



SGC

GATES foundation targets: update on cloning, expression and solubility

Levon Halabelian

SGC Toronto
Jan 12th 2022

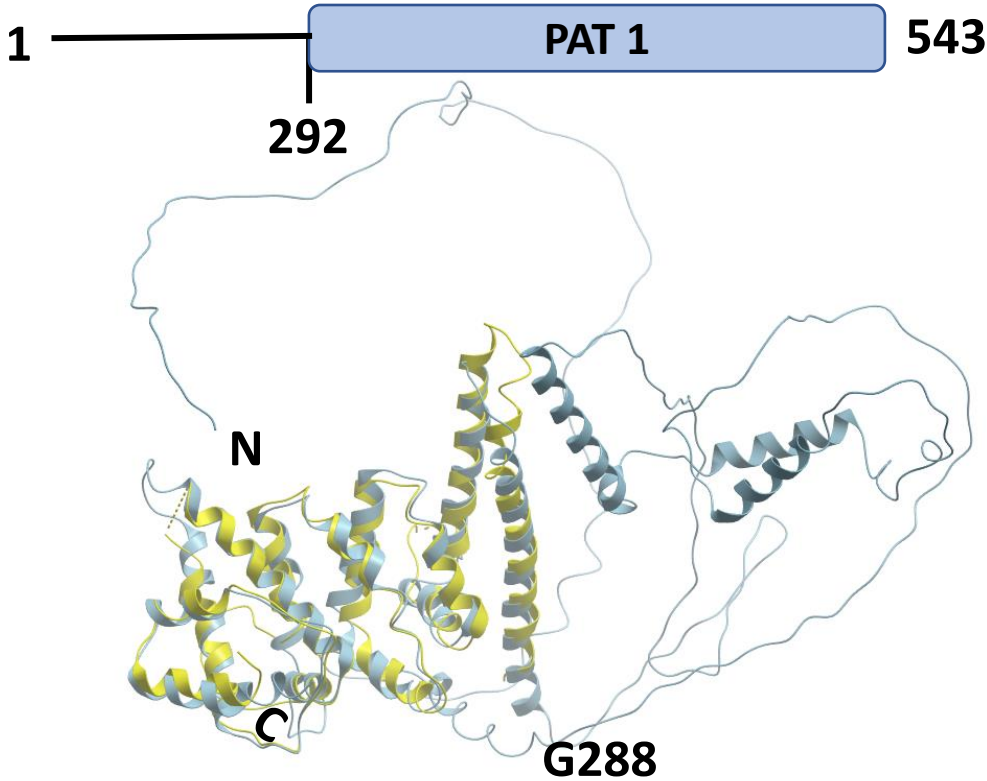


McGill

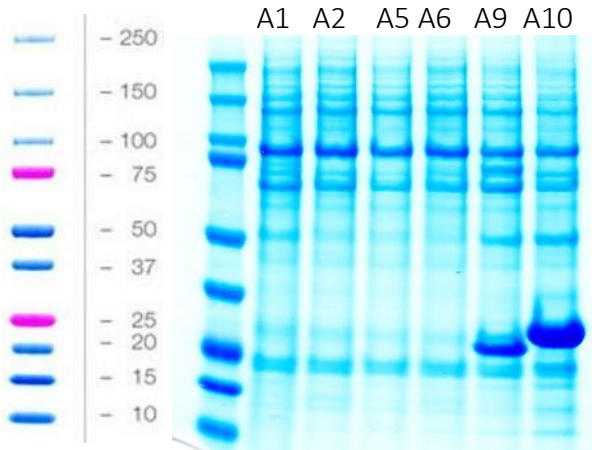


PatL2: RNA-binding protein that acts as a translational repressor

Testx in *sf9* insect cells
After Histag purification

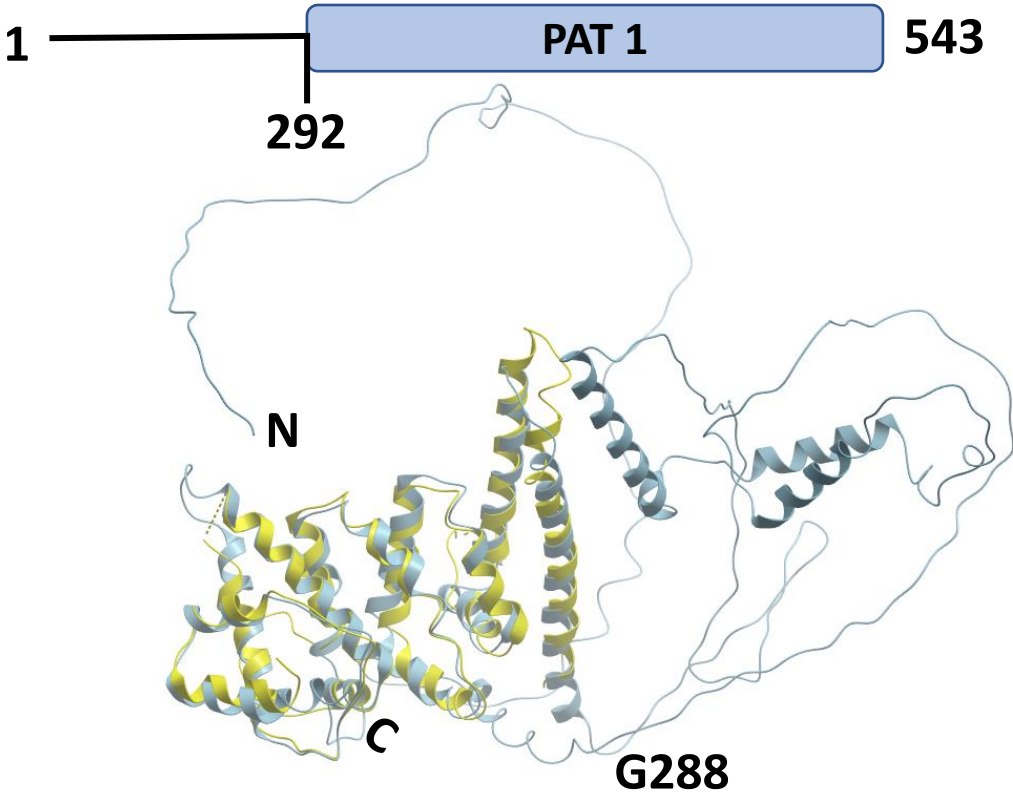


Overlay of predicted human PATL2_FL by AlphaFold (cyan) and human PatL1 C-termina domain (yellow) (PDB ID: 2XEQ). The C-terminal domains of Human PatL1 and PatL2 share 42% sequence identities.



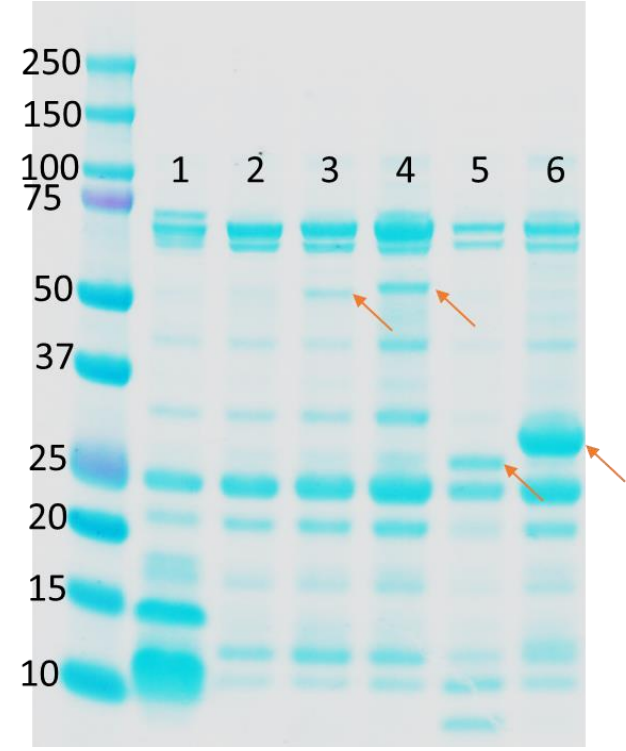
Well	Construct ID	Construct Name	Vector	MW
A01	PBC038-A01	PATL2_FL_BV_his	pFBOH-MHL	63657.38
A02	PBC038-A02	PATL2_FL_BV_bio	pFBD-BirA	65047.81
A05	PBC038-A05	PATL2_L89-Y543_BV_his	pFBOH-MHL	54179.27
A06	PBC038-A06	PATL2_L89-Y543_BV_bio	pFBD-BirA	55569.7
A09	PBC038-A09	PATL2_G288-Y543_BV_his	pFBOH-MHL	31161.92
A10	PBC038-A10	PATL2_G288-Y543_BV_bio	pFBD-BirA	32552.34

PatL2: RNA-binding protein that acts as a translational repressor



Overlay of predicted human PATL2_FL by AlphaFold (cyan) and human PatL1 C-termina domain (yellow) (PDB ID: 2XEQ). The C-terminal domains of Human PatL1 and PatL2 share 42% sequence identities.

