BioCreative IV-User Interactive Task

RLIMS-P Annotation Task

This document contains information about the annotation workflow for the Full BioCreative interactive task.

Annotation Workflow using RLIMS-P

1. **Go to** URL http://annotation.dbi.udel.edu/text_mining/rlimsp2/ (you can use one of the following browsers Chrome, Firefox or Safari)
2. **Login**: this is important in order to save your results. If successful your user name should appear in the menu on the upper right corner.
3. **Track time** it takes to go through the activity of annotating the abstracts using RLIMS-P. Do the same for the manually annotated set.
4. **Enter PMIDs** in PMID box (to be provided by organizers) or search based on keywords of interest.
5. **Validate Annotation for 30 PMID results in table.** The goal is to validate for each PMID i) substrate, kinase and sites at the abstract level for those with experimental information. Abstract level means that if the document mentions in multiple sentences that protein X is phosphorylated by kinase Y, you only select one of the instances for annotation; ii) UniProtKB accessions for the individual protein annotated (if possible).
   If the document provides a statement of a kinase-substrate-site in previous work you should not validate that information. Only for the things that the paper is about.
6. **Save output**:
   For your records save your annotation using the save button in evidence text/curation page. Alternatively, go to My Curation tab and save the complete result at the end of the activity.
7. **Record end time**

Annotation Workflow manual mode

1. **Track time** it takes to go through the activity of annotating all the abstracts
2. **Enter PMID list in PubMed**
3. **Add Annotation to template spreadsheet**:
   For each PMID provide **i)** tuples of substrate, kinase and sites at the abstract level. Abstract level means that if the abstract mentions in multiple sentences that protein X is phosphorylated by kinase Y, you only select one of the instances for annotation; **ii)** UniProtKB accessions for the individual proteins annotated as substrate and kinase (if possible).
4. **Save output**:
   Record all output in spreadsheet. Save all the information and submit back to Task Organizers when you are finished

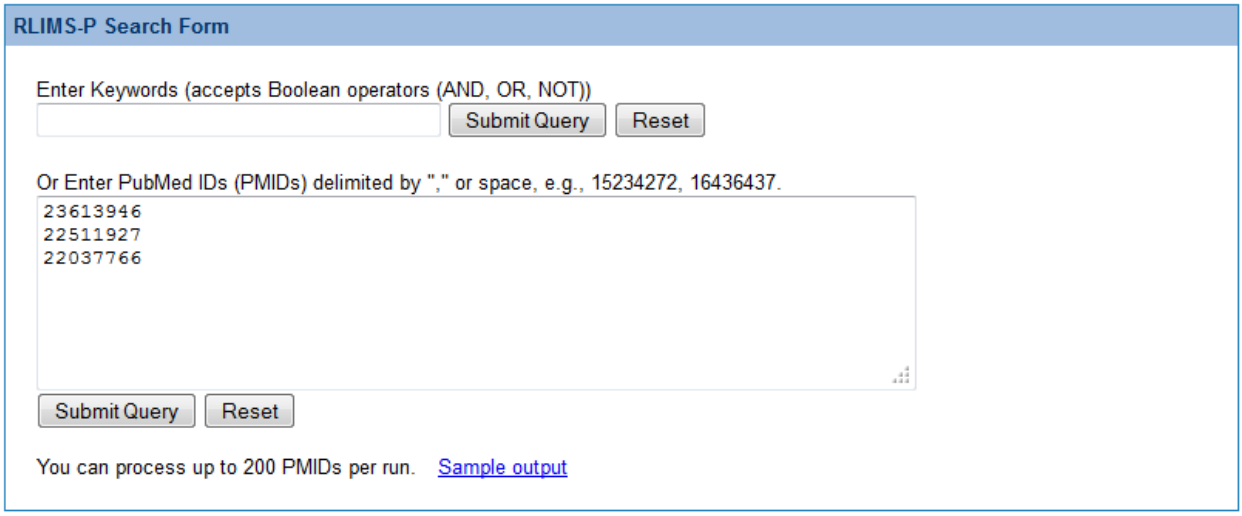
5. **Record end time**

At the end of the activity **Complete user survey (link to be added)**

You can use the examples below to practice and get familiar with the task and the system.

Example of RLIMS-P annotation Task

- Go to RLIMS-P page http://annotation.dbi.udel.edu/text_mining/rlimsp2/
- Log in
- Record starting time.
- Enter PMIDs in the corresponding box: 23613946, 22511927, 22037766



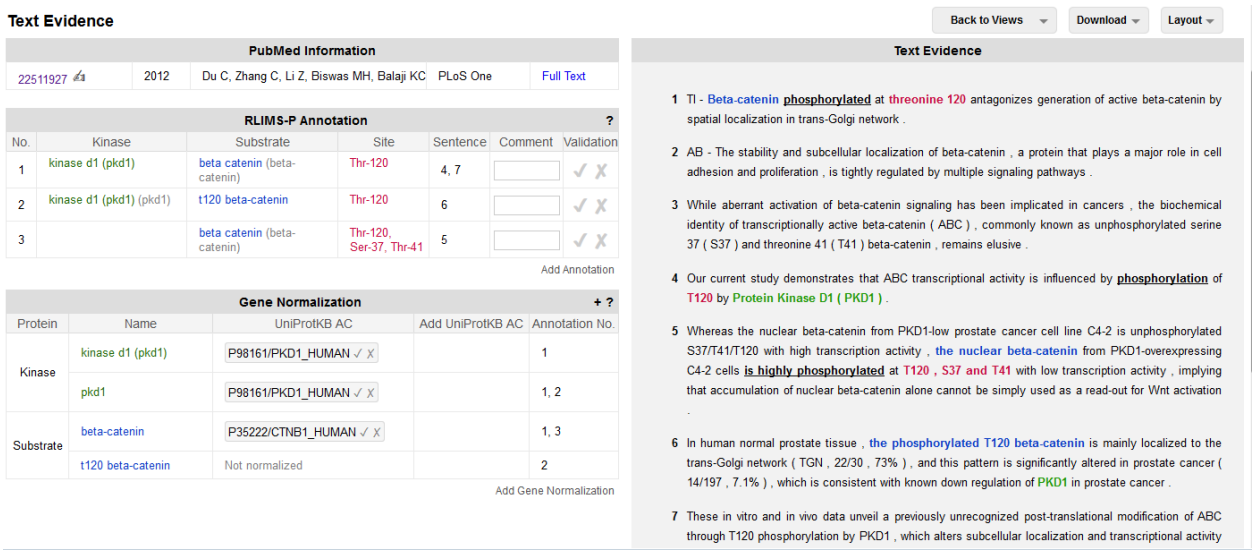
- To review annotation and edit select text evidence/curation icon. The default view of the table shows a summary of the kinases and substrates that are mentioned in the abstract. You could change the view if needed, but for curation you would need to go to Text Evidence/curation page.

