

This is the saved chat from the P4EU structural biology round table discussion 15/12/2023

09:39:18 From Alena Bogdanova to Everyone:

Eric likes to add A or S to the very end 😊

09:41:30 From Aljaž Gaber to Everyone:

2xGSS 😊

09:42:38 From Alena Bogdanova to Everyone:

GSAGSAAGSG we start with

09:44:00 From Anja Schütz to Everyone:

G4S linker is the first we try

09:44:18 From Ricardo Mauricio Gudino Carrillo to Everyone:

Will you put W in a linker?

09:45:02 From Juha Määttä to Everyone:

Replying to "Will you put W in a..."

Sometimes this would be good if POI lacks tryptophans

09:45:07 From Kelvin Lau to Everyone:

Reacted to "Sometimes this would..." with 👍

09:45:41 From Isabelle Stender to Everyone:

Hat auf "Sometimes this wou..." mit 👍 reagiert

09:46:59 From Juha Määttä to Everyone:

Replying to "Will you put W in a..."

But I haven't done this, does anyone know do they have any negative effects?

09:47:50 From Alena Bogdanova to Everyone:

Reacted to "Sometimes this would..." with 👍

09:48:38 From Peter Braun to Everyone:

why do computational approaches avoid W in proteins?

09:50:32 From Kelvin Lau to Everyone:

Replying to "why do computational..."

The I asked them it was due to certain folds and also for packing

09:50:37 From Sabine Suppmann to Everyone:

Nick can you paste the seq in the Chat?

09:50:41 From Kelvin Lau to Everyone:

Replying to "why do computational..."

But I am also not completely sure

09:50:47 From Sabine Suppmann to Everyone:

of the μ Phosphatase

09:53:55 From Nick Berrow to Everyone:

Replying to "Nick can you paste t..."

MTSTLPFSPQVSTPRSKFILNSYNQRRYTMGILPSPGMPALLSLVSLLSVLLMGCVA!ETG

09:54:20 From Sabine Suppmann to Everyone:

Replying to "Nick can you paste t..."

thanks

09:54:21 From Nick Berrow to Everyone:

Replying to "Nick can you paste t..."

but ...va!g seems to work just as well.

09:55:11 From Nick Berrow to Everyone:

Sorry should be: MGILPSPGMPALLSLVSLLSVLLMGCVA!ETG

09:56:12 From Simon Arnold Mortensen - EMBL Hamburg to Everyone:

This is a paper about heavy/light chain signal peptides for CHO cells.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4338144/>

09:57:54 From Alena Bogdanova to Everyone:

MBP

09:57:59 From Aljaž Gaber to Everyone:

sfGMP 😊

09:58:06 From Aljaž Gaber to Everyone:

sorry sfGFP

09:58:10 From Luigi Scietti to Everyone:

SUMO for E. Coli, or GST/MBP

09:58:25 From Sabine Suppmann to Everyone:

Replying to "Nick can you paste t..."

Solubility MBP for E coli; SumoStar for HEK/Insect

09:58:35 From Nick Berrow to Everyone:

has to be Sumo>Trx=Z>>>mbp>GST

09:59:07 From Nick Berrow to Everyone:

Replying to "This is a paper abou..."

thanks!

10:00:27 From Nick Berrow to Everyone:

Replying to "has to be Sumo>Trx=Z..."

obviously in E.coli. tend not to use fusions except FPs in eukaryotic systems.

10:02:48 From Alena Bogdanova to Everyone:

Eric!

10:03:22 From Aljaž Gaber to Everyone:

Does anyone know for a protease for C-terminal tag cleavage that doesn't leave many additional residues at the end? Like for example TEV for cleaving of N-terminal tags leaves just a G

10:03:45 From Luigi Scietti to Everyone:

Replying to "Does anyone know for..."

Not that I know unfortunately

10:04:08 From Aljaž Gaber to Everyone:

Replying to "Does anyone know for..."

Thanks Luigi. I searched far and wide with no success as well

10:04:17 From Luigi Scietti to Everyone:

Reacted to "Thanks Luigi. I sear..." with 👍

10:04:39 From Frederico Silva to Everyone:

We have a 2 protein complex where pelB was the way to get it done

10:04:52 From Isabelle Stender to Everyone:

MBP sometimes binds unspecific to your POI after cleavage, so I use this tag only for constructs that I don't plan to cleave

10:04:59 From Sabine Suppmann to Everyone:

Replying to "Does anyone know for..."