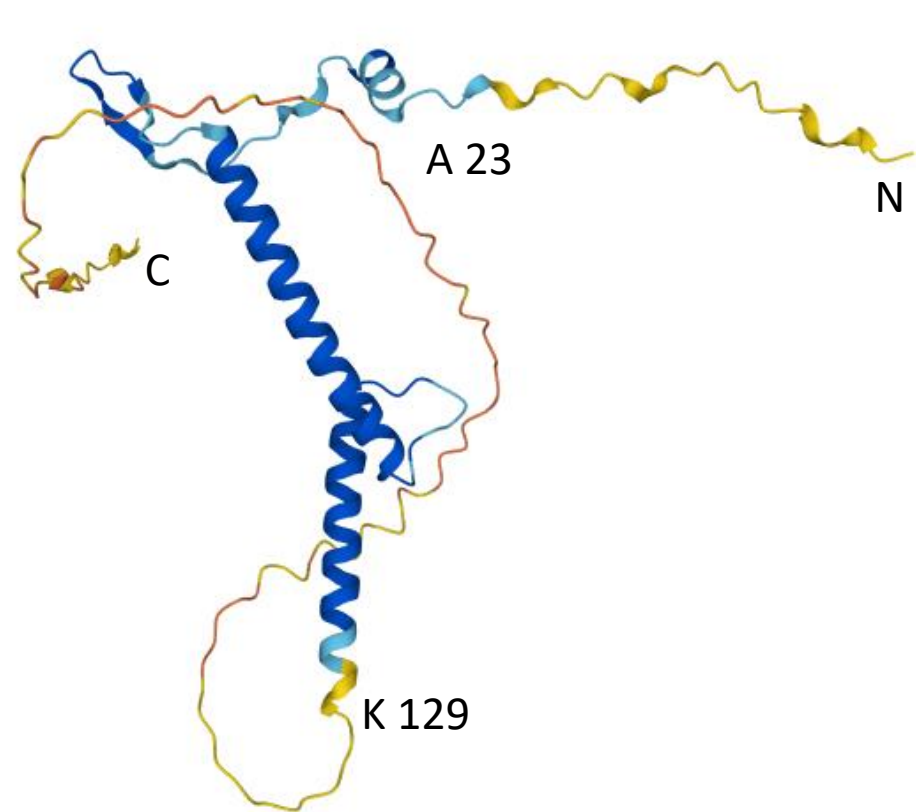
Test X in *E. coli*
After Histag purification



Well	Construct ID	Construct Name	Vector	MW
1	PBC038A03	PATL2_FL_Ec_his	pET28-MHL	63,657
2	PBC038A04	PATL2_FL_Ec_bio	p28BIOH-LIC	65,048
3	PBC038A07	PATL2_L89-Y543_Ec_his	pET28-MHL	54,179
4	PBC038A08	PATL2_L89-Y543_Ec_bio	p28BIOH-LIC	55,570
5	PBC038A11	PATL2_G288-Y543_Ec_his	pET28-MHL	31,162
6	PBC038A12	PATL2_G288-Y543_Ec_bio	p28BIOH-LIC	32,552

