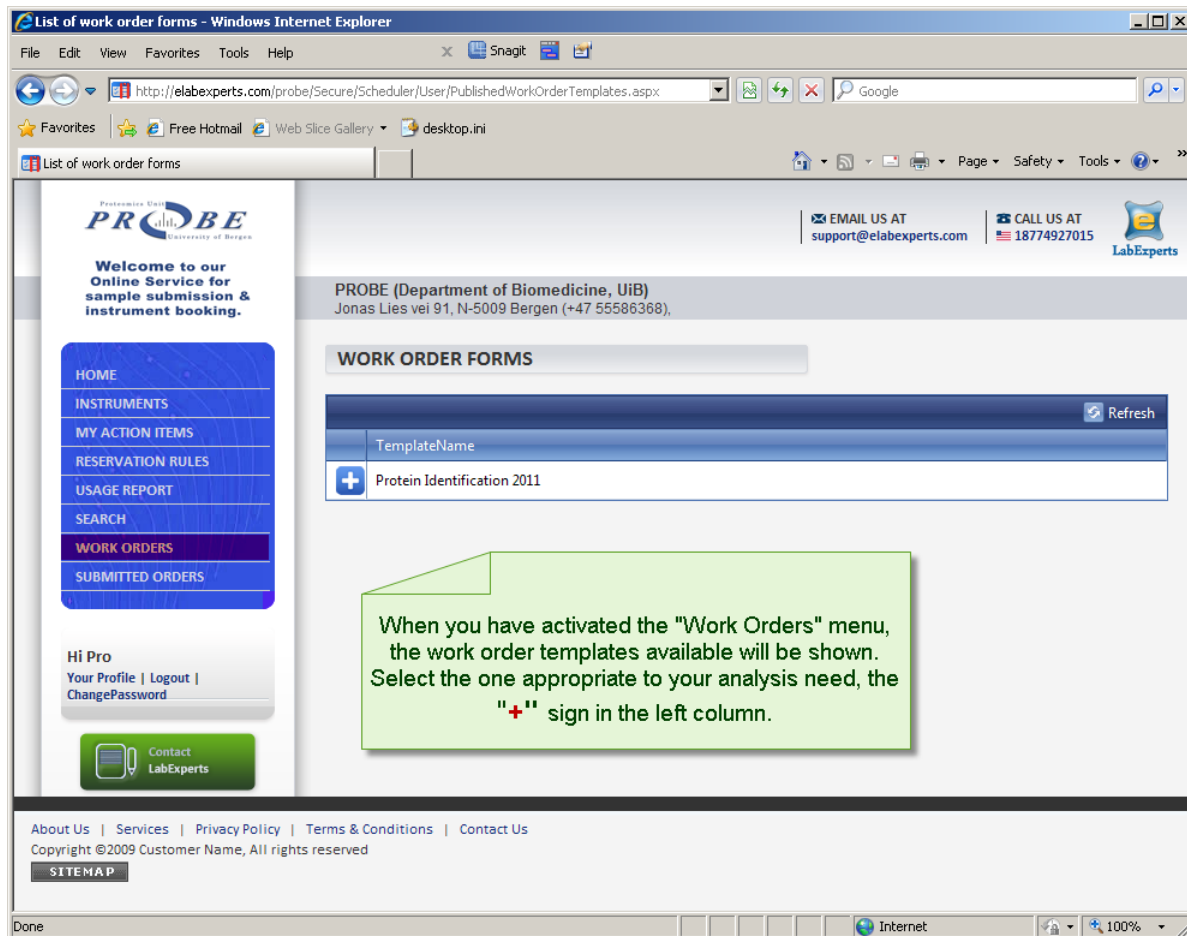


- submitting work orders


You need to log into our Booking System in order to submit samples for identification of proteins from SDS-PAGE or from in-solution. Fill in the work order form "Protein Identification 2011".



The screenshot shows a web browser window titled "List of work order forms - Windows Internet Explorer". The address bar shows the URL: <http://elabexperts.com/probe/Secure/Scheduler/User/PublishedWorkOrderTemplates.aspx>. The page content includes the PROBE logo and navigation menu on the left, contact information for PROBE (Department of Biomedicine, UiB) at the top right, and a table of work order forms. A green callout box highlights the "Protein Identification 2011" form.