Yes CPD cysteine Protease Domain can only be used in E,.coli, activated by InsP

10:05:12 From Luigi Scietti to Everyone:

Reacted to "Yes CPD cysteine Pro..." with 🌟

10:05:13 From Sabine Suppmann to Everyone:

Replying to "Does anyone know for..."

leaves 1 aminoacid at the C Terminus
10:05:30 From Aljaž Gaber to Everyone:
Replying to "Does anyone know for..."

Thanks Sabine!
10:05:36 From Sabine Suppmann to Everyone:
Replying to "Does anyone know for..."

beneficial for Ecoli nanobody Expression!
10:05:48 From M- Al to Everyone:
How to detect the formation of disulfide with mass spec
10:05:48 From Sabine Suppmann to Everyone:
Replying to "Does anyone know for..."

nanobody
10:05:49 From M- Al to Everyone:
?
10:06:25 From Nick Berrow to Everyone:
Replying to "Does anyone know for..."

I haven't heard of one-I think this topic came up in the forum. Remember for TEV the last aa of the site can be anything except P!

10:06:48 From Sabine Suppmann to Everyone:
or go for Pichia !

10:07:02 From Alena Bogdanova to Everyone:
Reacted to "or go for Pichia !" with 👍

10:07:26 From Isabelle Stender to Everyone:
no just in some cases

10:07:41 From Isabelle Stender to Everyone:
but then it's not possible to separate

10:08:04 From Isabelle Stender to Everyone:
yes, you have to run a control with mbp alone

10:08:12 From Aljaž Gaber to Everyone:
Thanks!

10:08:17 From Frederico Silva to Everyone:
Replying to "We have a 2 protein ..."

FleB
10:08:41 From Frederico Silva to Everyone:
Replying to "We have a 2 protein ..."

Correction, sorry!
10:10:11 From Kelvin Lau to Sabine Suppmann(Direct Message):
Hi Sabine, I just need to run to the lab for a bit. Do you want to continue taking some notes in the shared presentation

10:10:54 From Sabine Suppmann to Kelvin Lau(Direct Message):
yes

10:13:54 From Kelvin Lau to Sabine Suppmann(Direct Message):
Ok I'm back 😊

10:14:17 From Sabine Suppmann to Kelvin Lau(Direct Message):
Nothing missed

10:14:51 From Chris Cooper to Everyone:

An paper from some old colleagues studied some aspects of this (codon optimisation): doi:
10.1016/j.pep.2008.01.008

10:16:06 From Alena Bogdanova to Everyone:

Reacted to "An paper from some o..." with 👍

10:18:29 From M- Al to Everyone:

What is the difference between optimize mouse and optimize human sequence of the same protein for e coli expression?

10:19:18 From Kelvin Lau to Kim Remans(Direct Message):

What the supplier??

10:20:16 From Kelvin Lau to Kim Remans(Direct Message):

Maybe I am using that one :S

10:20:20 From Kim Remans to Kelvin Lau(Direct Message):

Twist :-)

10:20:56 From Kelvin Lau to Kim Remans(Direct Message):

Ok. I am sometimes using IDT...

10:22:51 From Isabelle Stender to Everyone:

you can use an e.coli strain with tRNA genes for rare codons

10:26:04 From Kelvin Lau to Everyone:

<https://star-protocols.cell.com/protocols/3094>

10:33:16 From Chris Cooper to Everyone:

Has anyone tried 1,6-Hexanediol to break open condensates formed from IDPs?

10:34:01 From Ricardo Mauricio Gudino Carrillo to Everyone:

Replying to "Has anyone tried 1,6..."

Yes I've tried, but did not work in our hands

10:34:08 From Chris Cooper to Everyone:

Reacted to "Yes I've tried, but ..." with 👍

10:34:39 From Ricardo Mauricio Gudino Carrillo to Everyone:

Replying to "Has anyone tried 1,6..."

It only help to prevent the LPS if the protein was not on that phase

10:35:00 From Isabelle Stender to Everyone:

in general for protein stability and in view of the fact that his tag is very popular: most of the proteins are imidazole sensitive

10:38:14 From Isabelle Stender to Everyone:

For a protein complex production, on which protein would you place the tag?

10:38:29 From Sabine Suppmann to Kelvin Lau(Direct Message):

in insect we have Multibac. BigBac and GoldenBac. What for HEK?

10:38:38 From Isabelle Stender to Everyone:

-> I choose the protein which is less soluble or hard to express

10:38:45 From Sabine Suppmann to Everyone:

in insect we have Multibac. BigBac and GoldenBac. What for HEK?

