

## PROTEIN SEARCH TUTORIAL

Users have three options to find a protein of interest: (a) search by protein identifier, (b) search by sequence (here ubiquitin), or (c) search by protein description. In addition, the search can be restricted to a single plant species by using the dropdown menu selection option below.

The image shows a screenshot of a web application's 'Protein Search' page. At the top is a navigation bar with links: HOME, PROTEIN SEARCH (highlighted with a dashed box), PTM SEARCH, PTM BLAST, EXPERIMENTS, PTM INFO, BROWSE/DOWNLOAD, SUBMIT, and RESOURCES. Below the navigation bar is a large white box containing the text 'Protein Search' and a 'Help pop-up' button. Underneath, there are three search options: 'SEARCH BY IDENTIFIER' (with a callout box 'a) Search Protein ID e.g. 'AT4G05320.1' or 'Q3EAA5' (UniProtKB)'), 'SEARCH BY SEQUENCE' (highlighted in blue, with a callout box 'b) Search protein sequence e.g. ubiquitin sequence'), and 'SEARCH BY DESCRIPTION' (with a callout box 'c) Search description e.g. 'polyubiquitin)'). The 'SEARCH BY SEQUENCE' option is selected, and its form is shown below. The form has two sections: 'Sequence' with the text 'MQIFVKLTGKITLEVSSDTIDNVKAKIQDKEGIPPDQQRLLF/' and a note 'String of amino acids (min 5 characters) Exact matches only returned'; and 'Species' with the text 'Arabidopsis thaliana' and a callout box 'Optional: restrict search to one species'. At the bottom right of the form is a 'SEARCH' button with a callout box 'Start query'.

HOME PROTEIN SEARCH PTM SEARCH PTM BLAST EXPERIMENTS PTM INFO BROWSE/DOWNLOAD SUBMIT RESOURCES

Protein Search [Help pop-up](#)

**a) Search Protein ID**  
e.g. 'AT4G05320.1' or 'Q3EAA5' (UniProtKB)

SEARCH BY IDENTIFIER

**SEARCH BY SEQUENCE**

SEARCH BY DESCRIPTION

**c) Search description**  
e.g. 'polyubiquitin'

**b) Search protein sequence**  
e.g. ubiquitin sequence

Sequence  
MQIFVKLTGKITLEVSSDTIDNVKAKIQDKEGIPPDQQRLLF/  
String of amino acids (min 5 characters)  
Exact matches only returned

Species  
Arabidopsis thaliana

**Optional: restrict search to one species**

SEARCH **Start query**

After pressing the 'search' button, any results will appear below the query box. All proteins fulfilling the criteria will be listed in the search results table. Note that the result table also includes protein splice forms! All columns can be sorted, including a description column, species abbreviation, cross-references protein identifiers of PLAZA or UniProtKB (requiring identical protein sequence) or the amount of PTM sites and types.

↕ : sort columns

## Search Results

↕ ID	↕ Description	↕ Species	↕ #PTMs	↕ #PTM Types	↕ PLAZA Gene ID	↕ Uniprot ID(s)
AT4G02890.3	Ubiquitin family protein	ath	268	5	AT4G02890	A0A178V886 J7FN14 Q3E7T8 Q8H159-2
AT4G02890.4	Ubiquitin family protein	ath 	268	5	AT4G02890	A0A178V886 J7FN14 Q3E7T8 Q8H159-2

Click for protein overview

<b>Species protein ID</b> (here Araport11) <b>NOTE: splice forms of protein are included!</b>	<b>Protein description</b>	<b>Species, here:</b> <i>Arabidopsis thaliana</i>	<b># PTM sites and types in protein</b>	<b>External IDs, cross-referenced if 100% protein identity</b>
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By clicking a protein identifier the PTM protein sequence overview is launched, as example we show here the protein encoded by polyubiquitin 10 (AT4G05320.1). Below a general protein info header with description and cross-references, a PTM table (left, green border), PTM protein sequence overview (top-right, red border) and protein domain/site table is provided (bottom-right, blue border). These are interactively connected to each other. For instance, by default all PTM checkboxes are selected in the PTM table. Removing a specific checkbox will remove the highlighting in the protein sequence overview. Note that a color legend can be displayed and also by hovering over a modified amino acid, the modification(s) will appear in a pop-up box. Similarly, a protein domain can be selected, e.g. here all ubiquitin domains were selected, and the domain will be underlined in the PTM protein sequence overview. In the PTM table additional information is found such as the type of PTM with corresponding protein position, the originating (plain) peptide identified by MS, the respective publication and a confidence color-coding. By clicking the MS study, the experiment overview is launched. If localization probabilities or differential abundance estimates (log2 fold change and significance) are available, these are displayed as well. Log2 fold changes are displayed in a heatmap-like gradient (green is upregulated, red is downregulated). In case the significance estimate was below the threshold employed in the respective study, this is also highlighted in green (note this was not the case here). The PTM table can be exported by clicking the 'Export results' button.