PROBE (Department of Biomedicine, UiB)
Jonas Lies vei 91, N-5009 Bergen (+47 55586368)

WORK ORDER FORMS

TemplateName
 Protein Identification 2011

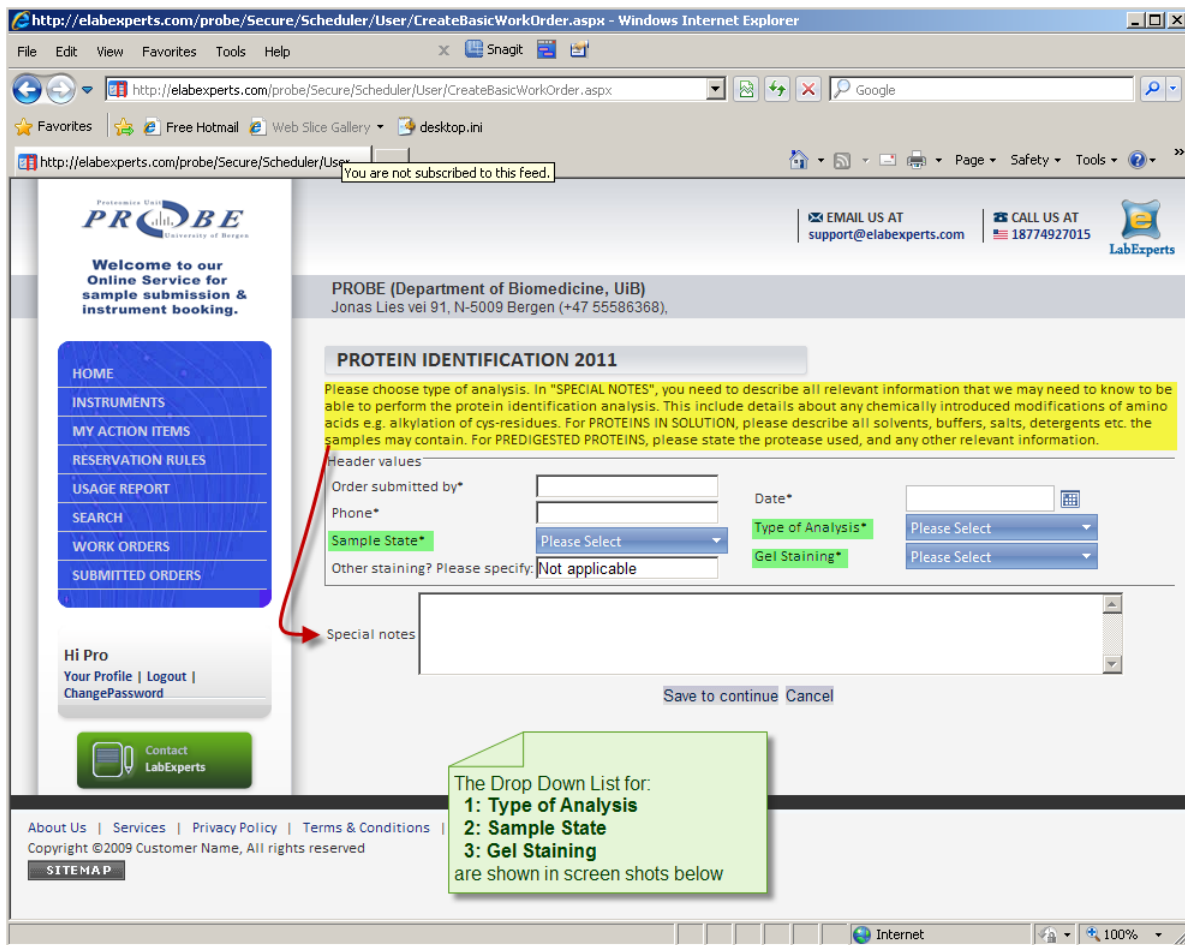
When you have activated the "Work Orders" menu, the work order templates available will be shown. Select the one appropriate to your analysis need, the "+" sign in the left column.

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SITEMAP

In the following, screenshots will guide you through this template...