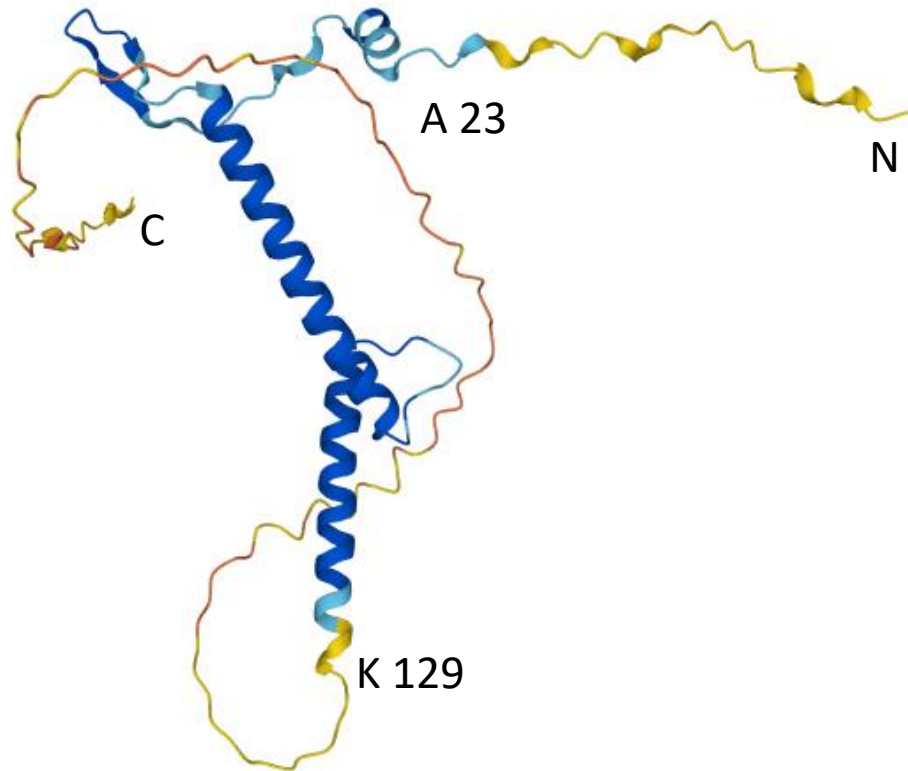
FIGLA: Germline specific transcription factor (Uniprot: Q6QHK4)

TestX with P2 in *sf9* insect cells
After Histag purification

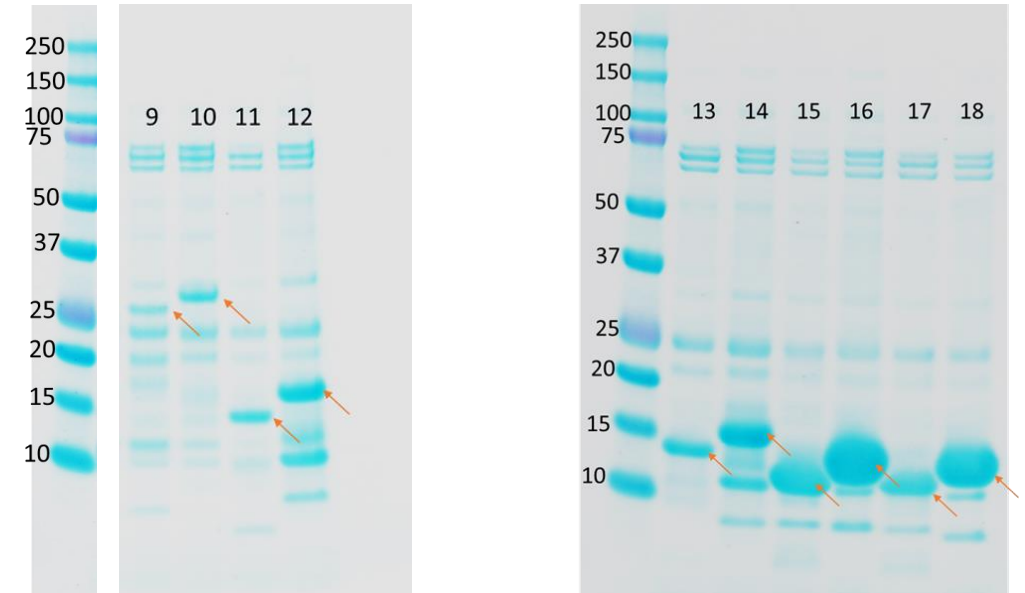


Well	Construct ID	Construct Name	Vector	MW
C2	PBC038-C02	FIGLA_FL_BV_his	pFBOH-MHL	26316.62
C3	PBC038C-03	FIGLA_FL_BV_bio	pFBD-BirA	27707.05

FIGLA: Germline specific transcription factor (Uniprot: Q6QHK4)



