

PTM BLAST TUTORIAL

PTM BLAST allows users to retrieve and compare PTMs in aligned protein sequences. There are two options to initiate PTM BLAST, either from (a) the PTM BLAST section or (b) any PTM Viewer plant protein overview page. In case of the PTM BLAST section (a), users can specify a protein sequence and indicate PTMs by their two-letter code abbreviation between brackets after the modified amino acid. For instance, phosphothreonine is indicated as T(Ph). Any PTM modification can be indicated as T(xx), or multiple can be indicated as T(Ph|Og). PTM BLAST can also be launched from any PTM Viewer protein overview (b). Here, the search is restricted to the protein sequence and its PTMs. However, a region from the protein sequence can be searched by indicating the start and end protein positions. This can be useful to PTM BLAST a protein region of interest, for instance a protein domain. After clicking 'BLAST SELECTED', a default protein BLAST (BLASTP) will be performed of the plain protein sequence. A progress message will appear and when the BLASTP job is finished the results can be viewed in a new tab by clicking 'DISPLAY BLAST RESULTS'.

A

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

SHOW PTM LIST ← Show PTM abbreviations

Lys modified by any PTM type ('xx')

BLAST Sequence ← Modified peptide sequence in FASTA format

```
>tr|B2CHJ6|B2CHJ6_WHEAT|Ribulose biphosphate carboxylase large chain OS=Triticum aestivum GN=rbcL PE=2 SV=1
MSPQTETKAGVGFKAGVKDYK(xx)LTYYPPEYET(ph|ub)KDTDILAAFRVSPQGPVPEEAGAAVAEE
SSTGTWTTVMTDGLTSLDRYKGRCYHIEPVAGEDSQWICYVAYPLDLFEEGSVTNMFTST
VGNVFGFK(ac)ALRALRLEDLRIPPTYSK(xx)TFQGPPHGIQVERDKLNKYGRPLLGGCTIKPKLGL
SAKINYGRACYECLRGGLDFTKDDENVNSQPFMRWRDRFVFCAEAIYKSQAETGEIKGHYL
NATAGTCEEMTK(su)RAVFARELGVPIVMHDYLTGGFTANTTLAHCYCRDNGLLHITHRAMHAY
IDRQKNHGMHFRVLAALRMSGGDHIHSGTVVGKLEGEREMTLGFVDLLRDDFIEKDRAR
GIFFTQDWVSMFVIVPVASGGIHWHPALTEIFGDDSVLQFGGGTLGHPWGNAPGAAAN
RVALFACVQARNIGRDLAREGNETTRAACKNSPELAAACEVWKATKFEFEPVDTTDK
```

Succinylated (su) Lys

BLAST SELECTED ← Run PTM BLAST

B Protein overview B2CHJ6 (bottom page)

BLAST

Perform a BLAST search for this sequence, or a part of this sequence (minimum 50 characters)

Select range to BLAST:

From: 1 To: 477 ← PTM BLAST whole sequence or region of it (e.g. a functional domain)

BLAST Sequence

```
MSPQTETKAGVGFKAGVKDYKLTYYTPEYETKDTDILAAFRVSPQGPVPEEAGAAVAEESSSTGTWTTVMTDGLTSLDRYKGRCYHIEPVAGEDSQWICYVAYPLDLFEEG
SVTNMFTSIVGNVFGFKALRALRLEDLRIPPTYSKTFQGPPHGIQVERDKLNKYGRPLLGGCTIKPKLGLSAKINYGRACYECLRGGLDFTKDDENVNSQPFMRWRDRFVFC
```

No manual insertion possible here, for that go to the 'PTM BLAST' (section A)

BLAST SELECTED ← 1. Run PTM BLAST

BLAST IN PROGRESS...