The screenshot displays a web interface for PTM analysis. It is divided into three main sections: a PTM table (left, green border), a protein sequence overview (top-right, red border), and a domains & sites table (bottom-right, blue border). Annotations highlight various interactive features and data points.

**PTM Table (Left, Green Border):** A table listing PTMs with columns for PTM Type, Mod AA, Pos, Peptide, Exp ID, Conf, Log2 FC, P/Q val, and Loc Prob. The table is annotated with a 'Sort' button and a 'Show confidence meta-data' button. A 'Checked: display PTM on overview' annotation points to a red circle icon. A 'Checked: Experiment ID # Click: experiment details Hover: title experiment' annotation points to the 'Exp ID' column. A 'Checked: Confidence estimate: High Medium Low Details: see further' annotation points to the 'Conf' column. A 'Peptide matches proteins encoded multiple gene loci On hover: display genes' annotation points to the 'Peptide' column.

**Protein sequence overview (Top-right, Red Border):** Shows the protein sequence with highlighted PTMs. A 'Highlighted PTMs' annotation points to the highlighted residues. A 'Checked: remove highlighting' annotation points to a button. A 'Checked: underline protein domain' annotation points to a button. A 'SHOW PTM COLOR LEGEND' button is also visible.

**Domains & Sites (Bottom-right, Blue Border):** A table showing Interpro Domains with columns for Show, IPR ID, Description, From, and To. A 'Checked: underline protein domain' annotation points to a button. A 'Protein domain or UniProtKB annotated sites' annotation points to the table header.

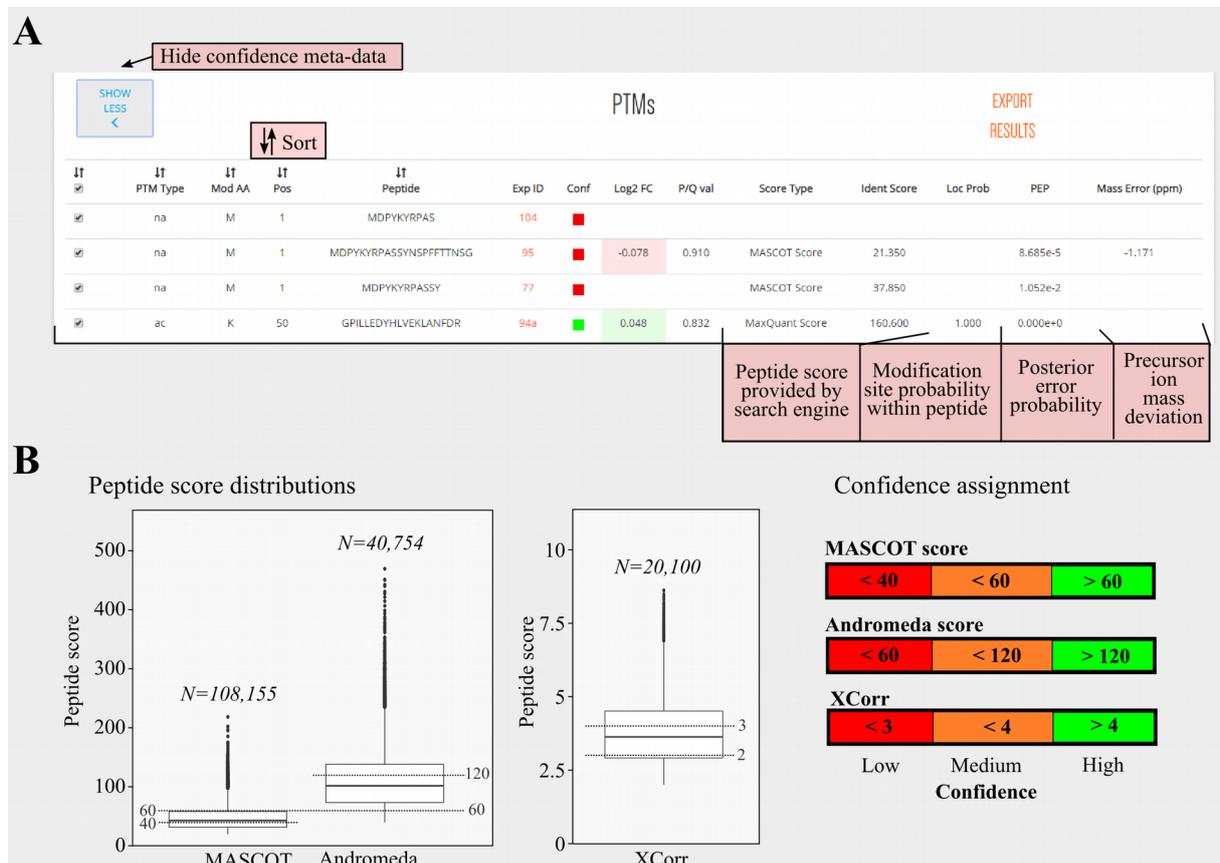
**Protein overview: PTM color legend (Bottom-right, Red Border):** A table showing PTM types and their corresponding colors. A 'Checked: underline protein domain' annotation points to a button.