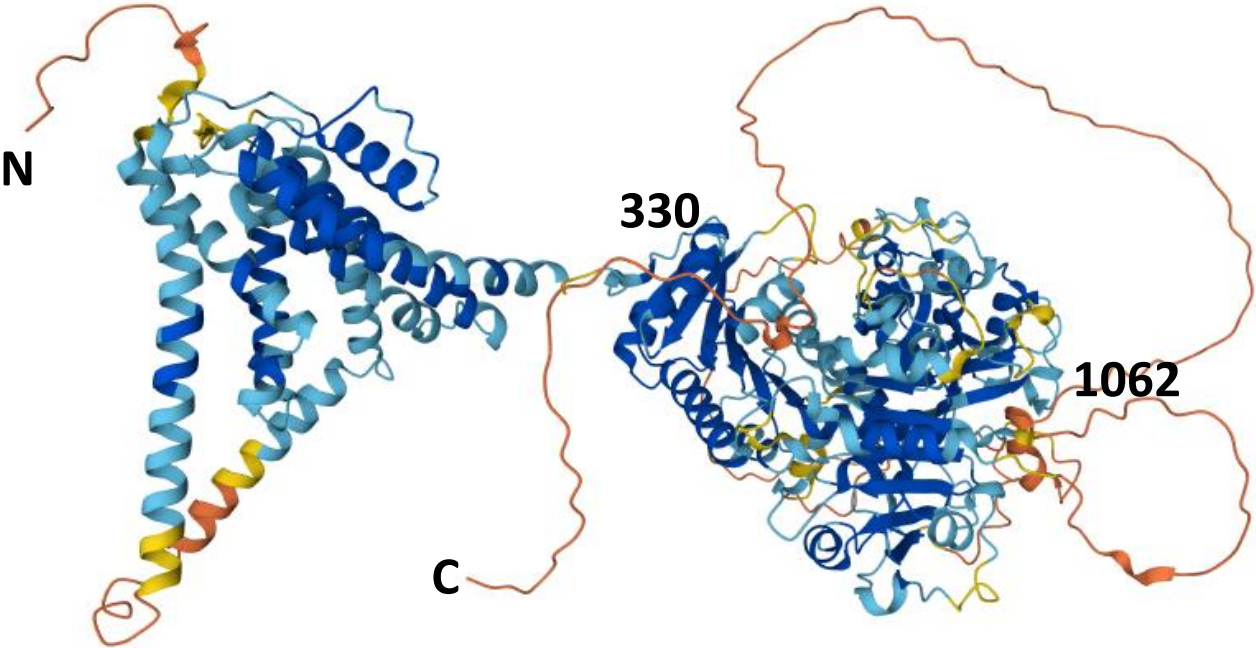
Test X in *E. coli* (After Histag purification)



Well	Construct ID	Construct Name	Vector	MW
9	PBC038B12	FIGLA_FL_Ec_his	pET28-MHL	26,317
10	PBC038C01	FIGLA_FL_Ec_bio	p28BIOH-LIC	26,626
11	PBC038C04	FIGLA_A23-S136_Ec_his	pET28-MHL	15,034
12	PBC038C05	FIGLA_A23-S136_Ec_bio	p28BIOH-LIC	15,343
13	PBC038C06	FIGLA_A41-S136_Ec_his	pET28-MHL	12,999
14	PBC038C07	FIGLA_A41-S136_Ec_bio	p28BIOH-LIC	14,389
15	PBC038C08	FIGLA_A56-S136_Ec_his	pET28-MHL	11,439
16	PBC038C09	FIGLA_A56-S136_Ec_bio	p28BIOH-LIC	12,829
17	PBC038C10	FIGLA_A56-S132_Ec_his	pET28-MHL	10,980
18	PBC038C11	FIGLA_A56-S132_Ec_bio	p28BIOH-LIC	12,370