Highlighted in the screenshot below, is a description of relevant information about your samples we need to know to perform the analysis you want to do. This information is submitted by using the text field **“Special notes”**. Information include details about any chemically introduced modifications of amino acids e.g. alkylation of cys-residues. For ***Proteins in solution*** you need to describe all solvents, buffers, salts, detergents etc. the samples may contain, and for ***Predigested Proteins*** we need to know the protease used.

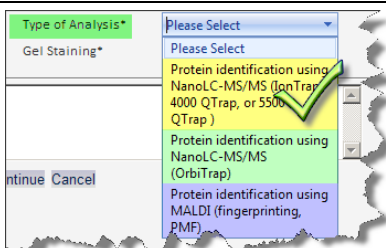
If your proteins originate from a species with no protein sequences in public databases, we can not identify your proteins, but may identify a few peptides caused by homology between species.



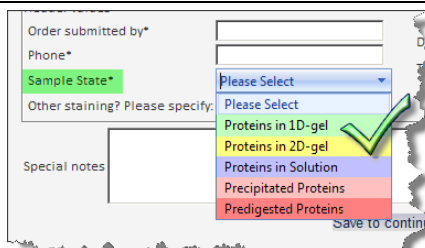
The screenshot shows the 'PROTEIN IDENTIFICATION 2011' form on the elabexperts.com website. The 'Special notes' field is highlighted in yellow. A red arrow points from the 'Special notes' field to a callout box. The callout box contains the text: 'The Drop Down List for: 1: Type of Analysis 2: Sample State 3: Gel Staining are shown in screen shots below'.

The screenshots below shows the “drop down list” where you have to choose:

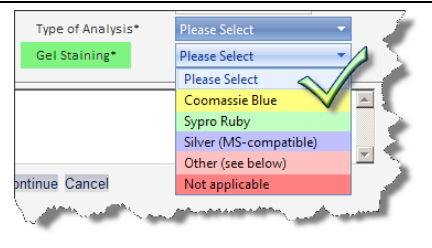
Type of Analysis (mass spec)



Sample State



Gel Staining



Type of Analysis

MALDI is best suited for protein digests of a single or just a few proteins. Gel-spots from 2D SDS-PAGE are therefore preferred. The sensitivity is very good, and peptides in low femtomoles can be detected and searched for protein identification. We use peptide mass fingerprinting (PMF) when identifying proteins with MALDI, but can also do fragmentation of peptides using post source decay (PSD).

ESI-MSMS is the preferred technique for complex protein samples, i.e. digest of cell lysates or bodyfluids, directly or after protein/peptide fractionation (SDS-PAGE, SCX, etc). For a successful mass spec analysis, we need to inject 1µg of proteins (approx. 20 pmoles). Regarding protein samples given as gel-spots (2D SDS-PAGE) or gel-lanes (1D SDS-PAGE), where the amount of protein may be difficult to suggest, a strong blue spot/lane is usually sufficient.

The **Orbitrap** is the most sensitive ESI-MSMS instrument, and may detect and fragment minute amounts of peptides. This instrument is preferred if there is a large dynamic range in protein concentration, and your interest is to detect peptides from the less abundant proteins. When the complexity is less, and sensitivity is not that much of an issue, an **IonTrap** or a **QTrap** will usually be sufficient.

Sample State

Here you need to select how you have treated your proteins, either in gel or in solution, precipitated or pre-digested.

Many detergents are not compatible with mass spec. For preparation of in-solution samples, avoid using Triton-X100, SDS, Tween 20, etc. However, if you need to use one of these detergents to solubilise your protein of interest, you need to remove it before the mass spec analysis. To achieve this, try to run your sample about 1 cm into 1D SDS-PAGE. The protein band may then be washed to remove detergents, etc. Other methods you can use are listed below, but we have no experience with their success in removing detergents.