- In Evidence page, inspect the result table for RLIMS-P annotation and the abstract. Validate only abstract-level information. This example is for PMID:22511927.

The information in this abstract about phosphorylation could be summarized as

beta catenin is phosphorylated at Thr-120, Ser-37 and Thr-41

beta catenin phosphorylation at Thr-120 by Protein kinase D1



Text Evidence

Back to Views | Download | Layout

PubMed Information

22511927	2012	Du C, Zhang C, Li Z, Biswas MH, Balaji KC	PLoS One	Full Text

RLIMS-P Annotation ?

No.	Kinase	Substrate	Site	Sentence	Comment	Validation
1	kinase d1 (pkd1)	beta catenin (beta-catenin)	Thr-120	4, 7		✓ ✗
2	kinase d1 (pkd1) (pkd1)	t120 beta-catenin	Thr-120	6		✓ ✗
3		beta catenin (beta-catenin)	Thr-120, Ser-37, Thr-41	5		✓ ✗

Add Annotation

Gene Normalization + ?

Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	kinase d1 (pkd1)	P98161/PKD1_HUMAN ✓ ✗		1
	pkd1	P98161/PKD1_HUMAN ✓ ✗		1, 2
Substrate	beta-catenin	P35222/CTNB1_HUMAN ✓ ✗		1, 3
	t120 beta-catenin	Not normalized		2

Add Gene Normalization

Text Evidence

1 TI - Beta-catenin phosphorylated at threonine 120 antagonizes generation of active beta-catenin by spatial localization in trans-Golgi network .

2 AB - The stability and subcellular localization of beta-catenin , a protein that plays a major role in cell adhesion and proliferation , is tightly regulated by multiple signaling pathways .

3 While aberrant activation of beta-catenin signaling has been implicated in cancers , the biochemical identity of transcriptionally active beta-catenin (ABC) , commonly known as unphosphorylated serine 37 (S37) and threonine 41 (T41) beta-catenin , remains elusive .

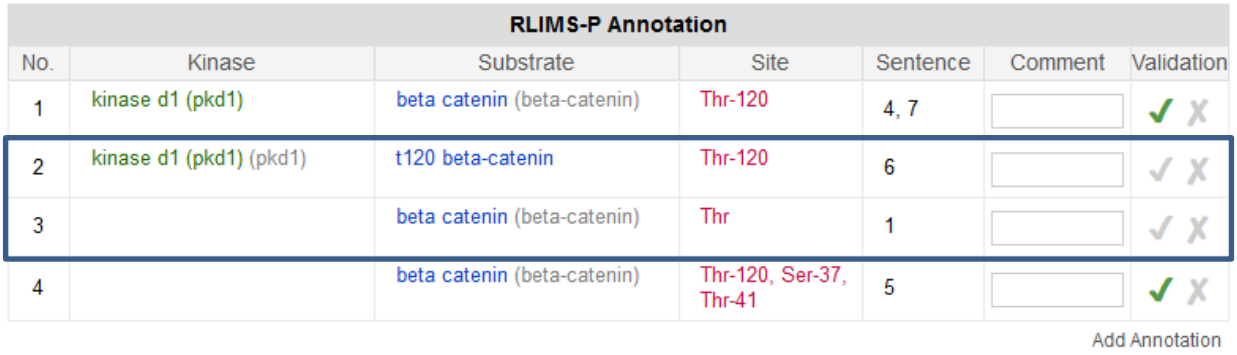
4 Our current study demonstrates that ABC transcriptional activity is influenced by phosphorylation of T120 by Protein Kinase D1 (PKD1) .

5 Whereas the nuclear beta-catenin from PKD1-low prostate cancer cell line C4-2 is unphosphorylated S37/T41/T120 with high transcription activity , the nuclear beta-catenin from PKD1-overexpressing C4-2 cells is highly phosphorylated at T120 , S37 and T41 with low transcription activity , implying that accumulation of nuclear beta-catenin alone cannot be simply used as a read-out for Wnt activation .

6 In human normal prostate tissue , the phosphorylated T120 beta-catenin is mainly localized to the trans-Golgi network (TGN , 22/30 , 73%) , and this pattern is significantly altered in prostate cancer (14/197 , 7.1%) , which is consistent with known down regulation of PKD1 in prostate cancer .

7 These in vitro and in vivo data unveil a previously unrecognized post-translational modification of ABC through T120 phosphorylation by PKD1 , which alters subcellular localization and transcriptional activity

Click on the check mark for the correct annotations (will turn green). Ignore annotations that do not add value such as those in No. 2 or No. 3 (in the box below).

