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Notes:

About Current Protocols ...

A protocol is a precise and detailed experiment or “method” for the study of a biochemistry, molecular biology, and/ or biomedical problem:

- helps speed up research by providing tried and tested procedures
- organized in a standardized format – easy to use in a laboratory environment
- step-by-step procedures
- includes materials and equipment needed
- figures and tables
- “notes” or “tips” provide troubleshooting suggestions.



Notes:

Features of Current Protocols product website currentprotocols.com ...

- Search and browse across all protocols
- Tools and calculators
- Video protocols
- New protocol alerts and RSS feeds
- All content on the site is open access
- Full text can be accessed on Wiley InterScience (with subscription)
- Ability to add supplier and user-generated protocols
- Rate and comment on protocols
- 'Ask the Experts' – free online protocol advice
- Troubleshooting forums
- Read the editors blog – for news, commentaries, and the latest developments in methods
- Personalize your experience with My CP.com
 - Upload your own protocol to share with the Current Protocols community
 - Organize your favorites

TOOLS & CALCULATORS

- ▶ [Bacterial Media Recipes Calculator](#)
- ▶ [Buffer Calculator](#)
- ▶ [Colorimetric Assay Calculation Tool](#)
- ▶ [Common Laboratory Recipes Calculator](#)
- ▶ [DNA-Protein Translator Tool](#)
- ▶ [DNA/RNA/Protein Molecular Weight Calculator](#)
- ▶ [G-Force/RPM Conversion Tool](#)
- ▶ [Hemocytometer Calculation Tool](#)
- ▶ [ImageJ Java Applet](#)
- ▶ [NEBcutter v. 2.0](#)
- ▶ [Polyacrylamide Gel Recipes Calculator](#)
- ▶ [Primer3Plus](#)
- ▶ [Radioactive Decay Calculator for Isotopes Commonly Used in Biomedical Research](#)
- ▶ [Solution Concentration Calculator](#)
- ▶ [Spectrophotometric Measurement of Nucleic Acids Calculator](#)
- ▶ [Units of Measurement Conversion Tool](#)

DISCUSSION BOARDS

- ▶ [General Discussion](#)
- ▶ [Ask the Experts: Molecular and Cell Biology](#)
- ▶ [Ask the Experts: Protein Science](#)

Video protocols available!

Watch peer-reviewed, high quality step-by-step video protocols for top lab procedures!

[See a list of all videos ▶](#)



FEATURED VIDEO

[Phase Contrast and Differential Interference Contrast \(DIC\) Microscopy](#)

Notes:

Home

From the homepage you can search or browse, access discussion boards, tools and calculators and the editors blog, view video protocols, and set up a new protocol alert or RSS feed

1... Browse by category directly from the homepage or click on the CATEGORIES tab from anywhere on the site.

2... You can search from the homepage or from any page on the site using the simple search text box.

3... View featured video protocol or link to full list.

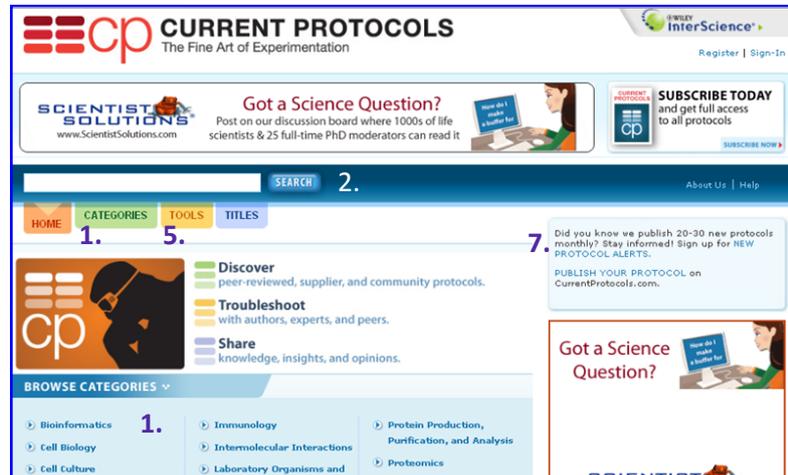
4... Link to BEYOND THE BENCH, the editors blog, on the right of all pages.