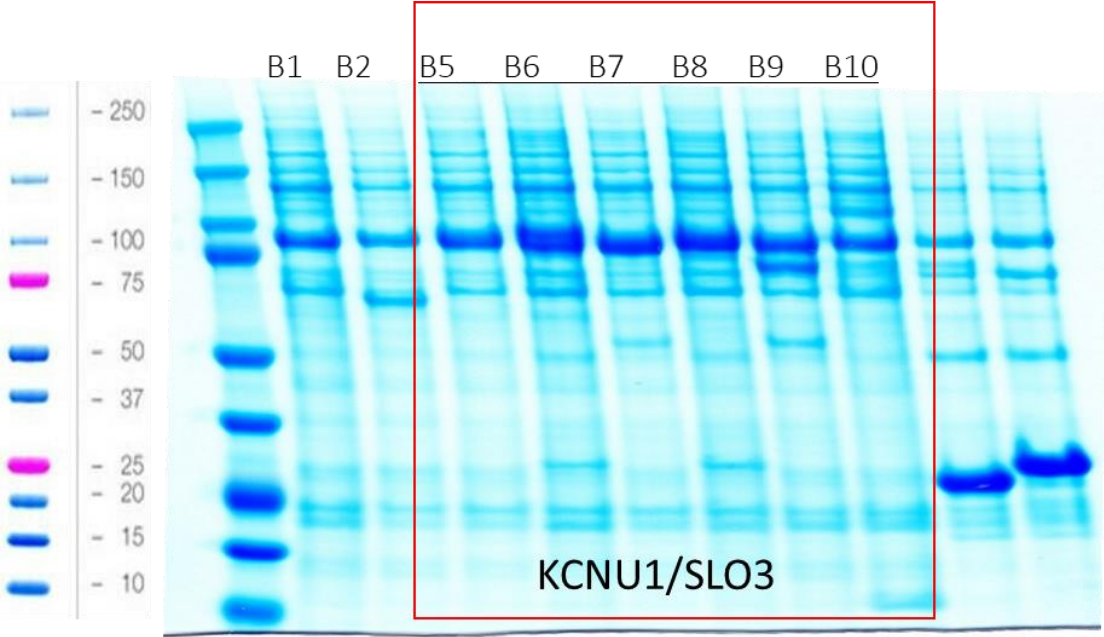
KCNU1/SLO3 - POTASSIUM CALCIUM-ACTIVATED CHANNEL SUBFAMILY U MEMBER 1

TestX in *sf9* insect cells
After Histag purification



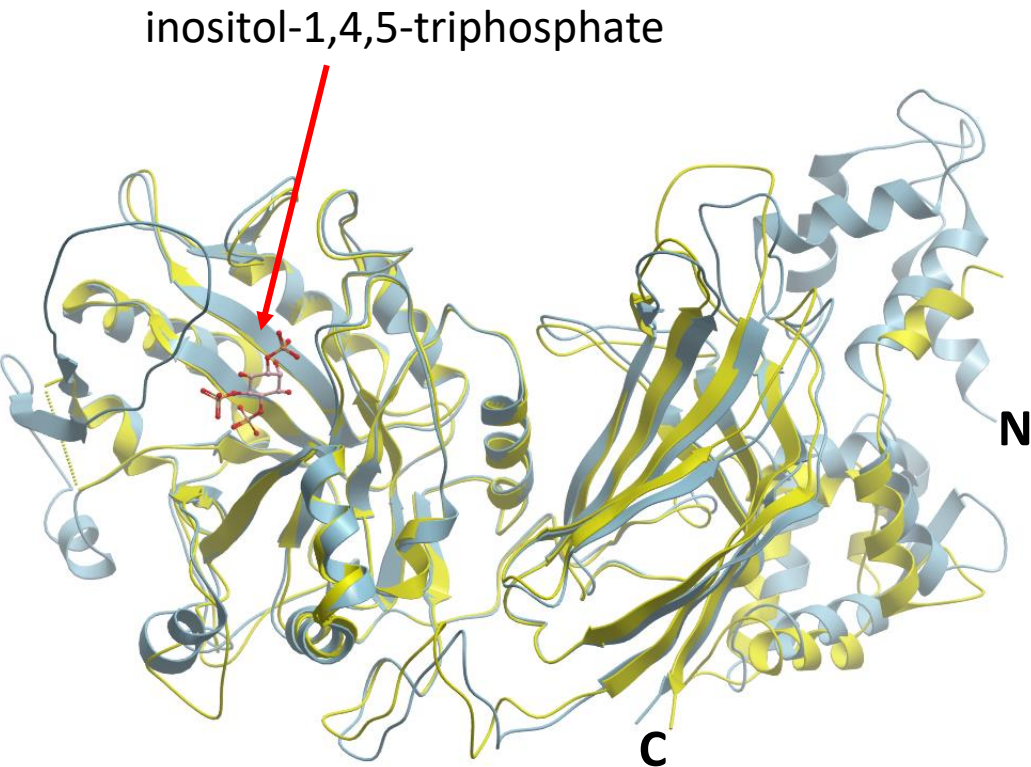
Transmembrane

cytoplasmic



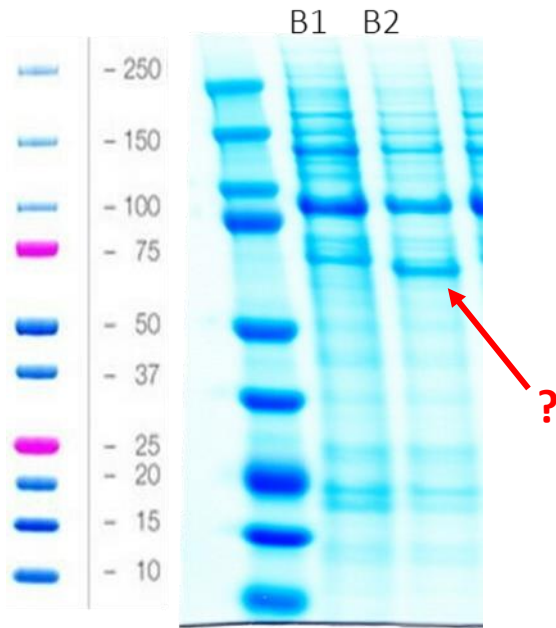
Well	Construct ID	Construct Name	Vector	MW
B5	PBC038B05	KCNU1_G330-S1062_BV_his	pFBOH-MHL	94118.52
B6	PBC038B06	KCNU1_G330-S1062_BV_bio	pFBD-BirA	95508.95
B7	PBC038B07	KCNU1_G330-S1062_LoopDel_BV_his	pFBOH-MHL	91987.41