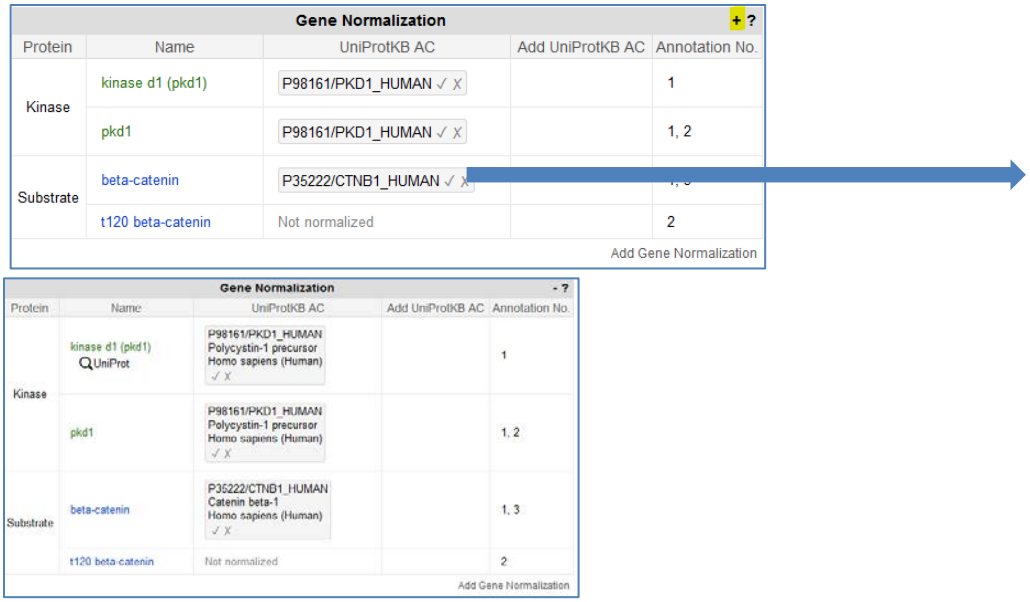


RLIMS-P Annotation

No.	Kinase	Substrate	Site	Sentence	Comment	Validation
1	kinase d1 (pkd1)	beta catenin (beta-catenin)	Thr-120	4, 7		✓ ✗
2	kinase d1 (pkd1) (pkd1)	t120 beta-catenin	Thr-120	6		✓ ✗
3		beta catenin (beta-catenin)	Thr	1		✓ ✗
4		beta catenin (beta-catenin)	Thr-120, Ser-37, Thr-41	5		✓ ✗

Add Annotation

- Inspect the Gene Normalization table. Validate when possible, add new if needed. For this activity you need to find species information for the individual proteins that you validated as kinase or substrate so you can link to the corresponding UniProtKB accession. Use the **+** icon to include the UniProtKB ID and protein name in the table. This usually helps to identify the entries.



Gene Normalization + ?

Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	kinase d1 (pkd1)	P98161/PKD1_HUMAN ✓ ✗		1
	pkd1	P98161/PKD1_HUMAN ✓ ✗		1, 2
Substrate	beta-catenin	P35222/CTNB1_HUMAN ✓ ✗		1, 3
	t120 beta-catenin	Not normalized		2

Add Gene Normalization

In many cases the evidence for finding the species is not in the abstract, so checking on full-length may be needed. For open access articles a link to full text is offered in the PubMed information section.

PubMed Information				
22511927	2012	Du C, Zhang C, Li Z, Biswas MH, Balaji KC	PLoS One	Full Text

In this example the abstract talks about *beta catenin* and *pkd1* in prostate cancer and uses C4-2 cell line which is *human*. The source of these proteins can be confirmed by looking the source of beta catenin in the full-text.

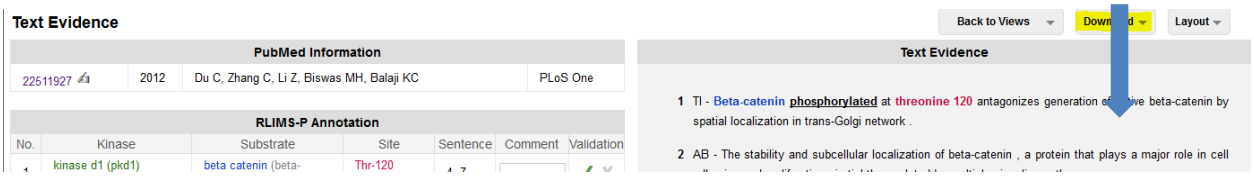
Then click on the "check" for PDK1_HUMAN and CTNB1_HUMAN. These accessions should turn green. If you have many UniProtKB accession options you don't need to validate them all. If you found the correct one just check it. Use the "x" only when all accessions are incorrectly assigned for a given kinase/substrate.

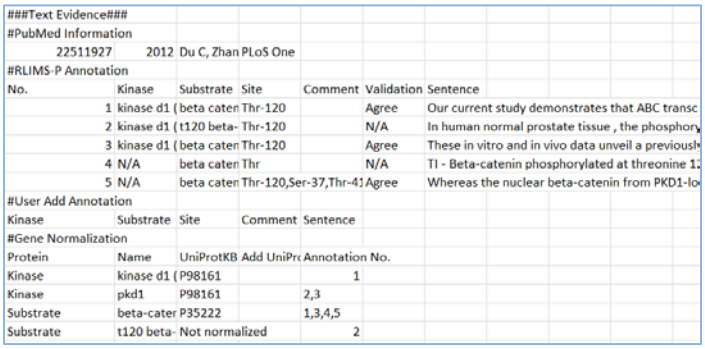
Gene Normalization + ?

Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	kinase d1 (pkd1)	P98161/PKD1_HUMAN ✓ ✗		1
	pkd1	P98161/PKD1_HUMAN ✓ ✗		1, 2
Substrate	beta-catenin	P35222/CTNB1_HUMAN ✓ ✗		1, 3
	t120 beta-catenin	Not normalized		2

Add Gene Normalization

Once you've finished you can download your result for your records, in a tab delimited format or BioC format.