5... Access Tools and Calculators from the TOOLS tab, and on the right of the screen on all pages.

6... Link to Discussion Boards on the right of the all pages.

7... Set up an email alert on the right of the screen or scroll to the bottom.

8... There you can also set up an RSS feed.



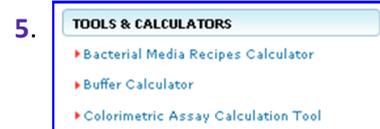
The screenshot shows the Current Protocols homepage. Callout 1 points to the 'CATEGORIES' tab. Callout 2 points to the search box. Callout 3 points to the 'Discover' section. Callout 4 points to the 'BEYOND THE BENCH' blog section. Callout 5 points to the 'TOOLS' tab. Callout 6 points to the 'DISCUSSION BOARDS' section. Callout 7 points to the 'SUBSCRIBE TODAY' button.



This section highlights video protocols. Callout 3 points to the text: "Watch peer-reviewed, high quality step-by-step video protocols for top lab procedures! See a list of all videos".



This section is titled 'BEYOND THE BENCH' and includes the text: "Read our editors' blog for news, commentaries, and the latest developments in methods in and out of the lab." Callout 4 points to this section.



This section lists various tools and calculators. Callout 5 points to the section header. The tools listed are: Bacterial Media Recipes Calculator, Buffer Calculator, and Colorimetric Assay Calculation Tool.



This section lists discussion boards. Callout 6 points to the section header. The boards listed are: General Discussion, Ask the Experts: Molecular and Cell Biology, and Ask the Experts: Protein Science.



The footer contains navigation links and copyright information. Callout 7 points to the 'New Protocol Alerts' link, and callout 8 points to the 'RSS' link. The footer text includes: "Purchasing & Subscribing Information | Tutorial | New Protocol Alerts | RSS | Current Protocols Editorial Board | Help | About Us | For Authors | For Librarians | For Advertisers | Website Advisory Panel | Privacy Policy | Copyright © 2000-2009 by John Wiley & Sons, Inc. or related companies. All rights reserved."

Notes:

Home > Registration and login

Registering on currentprotocols.com allows you to save and organize your favorites, and upload your own protocol to share with the Current Protocols community.

- 1... Click the Register link on the homepage.
- 2... On the registration page enter your email address and choose a password.
- 3... Enter your personal details.
- 4... Enter your country or location.
- 5... Complete the security check.
- 6... Check your details and click Register.

Once submitted you will receive an email asking you to authenticate your account.

7... After authenticating your account you can then log in.



User account

[Create new account](#) | [Log in](#) | [Request new password](#) | [Resend Authentication Mail](#)


 Current Protocols is part of Wiley InterScience. Create a free Wiley InterScience account below to upload your protocol, create a profile, or save your favorites. If you already have a Wiley InterScience account, log in using the tab above.

Email Address: *

An e-mail will be sent to you from Wiley InterScience with instructions on how to confirm your account.

Retype Email Address: *

 2.

Password: *

Retype Password: *

Please Note: passwords must be alphanumeric (no special characters) between 5 and 32 characters long.

Personal Details

First Name: *

 3.

Last Name: *

Country or Location: *

UNITED STATES **4.**

CAPTCHA

This question is for testing whether you are a human visitor and to prevent automated spam submissions.

5. What abjured



stop spam. read books.

[Register](#) **6.**

7.

[Create new account](#) | [Log in](#) | [Request new password](#) | [Resend Authentication Mail](#)


 Current Protocols is part of Wiley InterScience.

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Enter the e-mail address used in your Current Protocols or Wiley InterScience account.

Password: *

If you can't remember your Current Protocols or InterScience password, request a new password above.

[Log in](#)

Notes:



Home > Browsing

Browse by category directly from the homepage or click on the CATEGORIES tab from anywhere on the site.

- 1... Click on a category.
- 2... From the category page you can view recently added or featured protocols in that category.
- 3... You can refine your browsing further by selecting a sub category.
- 4... From the sub category results list you can browse the protocols within that category.
- 5... You can sort the results by date or alphabetically.
- 6... Or filter by **Current Protocols** only or **All Protocols** - which includes user and supplier generated protocols – where available.