B8	PBC038B08	KCNU1_G330-S1062_LoopDel_BV_bio	pFBD-BirA	93377.84
B9	PBC038B09	KCNU1_G330-S1062_LoopDel_BV_Chis	pFBOH-LIC-CHis	90819.16
B10	PBC038B10	KCNU1_G330-S1062_LoopDel_BV_sumo	pFBOH-SUMOstar-TEV	103373.1

PLC-zeta (UniProt: Q86YW0)



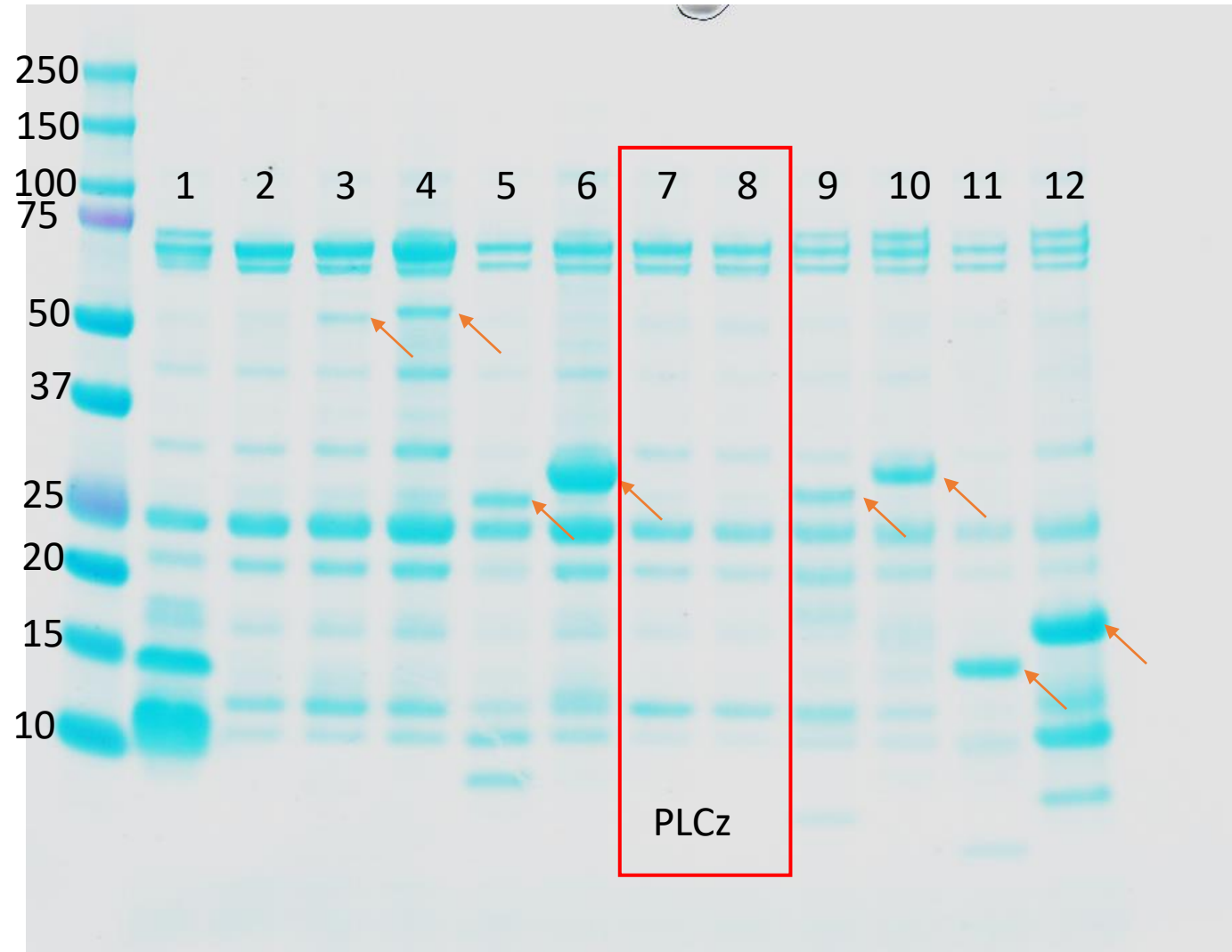
Overlay of predicted human PLC-zeta FL (cyan) and rat PLC-d1 (yellow) in complex with inositol-1,4,5-triphosphate (PDB ID: 1DJX).
P.S. Human PLC-zeta shares 42% sequence identity with *Rattus Norvegicus* PLC-d1.