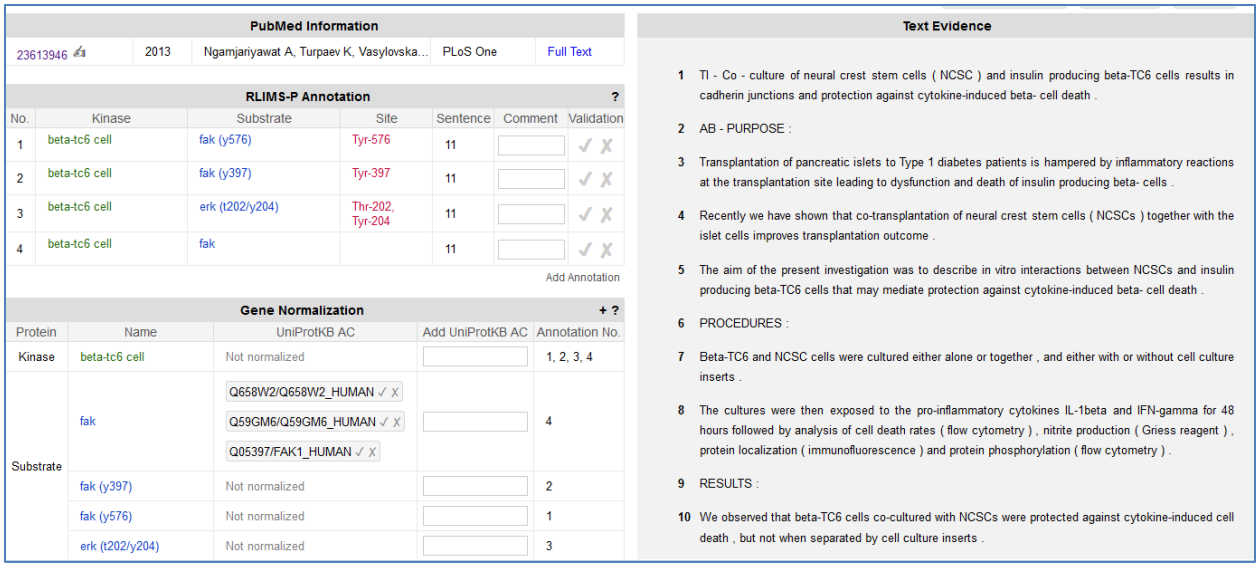




Return to your original results list by selecting *View by Summary* and the PMID that has been validated is now checked in the table

Always indicate wrong annotation.

Example: PMID 23613946



PubMed Information

23613946	2013	Ngamjariyawat A, Turpaev K, Vasylovska...	PLoS One	Full Text

RLIMS-P Annotation ?

No.	Kinase	Substrate	Site	Sentence	Comment	Validation
1	beta-tc6 cell	fak (y576)	Tyr-576	11		✓ ✗
2	beta-tc6 cell	fak (y397)	Tyr-397	11		✓ ✗
3	beta-tc6 cell	erk (t202/y204)	Thr-202, Tyr-204	11		✓ ✗
4	beta-tc6 cell	fak		11		✓ ✗

Add Annotation

Gene Normalization + ?

Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	beta-tc6 cell	Not normalized		1, 2, 3, 4
Substrate	fak	Q658W2/Q658W2_HUMAN ✓ ✗ Q59GM6/Q59GM6_HUMAN ✓ ✗ Q05397/FAK1_HUMAN ✓ ✗		4
	fak (y397)	Not normalized		2
	fak (y576)	Not normalized		1
	erk (t202/y204)	Not normalized		3

Text Evidence

1 TI - Co - culture of neural crest stem cells (NCSC) and insulin producing beta-TC6 cells results in cadherin junctions and protection against cytokine-induced beta- cell death .

2 AB - PURPOSE :

3 Transplantation of pancreatic islets to Type 1 diabetes patients is hampered by inflammatory reactions at the transplantation site leading to dysfunction and death of insulin producing beta- cells .

4 Recently we have shown that co-transplantation of neural crest stem cells (NCSCs) together with the islet cells improves transplantation outcome .

5 The aim of the present investigation was to describe in vitro interactions between NCSCs and insulin producing beta-TC6 cells that may mediate protection against cytokine-induced beta- cell death .

6 PROCEDURES :

7 Beta-TC6 and NCSC cells were cultured either alone or together , and either with or without cell culture inserts .

8 The cultures were then exposed to the pro-inflammatory cytokines IL-1beta and IFN-gamma for 48 hours followed by analysis of cell death rates (flow cytometry) , nitrite production (Griess reagent) , protein localization (immunofluorescence) and protein phosphorylation (flow cytometry) .

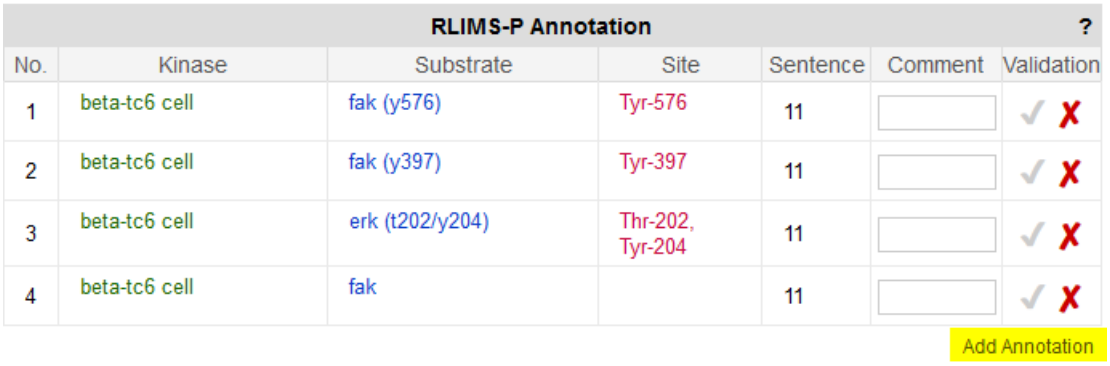
9 RESULTS :

10 We observed that beta-TC6 cells co-cultured with NCSCs were protected against cytokine-induced cell death , but not when separated by cell culture inserts .

The information in this abstract about phosphorylation could be summarized as

fak is phosphorylated at Tyr-576
fak is phosphorylated at Tyr-397
erk is phosphorylated at Thr-202 and Tyr-204

Although annotation of phosphorylation of fak and erk is correct in RLIMS-P output, the kinase information on beta-TC6 is not, as it is not a protein but the cell type. Check on X on validation column for all wrong statements. And use Add Annotation to enter the correct information.



RLIMS-P Annotation ?

No.	Kinase	Substrate	Site	Sentence	Comment	Validation
1	beta-tc6 cell	fak (y576)	Tyr-576	11		✓ ✗
2	beta-tc6 cell	fak (y397)	Tyr-397	11		✓ ✗
3	beta-tc6 cell	erk (t202/y204)	Thr-202, Tyr-204	11		✓ ✗
4	beta-tc6 cell	fak		11		✓ ✗

Add Annotation

And use Add Annotation to enter the correct information.