BROWSE CATEGORIES ▾

- ▶ Bioinformatics **1.**
- ▶ Immunology
- ▶ Protein Production, Purification, and Analysis
- ▶ Cell Biology
- ▶ Intermolecular Interactions
- ▶ Proteomics
- ▶ Cell Culture
- ▶ Laboratory Organisms and

Bioinformatics

BROWSE ▾

- ▶ Bioinformatics Fundamentals
- ▶ Biological Databases **3.**
- ▶ Cheminformatics
- ▶ DNA Analysis
- ▶ Expression Patterns
- ▶ Finding Genes
- ▶ Finding Similarities and Inferring Homologies
- ▶ Modeling Structures from Sequence
- ▶ Molecular modelling
- ▶ Phylogenetic trees
- ▶ Protein Analysis
- ▶ Proteomics
- ▶ Recognizing Functional Domains
- ▶ RNA Analysis

RSS Feed

RECENTLY ADDED **2.**

- ▶ Exome™ Horizon – Complete and Unified Bioinformatics learning platform
- ▶ Example of Use of TaqMan Real-Time RT-PCR to Analyze Bacterial Gene Transcript Levels: Haemophilus influenzae
- ▶ The UCSC Genome Browser: What Every Molecular Biologist Should Know
- ▶ The Importance of Biological Databases in Biological Discovery
- ▶ An Introduction to Sequence Similarity ("Homology") Searching

FEATURED PROTOCOLS **2.**

- ▶ Searching NCBI Databases Using Entrez
- ▶ Selection of a Platform for Mutation Detection

Bioinformatics

DNA Analysis **4.**

1 - 10 of 30 1 2 3 > >>

5. SORT BY: **ALPHABETICAL** DATE **6.** FILTER BY: **All Protocols** Current Protocols only

Assays for DNA Damage
Print Publication Date: November, 1999
Source: Current Protocols in Toxicology

Assembling Genomic DNA Sequences with PHRAP
Print Publication Date: March, 2007
Source: Current Protocols in Bioinformatics

Computer Manipulation of DNA and Protein Sequences
Print Publication Date: April, 1995
Source: Current Protocols in Molecular Biology



Notes:

Home > Searching

Search for specific terms or protocols directly from the homepage or from any page on the site.

1... Enter your search term.

2... On the search results page you can sort the results by date or alphabetically.

3... Filter by **Current Protocols** only or **All Protocols** - which includes user and supplier generated protocols – where available.

4... or Refine by category or Publication.

5... To view a protocol click on the title.

1.

[HOME](#) [CATEGORIES](#) [TOOLS](#) [TITLES](#)

Search Results

2. **SORT BY**

- ▶ Relevance
- ▶ Date
- ▶ Alphabetical

3. **REFINE THESE RESULTS**

All protocols [What's this?](#)
 Current Protocols only

9 matches found for **purification Escherichia coli**

[Preparation of Soluble Proteins from Escherichia coli](#)
Author(s): Paul T. Wingfield
Publication Date: August, 2005
Source: Current Protocols in Protein Science

[Selection of Escherichia coli Expression Systems](#)
Author(s): Alain Bernard, Mark Payton
Publication Date: June, 1995
Source: Current Protocols in Protein Science

IN CATEGORIES:

- ▶ Cell Culture (2)
- ▶ Microbiology (1) **4.**
- ▶ Molecular Biology (5)

IN PUBLICATIONS:

- ▶ Current Protocols in Molecular Biology (1)
- ▶ Current Protocols in Protein Science (8)

5. [Folding and Purification of Insoluble \(Inclusion Body\) Proteins from Escherichia coli](#)

Author(s): Paul T. Wingfield, Ira Palmer, Shu-Mei Liang

Publication Date: June, 1995

Source: Current Protocols in Protein Science

Notes:

Viewing content

All content on currentprotocols.com is free. The full text of the protocols is located on the [Wiley InterScience](http://www.interscience.wiley.com) web site and is available on subscription.

- 1... Here you can view the abstract.
- 2... Table of contents.
- 3... Materials list - which includes a link (at the bottom of the list) to a Suppliers guide.
- 4... Figures
- 5... click on figure, or the "View Image" link, to enlarge.
- 6... Right mouse click over the figure to save or copy
- 7... View a list of literature cited.
- 8... Click on the PDF or HTML links to view the full text.



