Regulation of CDK1 and CDK2 by the “cyclin-like” protein Spy1.

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Why investigate Spy1?

- Cyclin-dependent protein kinases (CDK) are involved in regulating cell division and are themselves regulated by protein association (usually cyclins) and phosphorylation [1].
- Spy proteins bind and activate CDK1 and CDK2 suggesting a role in regulating CDK1/2 activity [2]. Mainly, Spy proteins are involved with meiotic cell division (production of haploid cells)[3,4].
- However, Spy proteins have been found to be overexpressed in several cancers[5].
- Neuronal cells require Spy1 for appropriate cell fate (i.e. whether the cells should proliferate or differentiate) and inappropriate regulation by Spy1 of this step contributed to the development of glioma (cancer of glial cells)[6]

Hypothesis:

That Spy1 represents an alternative class of CDK activators to cyclins that differ in:

(i) their affinity for CDK subunits

(ii) mechanism of activation

(iii) substrate specificity

Large scale expression of CDK2 (expressed to test interaction with Spy1):

- Transformed Rosetta Strain E.coli with pGEX vector encoding CDK2-civ1 (GST-tagged) and incubated 10ml overnight.
- Added 10ml to 1 litre of LB broth. Once optical density > 8.0, then induced protein expression with IPTG.
- Crack E.coli cells and purified CDK2 using GST binding resin.
- CDK2 was purified at ≅ 1.6mg/ml (15ml in total)

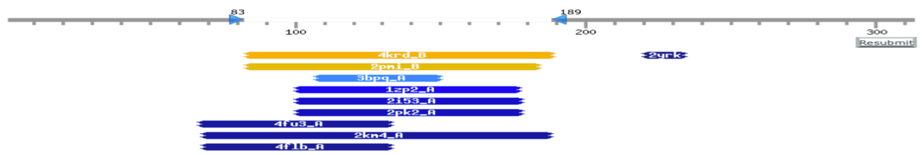

Figure 1. secondary structure prediction. Different constructs of Spy1 were decided upon using the Spy1 domain prediction.

Table 1:

Construct length (Residues)	Construct label.
1-206	1.2
1-313	1.3
27-189	1.4
27-206	1.5
27-313	1.6
64-189	1.7
64-206	1.8

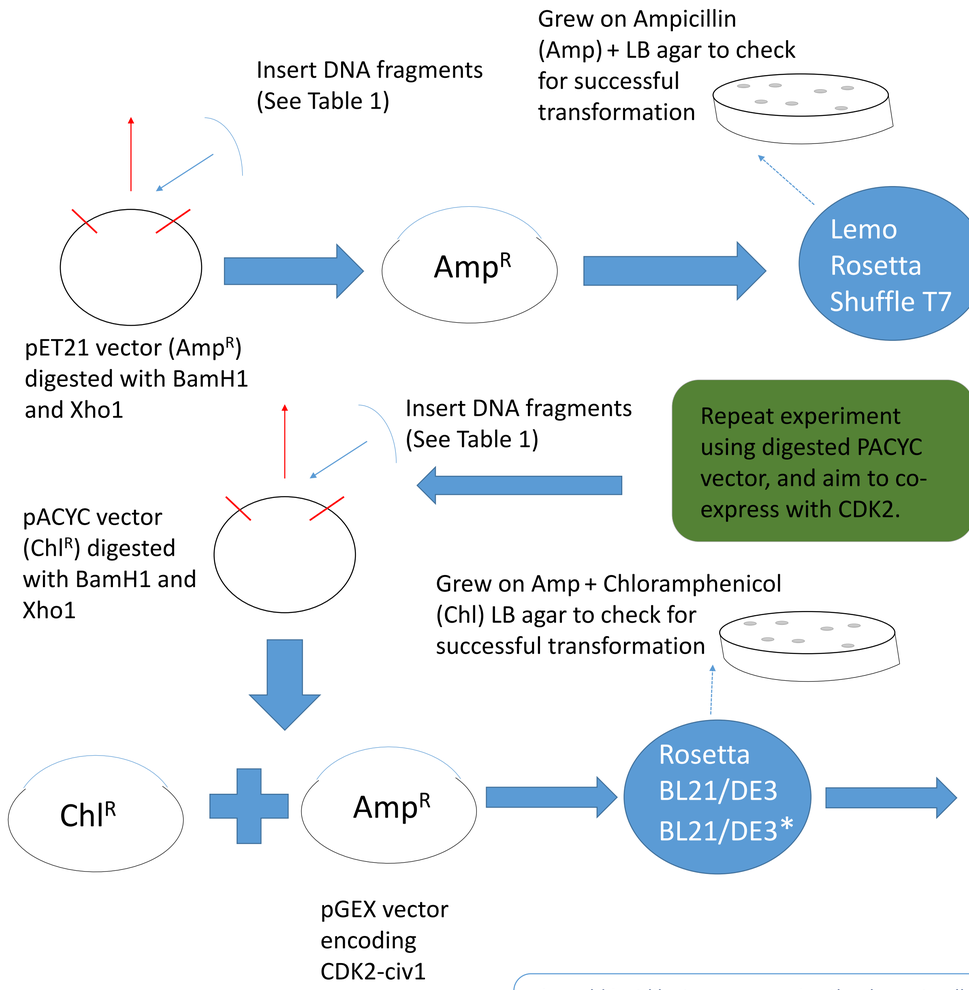

Figure 1

Figure 2. SDS-Page gel of pull downs showed Speedy protein expression was unsuccessful using the digested pET21 vector.

RESULT: After expression, another round of purification was under taken and the SDS-Page gels of the flow through and pull-downs showed successful expression and purification of CDK2 but no expression of the Spy1.8(64-206) construct. This proved that a soluble form of Spy1 can not be expressed in Rosetta strains, even when co-expressed with CDK2. Further research will look to express Spy1 in Insect cells.

I would just like to say a massive thank you to all the researchers at the NICR for all their support during my project, with a special thank you to Prof. Jane Endicott, Dr Mathew Martin and Svitlana Korolchuk. Also, thank you too Alice Bradbury for keeping me sane when Spy1 refused to express.

References: 1. Morgan, D.O. The cell cycle: principles of control, (Oxford University Press, 2007). **2.** Nebreda, A.R. Curr Opin Cell Biol 18, 192-8 (2006). **3.** Lenormand, JL. et al., EMBO J 18, 1869-77 (1999). **4.** Ferby, I., et al., Genes Dev 13, 2177-89 (1999) **5**. Golipour, A. et al. Cancer Res 68, 3591-600 (2008).**6**. Lubanska, D. et al. Cancer Cell 25, 64-76 (2014).