User Added Annotation

No.	Kinase	Substrate	Site	Sentence	Comment	Delete
5	Kinase	fak	Tyr-576	11	Comment	
6	Kinase	fak	Tyr-397	11	Comment	
7	Kinase	erk	Thr-202, Tyr-204	11	Comment	

Add Annotation

Now the gene normalization step for fak and erk. The abstract describes endogenous beta-TC6 cell line which is murine (mouse). So fak and erk should be mouse entries.

Gene Normalization

Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	beta-tc6 cell	Not normalized		1, 2, 3, 4
Substrate	fak	Q05397 ✓ ✗ Q59GM6 ✓ ✗ Q658W2 ✓ ✗		4
	fak (y397)	Not normalized		2
	fak (y576)	Not normalized		1
	erk (t202/y204) UniProt	Not normalized		3

Add UniProtKB Entry

Use the UniProt icon to search the database. Modify search box keywords in UniProt to include mouse.

In this example erk was not normalized as it represents a family of proteins. The full text describes use of antibodies for Phospho-ERK1/2(T202/Y204) so we don't know which protein it is.

In the case of fak, a search for fak and mouse yields fak1 and fak2 (Pyk2). Again here they use and antibody Y-397 which may react with fak2 (according to Invitrogen antibody specifications).

From http://tools.invitrogen.com/content/sfs/manuals/44625G_Rev1108.pdf: "Human FAK. Mouse, frog and fly FAK have not been tested but are expected to react. This antibody will cross-react with the corresponding autophosphorylation site on Proline-rich/Ca2+-activated tyrosine kinase (Pyk2), [pY402]. FAK [pY397] polyclonal antibody (Cat. #44-624G) does not cross-react with Pyk2."

In addition, the other antibody used for Y576 describes that it cross react with other activated receptors

Phospho-FAK (Tyr576/577) Antibody detects endogenous levels of FAK only when phosphorylated at tyrosine 576/577. This antibody may cross-react with other activated receptor tyrosine kinases.

Therefore, we cannot normalize in this case.

Go back to Summary to continue with next PMID (note that now two PMIDs are checked)

Summary Show all annotations — View by Summary — Download

Show Selected	PubMed ID	Protein Kinase	Phosphorylated Protein (Substrate)	No. of Sentences	Text Evidence/Curation
☐	23613946 ✓	beta-tc6 cell	fak (y576), fak (y397), erk (t202/y204), fak	1	✍
☐	22511927 ✓	kinase d1 (pkd1)	beta catenin, t120 beta-catenin	5	✍
☐	22037766	mink1	prickle	1	✍

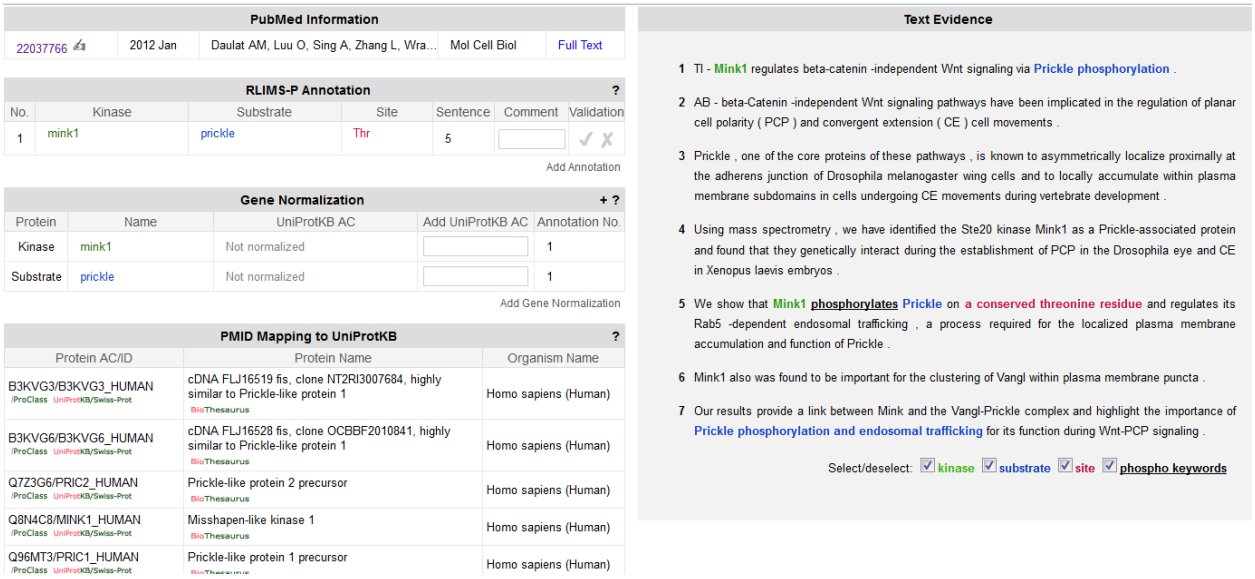
Annotation of PMID 22037766

Summary Show all annotations — View by Summary — Download

Show Selected	PubMed ID	Protein Kinase	Phosphorylated Protein (Substrate)	No. of Sentences	Text Evidence/Curation
☐	23613946 ✓	beta-tc6 cell	fak (y576), fak (y397), erk (t202/y204), fak	1	✍
☐	22511927 ✓	kinase d1 (pkd1)	beta catenin, t120 beta-catenin	5	✍
☐	22037766	mink1	prickle	1	✍

The phosphorylation information in this abstract can be summarized as:

Prickle phosphorylated by Mink1 on Thr



PubMed Information

22037766 ✍	2012 Jan	Daulat AM, Luu O, Sing A, Zhang L, Wra...	Mol Cell Biol	Full Text

RLIMS-P Annotation ?

No.	Kinase	Substrate	Site	Sentence	Comment	Validation
1	mink1	prickle	Thr	5		✓ ✗

Add Annotation

Gene Normalization + ?

Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	mink1	Not normalized		1
Substrate	prickle	Not normalized		1

Add Gene Normalization

PMID Mapping to UniProtKB ?