- Chloroform/MeOH-extraction (*example: Vertommen, et al. 2010. Planta 231:1113-1125, <http://ivaan.com/protocols/145.html>*)
- Protein precipitation protocols (*example: <http://wolfson.huji.ac.il/purification/Protocols/ProteinPrecipitation.html>*)
- Molecular weight cut-off filter (*example: Amicon ultrafiltration filters*)
- Reversed phase chromatography (*example: Macroporous RP-C18 columns for proteins (Agilent) and RP-C18 micro columns for peptides*)
- Liquid-liquid extraction (*ethyl acetate, Yeung, et al. 2008. Anal. Biochem. 382: 135-137*)

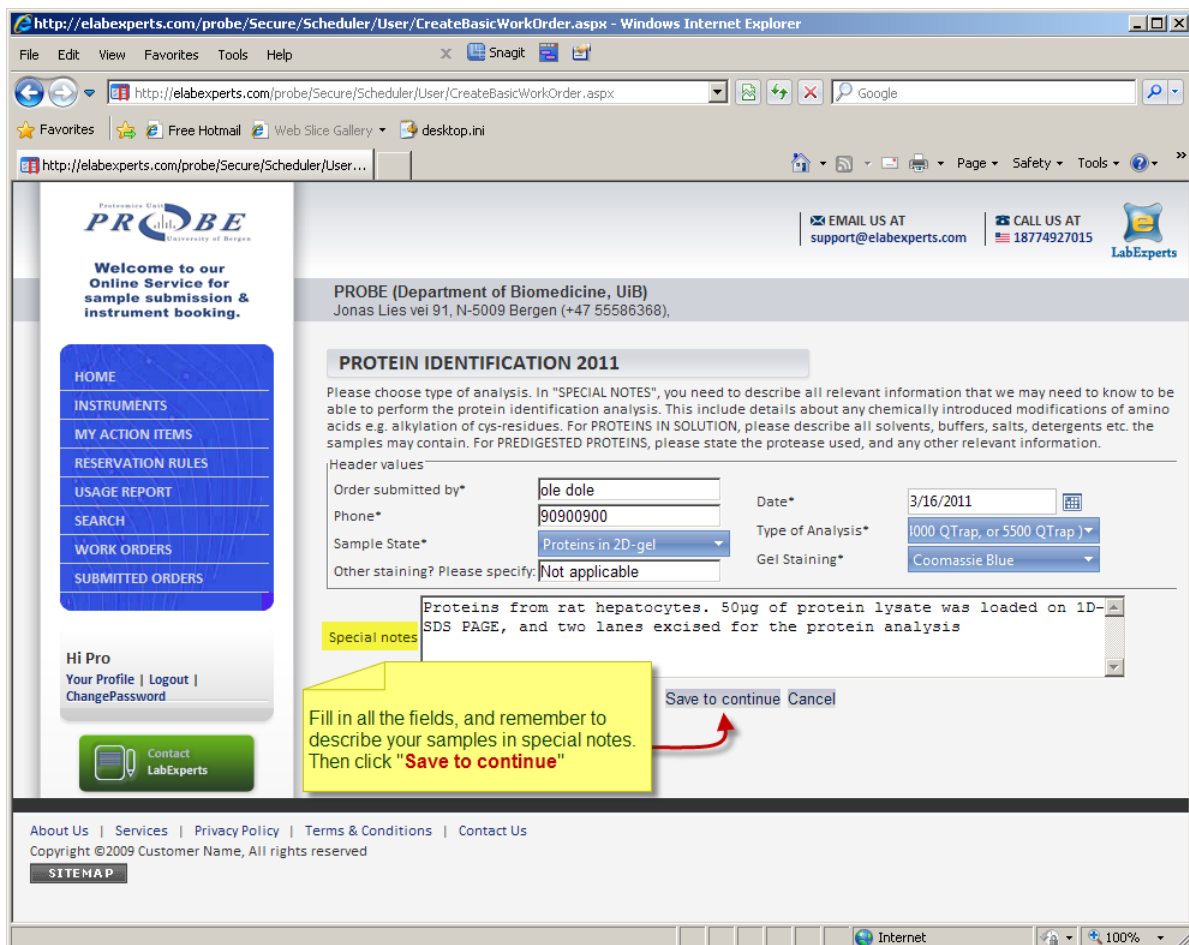
Detergents cause serious problems with chromatographic resolution and ion suppression in LC-MS. If there is a need to solubilise very hydrophobic peptides, there is some mass spec friendly detergents that may be used in low concentrations, 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) and N-octyl-β-glucopyranoside (OGP). CHAPS with a critical micelle concentration (CMC) of 0.4% may be used in electrospray instruments at a maximum of 1%, and OGP (CMC = 0.5%) at a maximum concentration of 0.01% (Funk, et al. 2005. RCM 19: 2986-2988). However, we have no knowledge of any

chromatographic separation/resolution problems when injecting samples containing these detergents.

