivity. *Current Protocols, 1*, e143. doi: 10.1002/cpz1.143

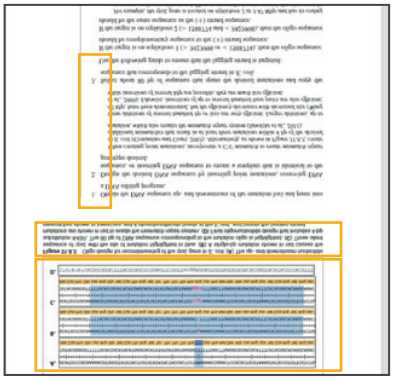
INTRODUCTION

Epithelial cells balance barrier function with transport of solutes and water between internal and external compartments. The bulk of transport is mediated by active transcellular

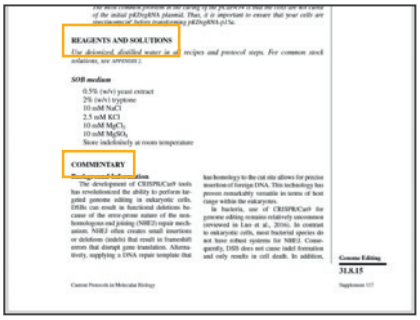
- **Basic, Alternate and Support Protocols** included as appropriate. Each protocol within the article has its own title, introduction and materials list with information about obtaining the reagents.



- **Protocol steps:** Numbered, step-by-step, written in active voice, and providing detailed instructions.
- Italicized **annotations** to steps with helpful hints, alternatives, and additional information.



- **Reagents and Solutions** section provides recipes for buffers that need to be prepared.
- **Commentary** section: Background Information, Critical Parameters, Troubleshooting, Anticipated Results, Time Considerations, Literature Cited.



REAGENTS AND SOLUTIONS

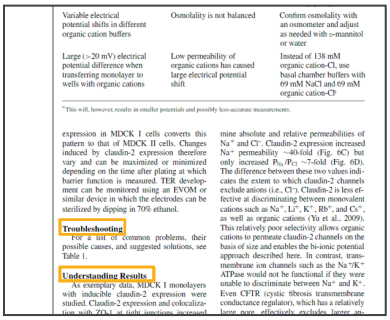
Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see APPENDIX 2.

SOB medium

0.5% (w/v) yeast extract
2% (w/v) tryptone
10 mM NaCl
2.5 mM KCl
10 mM $MgCl_2$
10 mM $MgSO_4$
Store indefinitely at room temperature

COMMENTARY

- **Troubleshooting:** what to do if things don't go as expected.
- **Understanding Results**



Variable electrical potential shifts in different organic cation buffers	Osmolality is not balanced	Confirm osmolality with an osmometer and adjust as needed with D-mannitol or water
Large (>20 mV) electrical potential difference when transferring monolayer to wells with organic cations	Low permeability of organic cations has caused large electrical potential shift	Instead of 138 mM organic cation-Cl, use basal chamber buffers with 69 mM NaCl and 69 mM organic cation-Cl

*This will, however, results in smaller potentials and possibly less-accurate measurements.

expression in MDCK I cells converts this pattern to that of MDCK II cells. Changes induced by claudin-2 expression therefore vary and can be maximized or minimized depending on the time after plating at which barrier function is measured. TER development can be monitored using an EVOM or similar device in which the electrodes can be sterilized by dipping in 70% ethanol.

Troubleshooting

For a list of common problems, their possible causes, and suggested solutions, see Table 1.

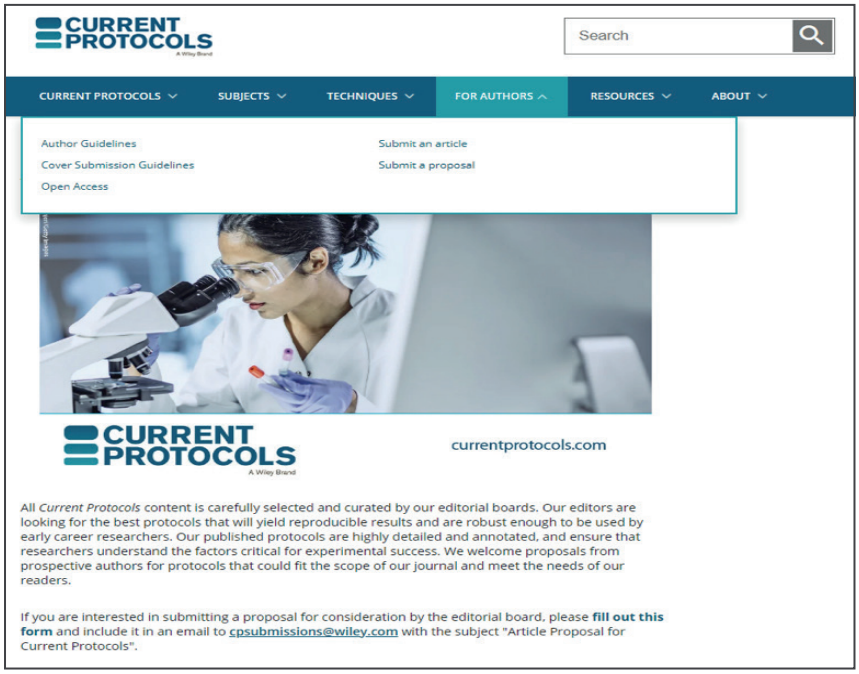
Understanding Results

As exemplary data, MDCK I monolayers with inducible claudin-2 expression were studied. Claudin-2 expression and colocaliza-

mine absolute and relative permeabilities of Na^+ and Cl^-. Claudin-2 expression increased Na^+ permeability ~40-fold (Fig. 6C) but only increased P_{Na}/P_{Cl} ~7-fold (Fig. 6D). The difference between these two values indicates the extent to which claudin-2 channels exclude anions (i.e., Cl^-). Claudin-2 is less effective at discriminating between monovalent cations such as Na^+, Li^+, K^+, Rb^+, and Cs^+, as well as organic cations (Yu et al., 2009). This relatively poor selectivity allows organic cations to permeate claudin-2 channels on the basis of size and enables the bi-ionic potential approach described here. In contrast, transmembrane ion channels such as the Na^+/K^+ ATPase would not be functional if they were unable to discriminate between Na^+ and K^+. Even CFTR (cystic fibrosis transmembrane conductance regulator), which has a relatively

How do I publish a protocol?

If you would like to submit a protocol for publication, visit the title in which you are interested. On the top navigation, click on the section **For Authors.** Within the drop-down menu, select **Submit a proposal.** The editorial board will consider your proposal, and if appropriate, will invite you to submit a manuscript describing the method in detail.



You may also submit your proposal by scanning the following QR code.

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