Protein AC/ID	Protein Name	Organism Name
B3KVG3/B3KVG3_HUMAN /ProClass UniProtKB/Swiss-Prot	cDNA FLJ16519 fis, clone NT2RI3007684, highly similar to Prickle-like protein 1 BioThesaurus	Homo sapiens (Human)
B3KVG6/B3KVG6_HUMAN /ProClass UniProtKB/Swiss-Prot	cDNA FLJ16528 fis, clone OCBBF2010841, highly similar to Prickle-like protein 1 BioThesaurus	Homo sapiens (Human)
Q7Z3G6/PRIC2_HUMAN /ProClass UniProtKB/Swiss-Prot	Prickle-like protein 2 precursor BioThesaurus	Homo sapiens (Human)
Q8N4C8/MINK1_HUMAN /ProClass UniProtKB/Swiss-Prot	Misshapen-like kinase 1 BioThesaurus	Homo sapiens (Human)
Q96MT3/PRIC1_HUMAN /ProClass UniProtKB/Swiss-Prot	Prickle-like protein 1 precursor	Homo sapiens (Human)

Text Evidence

1 TI - **Mink1** regulates beta-catenin -independent Wnt signaling via **Prickle phosphorylation** .

2 AB - beta-Catenin -independent Wnt signaling pathways have been implicated in the regulation of planar cell polarity (PCP) and convergent extension (CE) cell movements .

3 Prickle , one of the core proteins of these pathways , is known to asymmetrically localize proximally at the adherens junction of Drosophila melanogaster wing cells and to locally accumulate within plasma membrane subdomains in cells undergoing CE movements during vertebrate development .

4 Using mass spectrometry , we have identified the Ste20 kinase Mink1 as a Prickle-associated protein and found that they genetically interact during the establishment of PCP in the Drosophila eye and CE in Xenopus laevis embryos .

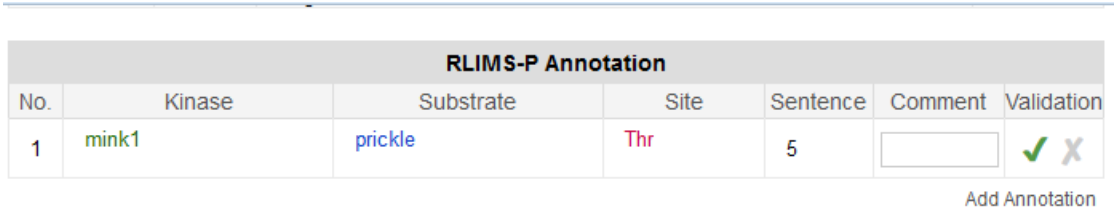
5 We show that **Mink1** **phosphorylates** **Prickle** on **a conserved threonine residue** and regulates its Rab5 -dependent endosomal trafficking , a process required for the localized plasma membrane accumulation and function of Prickle .

6 Mink1 also was found to be important for the clustering of Vangl within plasma membrane puncta .

7 Our results provide a link between Mink and the Vangl-Prickle complex and highlight the importance of **Prickle phosphorylation and endosomal trafficking** for its function during Wnt-PCP signaling .

Select/deselect: ☑ kinase ☑ substrate ☑ site ☑ phospho keywords

In this case the information provided by RLIMS-P coincides with that of the abstract level information, so it can be checked. The residue is not mentioned in the abstract only that it is a Thr.



RLIMS-P Annotation

No.	Kinase	Substrate	Site	Sentence	Comment	Validation
1	mink1	prickle	Thr	5		✓ ✗

Add Annotation

Now to the normalization business. In this case, GenNorm (the program use for normalization) was not able to find a UniProt accession for the proteins in this abstract. However, there are some entries

suggested via the UniProtKB bibliography mapping service (meaning that some database link this PMID to the entries suggested). This is provide additional help in finding the correct entry. To confirm that the proteins are human as suggested by the mapping, you would need to go to full-text.

Gene Normalization + ?

Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	mink1	Not normalized		1
Substrate	prickle	Not normalized		1

Add Gene Normalization

PMID Mapping to UniProtKB ?

Protein AC/ID	Protein Name	Organism Name
B3KVG3/B3KVG3_HUMAN /ProClass UniProtKB/Swiss-Prot	cDNA FLJ16519 fis, clone NT2RI3007684, highly similar to Prickle-like protein 1 BioThesaurus	Homo sapiens (Human)
B3KVG6/B3KVG6_HUMAN /ProClass UniProtKB/Swiss-Prot	cDNA FLJ16528 fis, clone OCBBF2010841, highly similar to Prickle-like protein 1 BioThesaurus	Homo sapiens (Human)
Q7Z3G6/PRIC2_HUMAN /ProClass UniProtKB/Swiss-Prot	Prickle-like protein 2 precursor BioThesaurus	Homo sapiens (Human)
Q8N4C8/MINK1_HUMAN /ProClass UniProtKB/Swiss-Prot	Misshapen-like kinase 1 BioThesaurus	Homo sapiens (Human)
Q96MT3/PRIC1_HUMAN /ProClass UniProtKB/Swiss-Prot	Prickle-like protein 1 precursor BioThesaurus	Homo sapiens (Human)

Consulting the full text, in *Materials and Methods* the information about the species, which is human, can be confirmed. Prickle refers to two proteins Prickle 1 and Prickle 2:

“cDNA for human *PRICKLE1* and *PRICKLE2”*

“The cDNA for Mink1 was obtained from clone MGC:21111.”

So we know that we can add the corresponding accessions in the normalization table. Since in this case they define that they are looking into both Prickle proteins you can add both accession in the box. You can ignore accession for redundant entries.

Gene Normalization				
Protein	Name	UniProtKB AC	Add UniProtKB AC	Annotation No.
Kinase	mink1	Not normalized	Q8N4C8,	1
Substrate	prickle	Not normalized	Q96MT3, Q7Z3...	1

PMID Mapping to UniProtKB		
Protein AC/ID	Protein Name	Organism Name
B3KVG3/B3KVG3_HUMAN ProClass UniProtKB/Swiss-Prot	cDNA FLJ16519 fis, clone NT2RI3007684, highly similar to Prickle-like protein 1 BioThesaurus	Homo sapiens (Human)
B3KVG6/B3KVG6_HUMAN ProClass UniProtKB/Swiss-Prot	cDNA FLJ16528 fis, clone OCBBF2010841, highly similar to Prickle-like protein 1 BioThesaurus	Homo sapiens (Human)
Q7Z3G6/PRIC2_HUMAN ProClass UniProtKB/Swiss-Prot	Prickle-like protein 2 precursor BioThesaurus	Homo sapiens (Human)
Q8N4C8/MINK1_HUMAN ProClass UniProtKB/Swiss-Prot	Misshapen-like kinase 1 BioThesaurus	Homo sapiens (Human)
Q96MT3/PRIC1_HUMAN ProClass UniProtKB/Swiss-Prot	Prickle-like protein 1 precursor BioThesaurus	Homo sapiens (Human)

Go back to summary view. After all PMIDs have been checked in the summary result page, then your task is done.