ID	PTM Type	Color
mo	Methionine Oxidation	Green
ac	Acetylation	Yellow
ub	Ubiquitination	Red
nt	N-terminal	Blue
ph	Phosphorylation	Orange
	Multiple types	Blue

Show	IPR ID	Description	From	To
<input checked="" type="checkbox"/>	IPR000626	Ubiquitin domain	1	76
<input checked="" type="checkbox"/>			77	152
<input checked="" type="checkbox"/>			153	228
<input checked="" type="checkbox"/>			229	304
<input checked="" type="checkbox"/>			305	380

PTM Type	Conf	Log2 FC	P/Q val	Loc Prob
mo	5a	0.032		
	5b	0.060		
ac	97			
ac	97			
ub	100			
ub	99			
	100			
ub	99			
	100			
ub	99			
	100			
ac	94a	-0.206	0.240	1.000
	97			
ub	3			1.000

Details of the confidence meta-data collected can be consulted by clicking 'SHOW CONFIDENCE'. Below, we can view the extended version (figure panel A) including these confidence estimates reported by experiments, including peptide scores, posterior error probability (PEP), modification site localization probability and/or precursor mass deviation. Peptide scores are measured by search engines and score how a tandem mass spectrum matches a peptide from the searched protein database. Most frequently reported scores (used search algorithms) are the MASCOT ion score (MASCOT, Perkins et al., 1999), the Andromeda score (built-in MaxQuant software suite, Cox et al. 2011) and the cross-correlation score (XCorr, originally for SEQUEST, Eng et al., 1994). Distributions of these scores can be consulted in the figure panel B below. For these three search engines minimal peptide score thresholds were used. MASCOT ion scores were required to be at least 20, Andromeda scores 40 and XCorr scores at least 2. Next to peptide scores, which are highly differing and dependent on the search algorithm used, the PEP provides a more unified confidence estimate and can be considered as a "local FDR" that expresses the chance that a given peptide-to-spectrum match was incorrect. Most PEP values reported here were measured by software such as MaxQuant (Cox and Mann 2008), Proteome Discoverer (Thermo Scientific) or post-processing algorithms such as Percolator (Käll et al., 2007). Lastly, beside peptide-level confidence measurements, modification localization probability within a peptide can be assessed by algorithms such as PhosphoRS (Taus et al., 2011) or the PTM Score implemented in MaxQuant (Olsen et al., 2006). Here, we required a modification site localization probability of at least 0.75, when reported. Based on the peptide scores provided, PTMs are categorized as being low, medium or high confident (figure panel B - right). Assessing reliability of PTMs is a crucial step as false positive identification may occur in mass spectrometry identification results. In this aspect, careful inspection of experimental details remains therefor advisable.



## REFERENCES

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