Folding and Purification of Insoluble (Inclusion Body) Proteins from Escherichia coli

Paul T. Wingfield¹, Ira Palmer¹, Shu-Mei Liang²

¹National Institutes of Health, Bethesda, Maryland
²North American Vaccine Corp., Beltsville, Maryland

Publication Name: Current Protocols in Protein Science
Unit Number: UNIT 6.5
DOI: 10.1002/0471140864.ps0605s00
Print Publication Date: June, 1995
Online Posting Date: May, 2001

USER RATINGS

Easy to Follow
Your rating: None (2 votes)

Achieved Expected Results
Your rating: None (1 vote)

Overall Rating
Your rating: None (1 vote)

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8.

GO TO THE FULL TEXT: [PDF](#) or [HTML](#) at Wiley Interscience

Are you the author of this protocol? [Login](#) or [register](#) and return to this page.
Learn more about the author(s): Paul Wingfield

ABSTRACT 1.

2. TABLE OF CONTENTS

3. MATERIALS

4. FIGURES

7. LITERATURE CITED

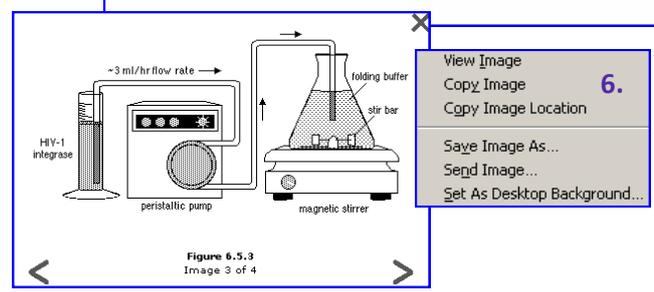
AUTHOR NOTES

ABSTRACT

Heterologous expression of recombinant proteins in E. coli often results in the formation of insoluble and inactive protein aggregates, commonly referred to as inclusion bodies. To obtain the native (i.e., correctly folded) and hence active form of the protein from such aggregates, four steps are usually followed: (1) the cells are lysed and the aggregates, (2) the cell wall and outer membrane components of the aggregates are removed, (3) the aggregates are solubilized (or extracted) with strong protein denaturants, and (4) the solubilized, denatured proteins are folded with concomitant oxidation of reduced cysteine residues into the correct disulfide bonds to obtain the native protein. This unit features three different approaches to the final step of protein folding and purification. In the first, guanidineHCl is used as the denaturant, after which the solubilized protein is folded (before purification) in an "oxido-shuffling" buffer system to increase the rate of protein oxidation. In the second, acetic acid is used to solubilize the protein which is then refolded in a "refolding" buffer before purification.

NOTE: All steps are carried at 4°C unless otherwise stated.
Looking for materials? [Suppliers Guide 3.](#)

Figure 6.5.3
Setup for folding of HIV-1 integrase by dilution into buffer.
[5. View Image](#)



Notes:

Home > Video Protocols

currentprotocols.com offers free video protocols.

1... From the homepage you can view the featured video protocol.

2... Click on the "See a list of all videos" link to see all available videos.

3... Click on image or title to view.

4... As with text protocols, you can ask questions or provide tips, tricks or improvements.

1. **Video protocols available!**
Watch peer-reviewed, high quality step-by-step video protocols for top lab procedures!
2. [See a list of all videos ▶](#)



FEATURED VIDEO
Phase Contrast and Differential Interference Contrast (DIC) Microscopy

Current Protocols Videos



3. **Alternate Aphid Feeding Chamber**

Duration: 1:50
[Play Video ▶](#)



Aphid Feeding Chamber

Duration: 2:06
[Play Video ▶](#)



Calibration of the needle with masses

Duration: 0:29
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Chromosome

Duration:
[Play Video ▶](#)



Coating glass

Duration:
[Play Video ▶](#)



Counting

Duration:



Alternate Aphid Feeding Chamber, CPMC UNIT 16B.1
Duration: 1:50
Video Seq: 2
RELATED PROTOCOLS:
[Aphid Transmission of Plant Viruses](#)

4. **Looking for Answers?**
Do you have tips, tricks, or improvements to share?
[JOIN THE CONVERSATION](#)

Notes:



Home > Tools and Calculators

The tools and calculators on currentprotocols.com are free and can be used online providing instant results.