You can download all the curated data by selecting in the upper right menu My Curation option

CeciA | My Curation | Sign out

And then select "Save Result"

Record end time.

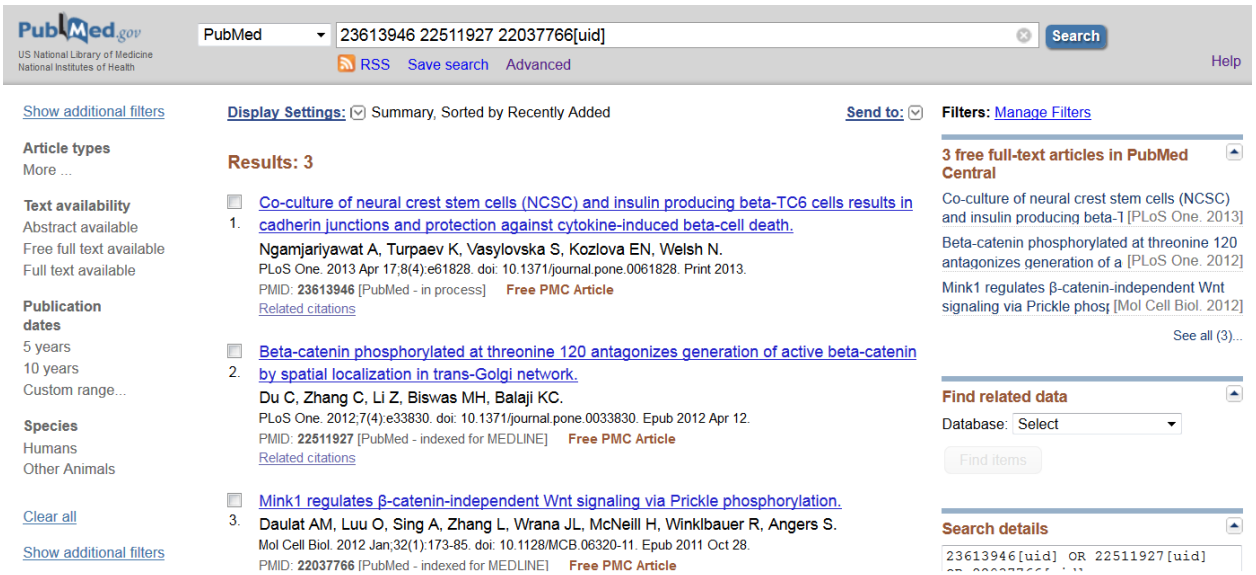
Please record time intervals if you do the task in steps, as we need the total time.

Example Manual Task

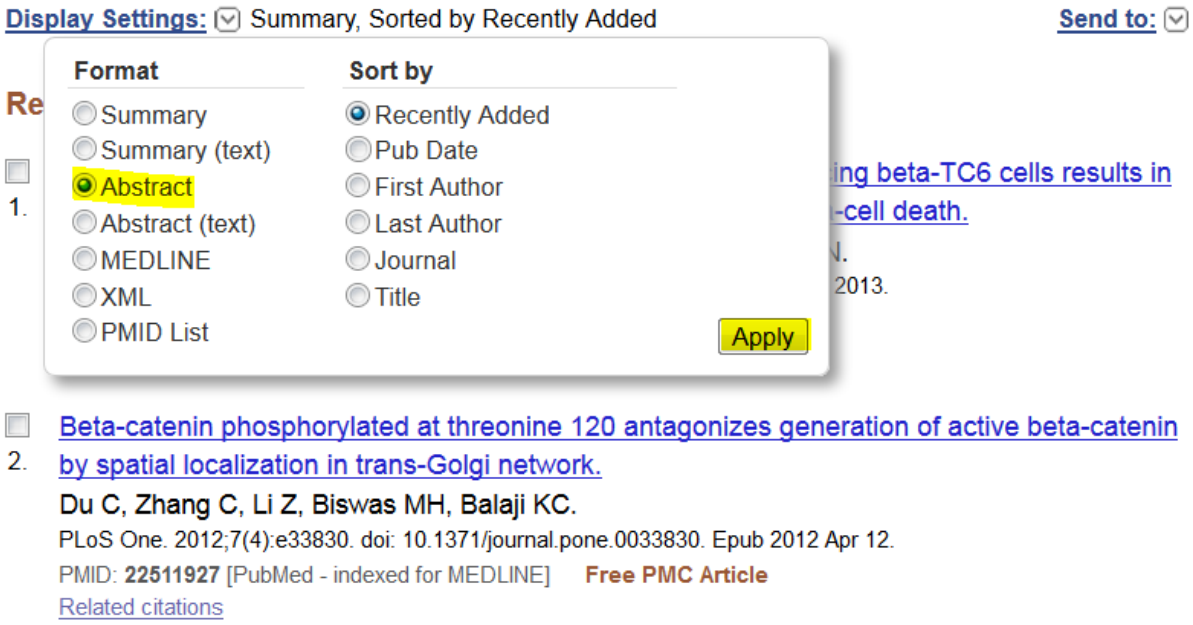
Let's use as example the same PMIDs (23613946, 22511927, 22037766). This is one suggested workflow but you could use your own ideas for this part as long as in the end you provide a file with:

PMID	Kinase	UniProtKB Kinase	Substrate	UniProtKB Substrate	Site (3 letter code-, sep commas)

- Go to Pubmed http://www.ncbi.nlm.nih.gov/pubmed
- Record start time
- Enter list of PMIDs separated by commas



- In Display settings select view Abstract or Abstract (text) and Apply



Now you have all the abstracts and you can read and find the information about protein phosphorylation

1. Co-culture of neural crest stem cells (NCSC) and insulin producing beta-TC6 cells results in cadherin junctions and protection against cytokine-induced beta-cell death.