10:39:05 From Joop van den Heuvel to Everyone:

Bac Mam

10:39:26 From Alena Bogdanova to Everyone:

Replying to "in insect we have Mu..."

We use same strategy to assemble

10:41:30 From Chris Cooper to Everyone:

my old colleagues managed to co-transfect ~10 plasmids in HEK for a large multi-protein complexe.

I think this was a well-behaved protein though

10:41:46 From Nick Berrow to Everyone:

Replying to "in insect we have Mu..."

I was tempted to make a GoldenMam suite but never got around to it. Anyone interested?
10:41:57 From Kelvin Lau to Everyone:

Reacted to "I was tempted to mak..." with 👍

10:42:39 From Nick Berrow to Everyone:

Has anyone tried P2A etc. sequences between proteins?

10:42:51 From Sabine Suppmann to Everyone:

Nick, I have started with David on GoldenBacMam

10:42:54 From Alena Bogdanova to Everyone:

Reacted to "I was tempted to mak..." with 👍

10:43:13 From Sabine Suppmann to Everyone:

but I will not follow-up on it, Maybe you?

10:43:13 From Juha Määttä to Everyone:

In our group we have used IRES

10:43:22 From Ondrej Vanek to Everyone:

Replying to "in insect we have Mu..."

I recall there is a MultiBac variant modified for mammalian cells called MultiMam, also from Imre Berger's group. Published ca 10 years ago.

10:43:42 From Nick Berrow to Everyone:

Replying to "Nick, I have started..."

Great! OK let me know if i can help.

10:44:16 From Juha Määttä to Everyone:

Replying to "In our group we have..."

Which worked quite well with two proteins

10:45:11 From Kelvin Lau to Everyone:

@Alena Bogdanova what P2A sequence?

10:45:54 From Alena Bogdanova to Everyone:

Replying to "@Alena Bogdanova wha..."

I do not remember now, but I will dig it out and sent to you.

10:45:59 From Kelvin Lau to Everyone:

Reacted to "I do not remember no..." with 👍

10:46:37 From Ondrej Vanek to Everyone:

I recall there is a MultiBac version modified for mammalian cells called MultiMam, also from Imre Berger's group. Published ca 10 years ago.

10:50:28 From Aljaž Gaber to Everyone:

I must leave because they scheduled an important but unexpected committee meeting at 11.00. Thanks to everyone for their contributions, and thanks for the opportunity to share and discuss our experiences.

Cheers!

10:50:53 From Frederico Silva to Everyone:

Reacted to "I must leave because..." with 👍

10:52:25 From Ondrej Vanek to Everyone:

Another approach from the same group I guess was to express the components of a protein complex as a polyprotein (single long polypeptide) with units separated by a protease cleavage site which itself comes only at the end of the polyprotein to chop it up to pieces - this way, you avoid co-transfection with many plasmids, everything is in one plasmid, one cell, the right stoichiometry etc. / published as well...

10:52:43 From Simon Arnold Mortensen - EMBL Hamburg to Everyone:

I also have to leave for a meeting at 11:00. Thanks for an interesting session. Have a nice weekend.

10:53:52 From Nick Berrow to Everyone:

Replying to "Another approach fro..."

interesting Ondrej-do you have the ref?

10:55:37 From Ondrej Vanek to Everyone:
Replying to "Another approach fro..."

https://doi.org/10.1007%2F978-1-62703-691-7_8

10:55:45 From Nick Berrow to Everyone:
Replying to "Another approach fro..."

thanks!

10:56:07 From Ondrej Vanek to Everyone:
Replying to "Another approach fro..."

Nie Y, Bellon-Echeverria I, Trowitzsch S, Bieniossek C, Berger I. Multiprotein complex production in insect cells by using polyproteins. *Methods Mol Biol.* 2014;1091:131-41. doi: 10.1007/978-1-62703-691-7_8. PMID: 24203328

10:57:09 From Chris Cooper to Everyone:
thanks all!

10:57:11 From Ricardo Mauricio Gudino Carrillo to Everyone:
Thank you for meeting!

10:57:13 From Isabelle Stender to Everyone:
Thank's a lot!

10:57:14 From Frederico Silva to Everyone:
Thank you all

10:57:14 From Emilie Song to Everyone:
Thank you everyone!!

10:57:16 From Kelvin Lau to Everyone:
Thanks!

10:57:18 From Vangelis Christodoulou to Everyone:
Thanks

10:57:18 From Alena Bogdanova to Everyone:
Thank you very much! I was a great idea! Let's repeat!

10:57:19 From Annabel Borg to Everyone:
thanks

10:57:25 From roger george to Everyone:
thanks