Gel staining

Select from the drop down list the stain you used to visualize your proteins, preferentially Coomassie Blue dyes (R-250 or G-250). We can be fairly certain that we will identify proteins if they give a nice strong blue band/spot in the gel. You can also use Sypro Ruby, or other type of sypro stains, since they are very mass spec friendly. If you need to use Silver staining, make sure you use the MS-compatible staining recipe without glutaraldehyde fixation. Because silver impedes trypsin activity, we need to remove all traces of silver before trypsin digestion. This is a slow process and protein losses may occur. We therefore don't recommend using this stain. If you have used a stain not on the list, choose "**other**", and specify in the text field below.

If your proteins are in-solution, select "**not applicable**".



http://elabexperts.com/probe/Secure/Scheduler/User/CreateBasicWorkOrder.aspx - Windows Internet Explorer


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EMAIL US AT support@elabexperts.com CALL US AT 18774927015

PR  **BE**
 University of Bergen

Welcome to our Online Service for sample submission & instrument booking.

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 Your Profile | Logout | ChangePassword

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PROBE (Department of Biomedicine, UiB)
 Jonas Lies vei 91, N-5009 Bergen (+47 55586368).

PROTEIN IDENTIFICATION 2011

Please choose type of analysis. In "SPECIAL NOTES", you need to describe all relevant information that we may need to know to be able to perform the protein identification analysis. This include details about any chemically introduced modifications of amino acids e.g. alkylation of cys-residues. For PROTEINS IN SOLUTION, please describe all solvents, buffers, salts, detergents etc. the samples may contain. For PREDIGESTED PROTEINS, please state the protease used, and any other relevant information.

Header values

Order submitted by*	<input type="text" value="ole dole"/>	Date*	<input type="text" value="3/16/2011"/>
Phone*	<input type="text" value="90900900"/>	Type of Analysis*	<input type="text" value="1000 QTrap, or 5500 QTrap"/>
Sample State*	<input type="text" value="Proteins in 2D-gel"/>	Gel Staining*	<input type="text" value="Coomassie Blue"/>
Other staining? Please specify:	<input type="text" value="Not applicable"/>		