Nikolaidou-Neokl, Tumaev K, Vasylovska S, Hertox Ek, Welsh N.

Abstract

PURPOSE: Transplantation of pancreatic islets to Type 1 diabetes patients is hampered by inflammatory reactions at the transplantation site leading to dysfunction and death of insulin producing beta-cells. Recently, we have shown that co-transplantation of neural crest stem cells (NCSCs) together with the islet cells improves transplantation outcome. The aim of the present investigation was to describe in vitro interactions between NCSCs and insulin producing beta-TC6 cells that may mediate protection against cytokine-induced beta-cell death.

PROCEDURES: Beta-TC6 and NCSC cells were cultured either alone or together, and either with or without cell culture inserts. The cultures were then exposed to the pro-inflammatory cytokines IL-1β and IFN-γ for 48 hours followed by analysis of cell death rates (flow cytometry), nitrite production (Griess reagent), protein localization (immunofluorescence) and protein phosphorylation (flow cytometry).

RESULTS: We observed that beta-TC6 cells co-cultured with NCSCs were protected against cytokine-induced cell death, but not when separated by cell culture inserts. This occurred in parallel with (i) augmented production of nitrite from beta-TC6 cells, indicating that increased cell survival allows a sustained production of nitric oxide; (ii) NCSC-derived laminin production; (iii) decreased phospho-FAK staining in beta-TC6 cell focal adhesions, and (iv) decreased beta-TC6 cell phosphorylation of ERK(T202/Y204), FAK(Y397) and FAK(Y576). Furthermore, co-culture also resulted in cadherin and beta-catenin accumulations at the NCSC:beta-TC6 cell junctions. Finally, the gap junction inhibitor carbenoxolone did not affect cytokine-induced beta-cell death during co-culture with NCSCs.

CONCLUSION: In summary, direct contacts, but not soluble factors, promote improved beta-TC6 viability when co-cultured with NCSCs. We hypothesize that cadherin junctions between NCSC and beta-TC6 cells promote powerful signals that maintain beta-cell survival even though ERK and FAK signaling are suppressed. It may be fruitful to strategies to improve islet transplantation outcome may benefit from attempts to increase beta-cell cadherin junctions to neighboring cells.

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Related citations

Publication Types

2. Beta-catenin phosphorylated at threonine T120 antagonizes generation of active beta-catenin by spatial localization in trans-Golgi network.

Du D, Zhang C, Li S, Strauss MH, Reid MC.

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Abstract

The stability and subcellular localization of beta-catenin, a protein that plays a major role in cell adhesion and proliferation, is tightly regulated by multiple signaling pathways. While aberrant activation of beta-catenin signaling has been implicated in cancers, the biochemical identity of transcriptionally active beta-catenin (ABC), commonly known as unphosphorylated serine 37 (S37) and threonine 41 (T41) β-catenin, remains elusive. Our current study demonstrates that ABC transcriptional activity is influenced by phosphorylation of T120 by Protein Kinase D1 (PKD1). Whereas the nuclear β-catenin from PKD1-low prostate cancer cell line C4-2 is unphosphorylated S37/T41/T120 with high transcription activity, the nuclear β-catenin from PKD1-overexpressing C4-2 cells is highly phosphorylated at T120, S37 and T41 with low transcription activity, implying that accumulation of nuclear β-catenin alone cannot be simply used as a read-out for Wnt activation. In human normal prostate tissue, the phosphorylated T120 β-catenin is mainly localized to the trans-Golgi network (TGN, 22/30, 73%), and this pattern is significantly altered in prostate cancer (14/167, 7.1%), which is consistent with known down regulation of PKD1 in prostate cancer. These in vitro and in vivo data unveil a previously unrecognized post-translational modification of ABC through T120 phosphorylation by PKD1, which alters subcellular localization and transcriptional activity of β-catenin. Our results suggest the view that β-catenin signaling activity is regulated by spatial compartmentation and post-translational modifications and protein level of β-catenin alone is insufficient to count signaling activity.

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Related citations

Publication Types, MeSH Terms, Substances

3. Mink1 regulates β-catenin-independent Wnt signaling via Prickle phosphorylation.

Rocheleau Lucy D, Shin J, Zhang L, Wrana JL, McNeill H, Winklbauer R, Jones S.

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Abstract

β-Catenin-independent Wnt signaling pathways have been implicated in the regulation of planar cell polarity (PCP) and convergent extension (CE) cell movements. Prickle, one of the core proteins of these pathways, is known to asymmetrically localize proximally at the adherens junction of Drosophila melanogaster wing cells and to locally accumulate within plasma membrane subdomains in cells undergoing CE movements during vertebrate development. Using mass spectrometry, we have identified the Ste20 kinase Mink1 as a Prickle-associated protein and found that they genetically interact during the establishment of PCP in the Drosophila eye and CE in Xenopus laevis embryos. We show that Mink1 phosphorylates Prickle on a conserved threonine residue and regulates its Rab5-dependent endosomal trafficking, a process required for the localized plasma membrane accumulation and function of Prickle. Mink1 also was found to be important for the duration of Dvl1 within plasma membrane

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PMID	Kinase	UniProtKB Kinase	Substrate	UniProtKB Substra	Site (3 letter code-, separated by comm	Comment	Sentence
22511927			beta-catenin	P35222	Thr-120,Ser-37,Thr-41		
	Protein Kinase D1 (PKD1)	P98161	ABC	P35222	Thr-120	ABC is active beta-catenin	Our current study demonstrates that AE
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			erk		Thr-202,Tyr-204	cannot be normalized, use an	This occurred in parallel with (i) augmen
			fak		Tyr-576	cannot be normalized, use an	This occurred in parallel with (i) augmen
22037766	mink1	Q8N4C8	prickle	Q96MT3,Q7Z3G6	Thr	pricke include prickle 1 and p	We show that Mink1 phosphorylates Pr

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