DISPLAY BLAST RESULTS ← 3. Display results in new tab

2. PTM BLAST is running - please wait

The PTM BLAST results are organized in two interactive boxes. In the top PTM BLAST result table, significant BLASTP hits are displayed and sorted according to number of aligned PTMs. A distinction is made between aligned PTMs, which are aligned amino acid residues modified by the same or different PTM types, and PTM matches, where amino acids are aligned and modified by the same PTM type. On the left-hand side of the table, PTM BLAST hits can be selected. By default the top three PTM BLAST results, *i.e.* those with the most PTM alignments, are displayed. Alignment of proteins and their PTMs are found in the bottom sequence alignment box. Here, for each PTM BLAST hit the BLASTP protein alignment is given and protein sequences are decorated with their PTMs. This display is similar to the protein overview, where PTMs are highlighted in color according their PTM type(s). A color legend can be found below of the page. In case PTM type(s) matched at a certain aligned amino acid residue, the middle row will also be highlighted to indicate a PTM match. Note that above in the sequence alignment box there is an option to solely display peptides matching protein products from a single gene. In case of a PTM BLAST alignment between plant proteins from the same species, this can filter out PTMs derived from peptides that match both proteins ambiguously. Both the PTM BLAST results and sequence alignment box are scrollable, thus to view more selected alignments make sure to scroll down in the sequence alignment box.

Blast Results

Most significant PTM BLAST results are shown here. The number of aligned PTMs is shown per PTM BLAST hit

Make a selection of the checkboxes on the left to show the alignment below. Note that ambiguous peptides matching multiple proteins can be omitted

PTM BLAST result table

Show representative proteins only
Where results contain multiple splice forms of the same gene, show the representative protein only

Aligned PTMs (any type)

Aligned PTMs (same type)

| ↑ Show | ↓ Hit # | ↑ Protein ID | ↓ Description | ↑ Species | ↓ Total PTM sites | ↓ PTM Alignments | ↓ PTM Matches | ↓ e-Value |
|-------------------------------------|---------|--|-----------------------------------|-----------|-------------------|------------------|---------------|--------------|
| <input checked="" type="checkbox"/> | 5 | ATCG00490.1 A0A1B1W4U8 | ribulose-bisphosphate carboxylase | ath | 129 | 19 | 14 | 0 |
| <input checked="" type="checkbox"/> | 2 | LOC_Os10g21268.1 | protein_coding | osa | 18 | 14 | 8 | 0 |
| <input checked="" type="checkbox"/> | 7 | TRIAE_CS42_1AL_TGACv1_007391 _AA0055320.1 A0A1D550G6 | protein_coding | tae | 8 | 8 | 8 | 2.03054E-164 |
| <input type="checkbox"/> | 13 | TRIAE_CS42_1DL_TGACv1_062236 _AA0211160.1 A0A1D6RFV3 | protein_coding | tae | 7 | 7 | 7 | 9.16188E-131 |

If selected: alignment is shown below
3 best PTM alignments are shown by default

BLASTP E-val

Sequence Alignments

Highlight unique peptides only
Highlight only where the peptides containing PTMs are only found in one gene

Only show peptides unique matching proteins encoded from a single gene

ATCG00490.1 (A0A1B1W4U8) : e-Value 0

| Query protein | Aligned protein | Protein position |
|----------------|---|------------------|
| B2CH36 1 | MSPQTEKAGVGFKAGVKDYKLTYYTPEYETKDDILAAFRVSPQGPVPEEAGAAVAAE | 60 |
| -- | MSPQTEKAVGFKAGVKYKLTYYTPEYETKDDILAAFRV+PQGPVPEEAGAAVAAE | -- |
| A0A1B1W4U8 1 | MSPQTEKAGVGFKAGVKYKLTYYTPEYETKDDILAAFRVTPQGPVPEEAGAAVAAE | 60 |
| B2CH36 61 | SSTGTWTTVWDGLTSLDRYKGRCYHIEPVAGEDSQWICYVAYPLDLFEEGSVTNMFTSI | 120 |
| -- | SSTGTWTTVWDGLTSLDRYKGRCYHIEPVGE++Q+IYVAYPLDLFEEGSVTNMFTSI | -- |
| A0A1B1W4U8 61 | SSTGTWTTVWDGLTSLDRYKGRCYHIEPVGEEIQFIAYAYPLDLFEEGSVTNMFTSI | 120 |
| B2CH36 121 | VGNVFGFALRALRLEDLRIPPTYSKTFQGPPIQVERDKLNYGRPLLGCITIPPLGL | 180 |
| -- | VGNVFGFKALRALRLEDLRIPPYHIFQGPPIQVERDKLNYGRPLLGCITIPPLGL | -- |
| A0A1B1W4U8 121 | VGNVFGFKALRALRLEDLRIPPYHIFQGPPIQVERDKLNYGRPLLGCITIPPLGL | 180 |

BLASTP alignment

PTMs are highlighting according type(s)
PTM color legend is available below, or hover over

Aligned acetylated lysines

Scroll down to display other selected alignments