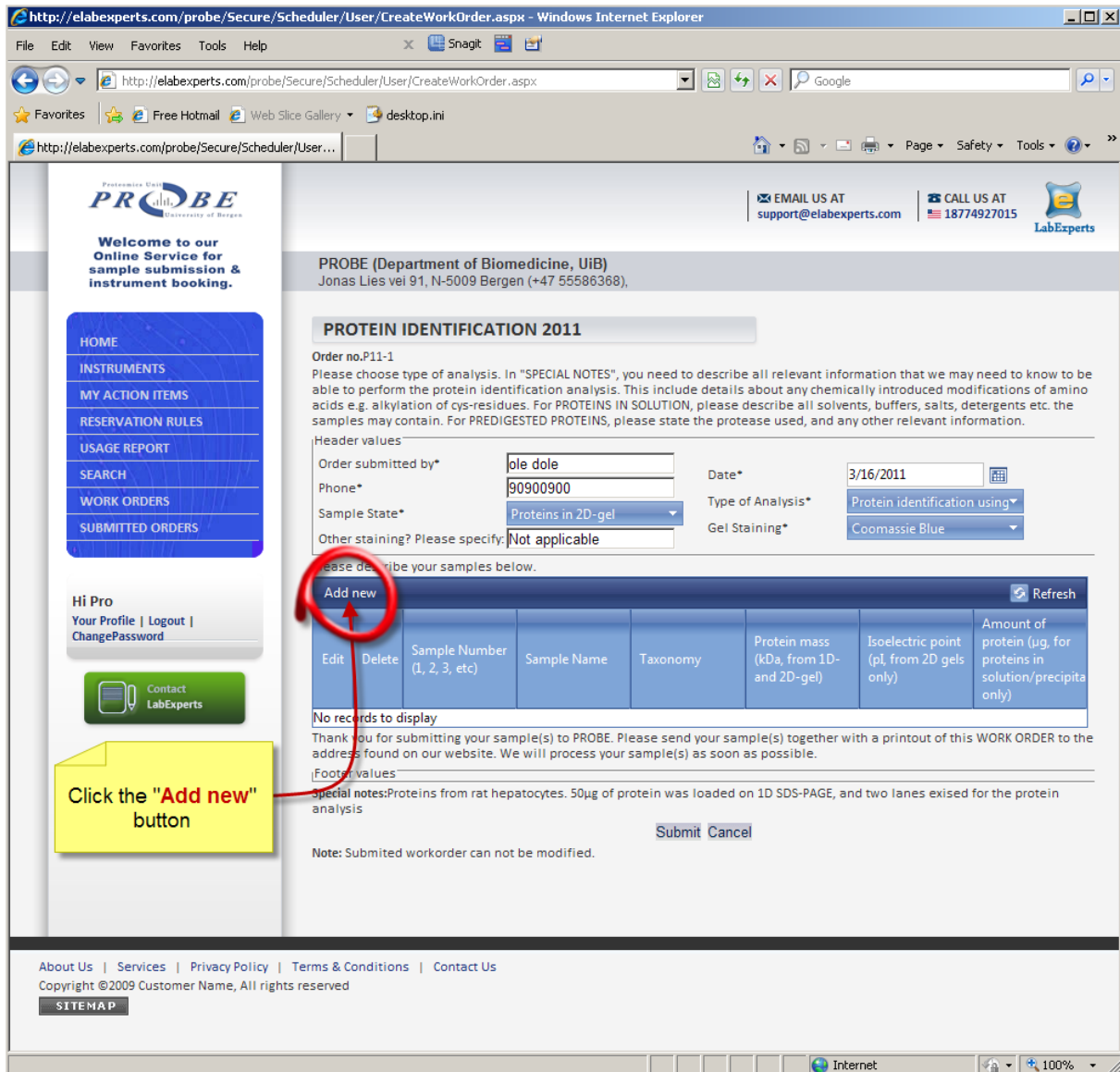
Special notes
 Proteins from rat hepatocytes. 50µg of protein lysate was loaded on 1D-SDS PAGE, and two lanes excised for the protein analysis

Fill in all the fields, and remember to describe your samples in special notes. Then click "**Save to continue**"

Save to continue Cancel

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 SITEMAP

OK, not far to go now. Next, give finally some information concerning each sample. When you click **“Save to continue”**, the following screen appears:



The screenshot shows a web browser window with the URL <http://elabexperts.com/probe/Secure/Scheduler/User/CreateWorkOrder.aspx>. The page header includes the PROBE logo and contact information: "PROBE (Department of Biomedicine, UIB) Jonas Lies vei 91, N-5009 Bergen (+47 55586368)".

The main content area is titled "PROTEIN IDENTIFICATION 2011" and includes the following form fields:

- Order no.: P11-1
- Header values:
 - Order submitted by*:
 - Phone*:
 - Sample State*:
 - Other staining? Please specify:
 - Date*:
 - Type of Analysis*:
 - Gel Staining*:

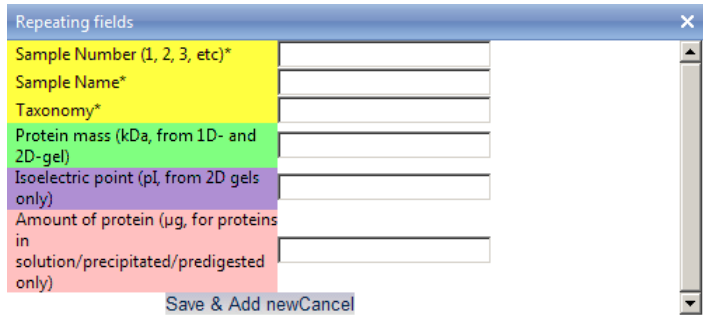
Below the form is a table with the following columns: Edit, Delete, Sample Number (1, 2, 3, etc), Sample Name, Taxonomy, Protein mass (kDa, from 1D- and 2D-gel), Isoelectric point (pI, from 2D gels only), and Amount of protein (µg, for proteins in solution/precipita only). The table currently displays "No records to display".