TestX in *sf9* insect cells
After Histag purification



Well	Construct ID	Construct Name	Vector	MW
B01	PBC038-B01	PLCz_FL_BV_his	pFBOH-MHL	72604.77
B02	PBC038C-B02	PLCz_FL_BV_bio	pFBD-BirA	73995.2

Test X in *E. coli*



BL21-DE3-pRARE2
3 ml TB 18°C for 16hrs
Purified on Ni-NTA

Well	Construct ID	Construct Name	Vector	MW
1	PBC038A03	PATL2_FL_Ec_his	pET28-MHL	63,657
2	PBC038A04	PATL2_FL_Ec_bio	p28BIOH-LIC	65,048
3	PBC038A07	PATL2_L89-Y543_Ec_his	pET28-MHL	54,179
4	PBC038A08	PATL2_L89-Y543_Ec_bio	p28BIOH-LIC	55,570
5	PBC038A11	PATL2_G288-Y543_Ec_his	pET28-MHL	31,162
6	PBC038A12	PATL2_G288-Y543_Ec_bio	p28BIOH-LIC	32,552
7	PBC038B03	PLCz_FL_Ec_his	pET28-MHL	72,605
8	PBC038B04	PLCz_FL_Ec_bio	p28BIOH-LIC	73,995
9	PBC038B12	FIGLA_FL_Ec_his	pET28-MHL	26,317
10	PBC038C01	FIGLA_FL_Ec_bio	p28BIOH-LIC	26,626
11	PBC038C04	FIGLA_A23-S136_Ec_his	pET28-MHL	15,034
12	PBC038C05	FIGLA_A23-S136_Ec_bio	p28BIOH-LIC	15,343

ACKNOWLEDGEMENTS

Constructs cloning & E.Coli test expression:

Peter Loppnau

Aled Edwards
Cheryl Arrowsmith

Insect cells expression:

Alma Seitova & Ashley Hutchinson

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FUNDING PARTNERS

The Structural Genomics Consortium is a registered charity (no: 1097737) that receives funds from AbbVie, Bayer AG, Boehringer Ingelheim, Genentech, Genome Canada through Ontario Genomics Institute [OGI-196], the EU and EFPIA through the Innovative Medicines Initiative 2 Joint Undertaking [EubOPEN grant 875510], Janssen, Merck KGaA (aka EMD in Canada and US), Pfizer, Takeda and the Wellcome Trust [106169/ZZ14/Z].