

Subject: RE: One question left
From: Maria Lluch Senar <maria.lluch@crg.eu>
Date: 17/11/16 16:29
To: Katerina Kirsanova <catherine.kirsanova@crg.eu>, Luis Serrano Pubul <luis.serrano@crg.eu>

In the MS database you put the sequence:

MSLPTSWVLVNAPSSLRKTMFCSRLFAPVVKMTSLSVRPRGLSCVSTKTPSVRCPALPWGSWGLISTSANLSMVC

The in silico peptides considered in the Mascot search would be (if the digestion is total):

-MSLPTSWVLVNAPSSLRK
-TMFCSRLFAPVVK
-TSLSVRPRGLSCVSTK
-TPSVRCPALPWGSWGLISTSANLSMVCPLPVTGVYYSLWVK
-MALVSSPVLTNIV

If the real protein that you have in Myco is the shorter version:

MVCPLPVTGVYYSLWVKMALVSSPVLTNIV

The peptide MALVSSPVLTNIV will could be still identified. If you do not identify the rest of the peptides it could be because the protein is shorter then, as I explained, TSS information have to be considered for properly annotation.

By using the longest version in MS database you know if the protein exist but not the length, you need to use RNAseq data.

From: Katerina Kirsanova
Sent: jueves, 17 de noviembre de 2016 16:16
To: Maria Lluch Senar <maria.lluch@crg.eu>; Luis Serrano Pubul <luis.serrano@crg.eu>
Subject: Re: One question left

El 11/17/16 a las 15:42, Maria Lluch Senar escribió:

Hi Katerina

In MS if you include the longest version of the ORF, all the putative peptides

I mean if the seq is shorter then you may happen to have lost the first pep as it would be different, you'll get only the longest, won't you?

For instance, if you have

MSLPTSWVLVNAPSSLRKTMFCSRLFAPVVKMTSLSVRPRGLSCVSTKTPSVRCPALPWGSWGLISTSANLSMVC

& it's inner part translated from the inner ORF, say

MVCPLPVTGVYYSLWVKMALVSSPVLTNIV

you won't never get in mass spec 1st peptide MVCPLPVTGVYYSLWVK (unless it suggested as an in-silico referrer)
of the 2dn guy, will you?

as they are checking only for the whole TPSVRCPALPWGSWGLISTSANLSMVCPLPVTGVYYSLWVK
aren't they? (I do not know)

So if the 2nd guy does exist, but they look only for peps of the 1st guy, say, find only the last peps,
common for both —

how do you know which one is real, i.e. that 2nd is may be more real whereas you do not see its 1st
pep

(which would be lighter and therefore missed in mass spec unless it's a referrer)?

The digestion results would be the same if the frame is the same.

Mascot does in silico digestion.

Cheers

Maria

-----Original Message-----

From: Katerina Kirsanova

Sent: jueves, 17 de noviembre de 2016 15:17

To: Maria Lluch Senar <maria.lluch@crg.eu>; Luis Serrano Pubul <luis.serrano@crg.eu>

Subject: One question left

Hi Maria,

Also, **to our chat today**, I didn't ask - with these inner ORFs inside the long
Do proteomic people make the protein digestion in-silico for inner frames too?

For the inner ORFs the digestion results (seqs/list of peptides) would be diff
And if they don't make as well this in-silico digestion peps for translated i

Cheers, -

Catherine