A red circle highlights the "Add new" button in the top left corner of the table area. A yellow callout box with the text "Click the 'Add new' button" points to this button.

At the bottom of the page, there is a footer with links for "About Us", "Services", "Privacy Policy", "Terms & Conditions", and "Contact Us", along with a "SITEMAP" button.

Click the **“Add new”** button to activate the sample information menu. The screenshots below show in detail how to add two samples to the work order.

1:



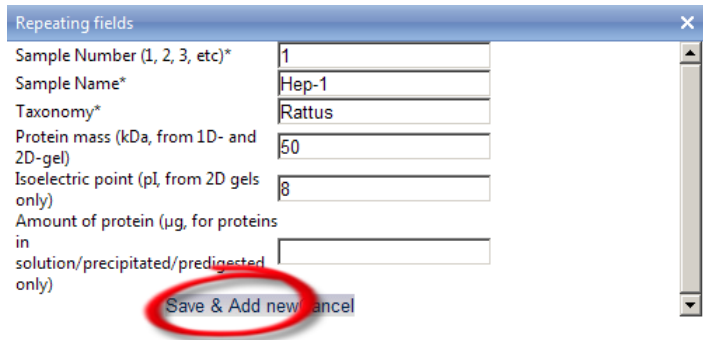
Repeating fields

Sample Number (1, 2, 3, etc)*	
Sample Name*	
Taxonomy*	
Protein mass (kDa, from 1D- and 2D-gel)	
Isoelectric point (pI, from 2D gels only)	
Amount of protein (µg, for proteins in solution/precipitated/predigested only)	

Save & Add new Cancel

Yellow highlighted fields are mandatory.

2:



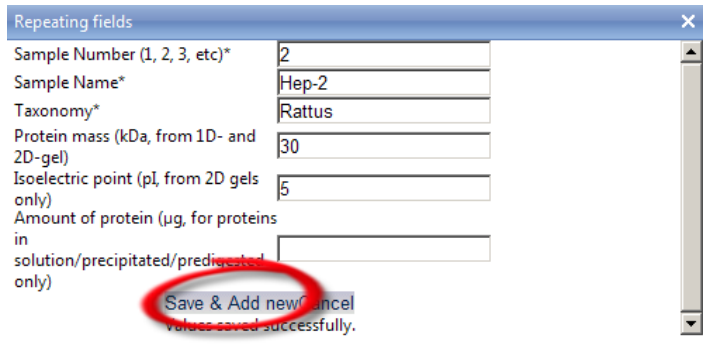
Repeating fields

Sample Number (1, 2, 3, etc)*	1
Sample Name*	Hep-1
Taxonomy*	Rattus
Protein mass (kDa, from 1D- and 2D-gel)	50
Isoelectric point (pI, from 2D gels only)	8
Amount of protein (µg, for proteins in solution/precipitated/predigested only)	

Save & Add new Cancel

Fill in necessary information and click **“Save and Add new”**

3:



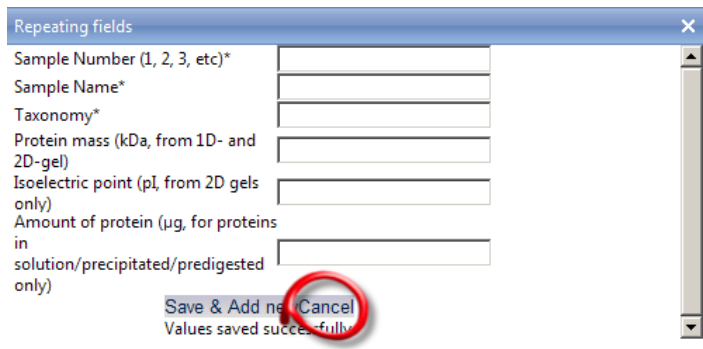
Repeating fields

Sample Number (1, 2, 3, etc)*	2
Sample Name*	Hep-2
Taxonomy*	Rattus
Protein mass (kDa, from 1D- and 2D-gel)	30
Isoelectric point (pI, from 2D gels only)	5
Amount of protein (µg, for proteins in solution/precipitated/predigested only)	

Save & Add new Cancel
 Values saved successfully.