1... Access tools and calculators from the TOOLS tab, and on the right of the screen on all pages.

2... Click on the tool or calculator.

Here is an example using the buffer calculator.

3... Choose a buffer from the drop-down menu.

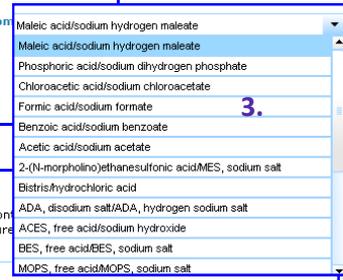
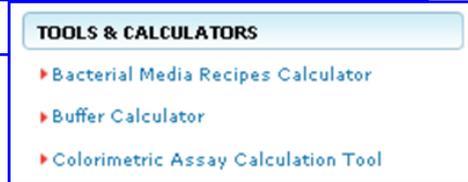
4... Adjust the bars to get concentration, volume, pH, and temperature.

5... The resulting recipe is displayed below.



Tools and Calculators

- ▶ Bacterial Media Recipes Calculator
- ▶ Buffer Calculator **2.**
- ▶ Colorimetric Assay Calculation Tool
- ▶ Common Laboratory Recipes Calculator
- ▶ DNA-Protein Translator Tool
- ▶ DNA/RNA/Protein Molecular Weight Calculator
- ▶ G-Force/RPM Conversion Tool
- ▶ Hemacytometer Calculation Tool
- ▶ ImageJ Java Applet
- ▶ NEBcutter v. 2.0
- ▶ Polyacrylamide Gel Recipes Calculator
- ▶ Primer3Plus
- ▶ Radioactive Decay Calculator for Isotopes Commonly Used in Biology
- ▶ Solution Concentration Calculator
- ▶ Spectrophotometric Measurement of Nucleic Acids Calculator
- ▶ Units of Measurement Conversion Tool



Buffer Calculator

Provides recipes for the preparation of buffers over a concentration range of 0.001 to 2000 mol/l. It also enables scaling for volume and correction for temperature. The following table lists the buffer systems used in order of ascending pKa's.

TO PREPARE Buffer: Maleic acid/sodium hydrogen maleate **3.** with pKa: 2.00

Concentration (mol/l): 0.005 2 0.005 **4.** Volume (ml): 100 2000 100

pH: 1 3 1 **4.** Temperature of usage (C°): 0 60 0

FOLLOW THE RECIPE **5.** 0.0004645

Ingredient	Stock concentration (mol/l)	Volume (ml)
Maleic acid	0.005 <input type="text"/> 5 <input type="text"/> 1 5.	0.4536
Sodium hydrogen maleate	0.005 <input type="text"/> 5 <input type="text"/> 1 5.	0.04645

Add water up to: 100 ml

Check pH and correct it if necessary



Notes:

Home > MyCP.com

Save and organize your favorites, and upload your own protocol to share with the Current Protocols community. To access this function you must be registered and logged in.

- 1... Log in
- 2... When logged in to currentprotocols.com you will see a list of your recently viewed protocols on the right hand side.
- 3... These can be quickly added to your favorites.
- 4... Click the MyCP.com link to manage your favorites, edit your profile and upload a protocol.
- 5... Add or delete protocols and tools from your favorites list.
- 6... Upload your own protocol.
- 7... Edit your profile and login information.