Repeat for the other samples..

4:



Repeating fields

Sample Number (1, 2, 3, etc)*	
Sample Name*	
Taxonomy*	
Protein mass (kDa, from 1D- and 2D-gel)	
Isoelectric point (pI, from 2D gels only)	
Amount of protein (µg, for proteins in solution/precipitated/predigested only)	

Save & Add new Cancel
 Values saved successfully.

When added the last sample, click **“Cancel”**

You can **edit** or **delete** single samples, or **add** more before submitting the work order. After that you will not be able to change the work order.

http://elabexperts.com/probe/Secure/Scheduler/User/CreateWorkOrder.aspx - Windows Internet Explorer

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http://elabexperts.com/probe/Secure/Scheduler/User/CreateWorkOrder.aspx

Proteomics Unit
PROBE
 University of Bergen

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PROBE (Department of Biomedicine, UiB)
 Jonas Lies vei 91, N-5009 Bergen (+47 55586368)

PROTEIN IDENTIFICATION 2011

Order no.P11-1
 Please choose type of analysis. In "SPECIAL NOTES", you need to describe all relevant information that we may need to know to be able to perform the protein identification analysis. This include details about any chemically introduced modifications of amino acids e.g. alkylation of cys-residues. For PROTEINS IN SOLUTION, please describe all solvents, buffers, salts, detergents etc. the samples may contain. For PREDIGESTED PROTEINS, please state the protease used, and any other relevant information.

Header values:

Order submitted by* Date*
 Phone* Type of Analysis*
 Sample State* Gel Staining*
 Other staining? Please specify:

Please describe your samples below.

Add new

Edit	Delete	Sample Number (1, 2, 3, etc)	Sample Name	Taxonomy	Protein mass (kDa, from 1D- and 2D-gel)	Isoelectric point (pI, from 2D gels only)	Amount of protein (µg, for proteins in solution/precipitate only)
		1	Hep-1	Rattus	50	8	
		2	Hep-2	Rattus	30	5	

Thank you for submitting your sample(s) to PROBE. Please send your sample(s) together with a printout of the WORK ORDER to the following address. We will process your sample(s) as soon as possible.

Special notes:Proteins from rat hepatocytes. 50µg of protein was loaded on SDS-PAGE for protein analysis

Note: Submitted workorder can not be modified.

Submit Cancel

Click "Submit" and we will receive your work order

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 SITEMAP

Thank you for submitting your sample(s) to PROBE. Please send your sample(s) together with a printout of the WORK ORDER to the following address. We will process your sample(s) as soon as possible.

PROBE
 Dept of Biomedicine
 University of Bergen
 Jonas Lies Vei 91

PROBE
 Inst. for Biomedisin
 Universitetet i Bergen
 Jonas Lies Vei 91

N-5009 Bergen
 Norway

5009 Bergen

Creating a Usage Report

The booking system allows the user to see how many expenses that have accumulated in a certain time period.

INSTRUMENT USAGE REPORT

Select instruments: [Dropdown menu]

Report Information
 Report name: Report Feb-March 2011
 Date from: 2/1/2011
 Date to: 3/31/2011

Actual usage tracked: **Display usage**

Instrument	Group	User	Hourly fee (kr)	hour fee (kr)	Weekend fee(kr)			
In-gel protein digestion	PROBE	Pro Be	0	0	0	0	2000.00	03/25/2011 03:30 - 03/25/2011 03:30
In-solution protein digestion	PROBE	Pro Be	0	0	0	0	400.00	03/01/2011 00:00 - 03/01/2011 00:00
Peptide SCX-separation	PROBE	Pro Be	0	0	0	0	1500.00	03/04/2011 00:00 - 03/04/2011 00:00
Desalting SCX-fractions	PROBE	Pro Be	0	0	0	0		

Export to: [Word] [Excel] [PDF] [Save] [Clear]

Check the instrument (if any) of which you want to see a report

Add a "Report name", and choose the dates where you want to see instrument usage or other expenses, and click "Display usage"

The list of bookings and/or expenses that appear can be exported to Word, Excel, or PDF. Just click on one of the icons.