1. Register | Sign-In

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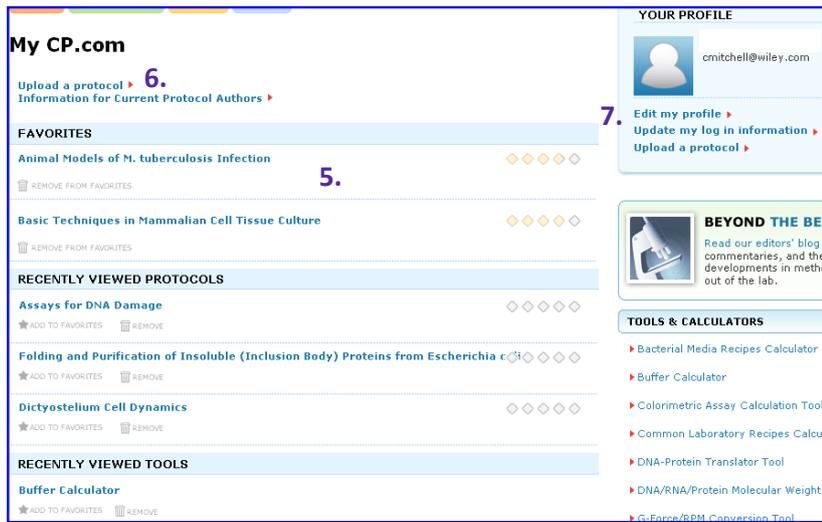
- ▶ Assays for DNA Damage **3.** ADD TO FAVORITES REMOVE
- ▶ Folding and Purification of Insoluble (Inclusion Body) Proteins from Escherichia coli ADD TO FAVORITES REMOVE
- ▶ Dictyostelium Cell Dynamics ADD TO FAVORITES REMOVE

DELETE THIS LIST

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Did you know we publish 20-30 new protocols monthly? Stay informed! Sign up for **NEW PROTOCOL ALERTS.**



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Information for Current Protocol Authors ▶

7.

FAVORITES

- Animal Models of *M. tuberculosis* Infection **5.** REMOVE FROM FAVORITES
- Basic Techniques in Mammalian Cell Tissue Culture REMOVE FROM FAVORITES

RECENTLY VIEWED PROTOCOLS

- Assays for DNA Damage ADD TO FAVORITES REMOVE
- Folding and Purification of Insoluble (Inclusion Body) Proteins from Escherichia coli ADD TO FAVORITES REMOVE
- Dictyostelium Cell Dynamics ADD TO FAVORITES REMOVE

RECENTLY VIEWED TOOLS

- Buffer Calculator ADD TO FAVORITES REMOVE

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cmitchell@wiley.com

- Edit my profile ▶
- Update my log in information ▶
- Upload a protocol ▶

BEYOND THE BENCH

Read our editors' blog commentaries, and the developments in methods out of the lab.

TOOLS & CALCULATORS

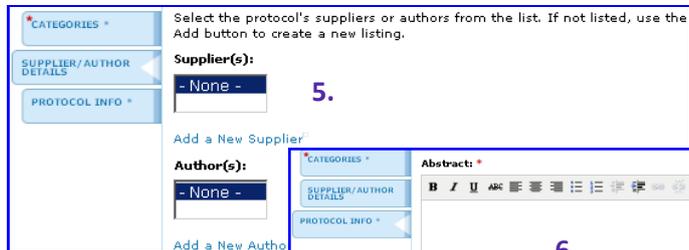
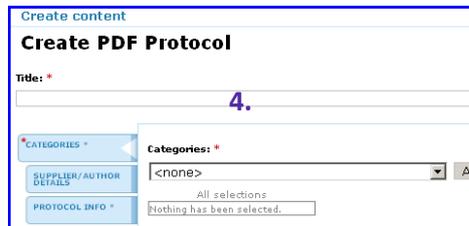
- Bacterial Media Recipes Calculator
- Buffer Calculator
- Colorimetric Assay Calculation Tool
- Common Laboratory Recipes Calculator
- DNA-Protein Translator Tool
- DNA/RNA/Protein Molecular Weight
- G-Force/RPM Conversion Tool

Notes:

Home > MyCP.com > Upload a protocol

Upload your own protocol to share with the Current Protocols community.

- 1... Click on the “Upload a protocol” link
 - 2... On the Share Your Protocol screen you will find submission guidelines for User Protocols and Supplier Protocols.
 - 3... Click on “Publish your protocol” to upload your protocol .
 - 4... Fill in title and select categories.
 - 5... Complete author details.
 - 6... Add an abstract and upload your protocol file.
 - 7... When ready, click the Save & Preview button.
- Once you have uploaded and saved your protocol it will be submitted to our editorial team for approval. If approved it will then be publicly viewable.



Notes: