p422: Abl kinase domain truncations, 96-well expression testing 11/06/2013

Expressed and Ni-affinity purified Abl kinase fragments:

Ordered by start and end amino acid. Duplicates have been grouped. Concentrations in ng/μl as measured by Caliper microfluidic system. Total volume of each elute was 80 μl.

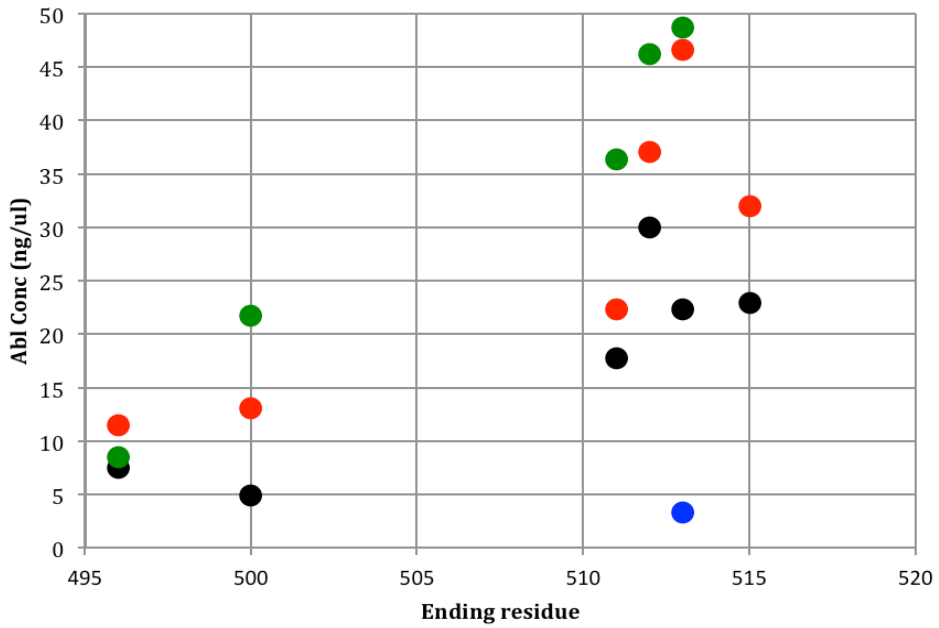
Well Label	AAstart	AAend	Abl Conc. (ng/μl)	YopH conc
A07	228	496	8	11
A08	228	500	5	6
A09	228	511	18	5
A10	228	512	30	5
A11	228	513	22	5
A12	228	515	23	0
B04	229	496	11	29
A01	229	500	9	9
B05	229	500	11	9
C12	229	500	19	23
D02	229	500	14	17
G10	229	500	14	13
H10	229	500	9	9
B06	229	511	22	7
A02	229	512	39	6
B07	229	512	23	4
D11	229	512	42	11
G02	229	512	42	7
H11	229	512	38	10
B08	229	513	47	10
A03	229	515	40	5
B09	229	515	22	5
D06	229	515	34	7
E10	229	515	30	4
F12	229	515	35	7
H12	229	515	31	5
C02	230	496	8	10
C03	230	500	22	10
C04	230	511	36	6
C05	230	512	46	10
C06	230	513	49	8
F11	241	513	3	20

Results for the duplicates show acceptable consistency.

YopH levels very quite dramatically from almost nothing (e.g. in A12 – in fact there is a band barely visible, but it is below the detection threshold used here [4-5 ng/µl]), to being at equal concentration to the kinase (e.g. C12).

For the best expressed truncations (e.g. 229-512, 229-513), YopH is present at about 25% the concentration of the Abl kinase domain.

Expression Levels.



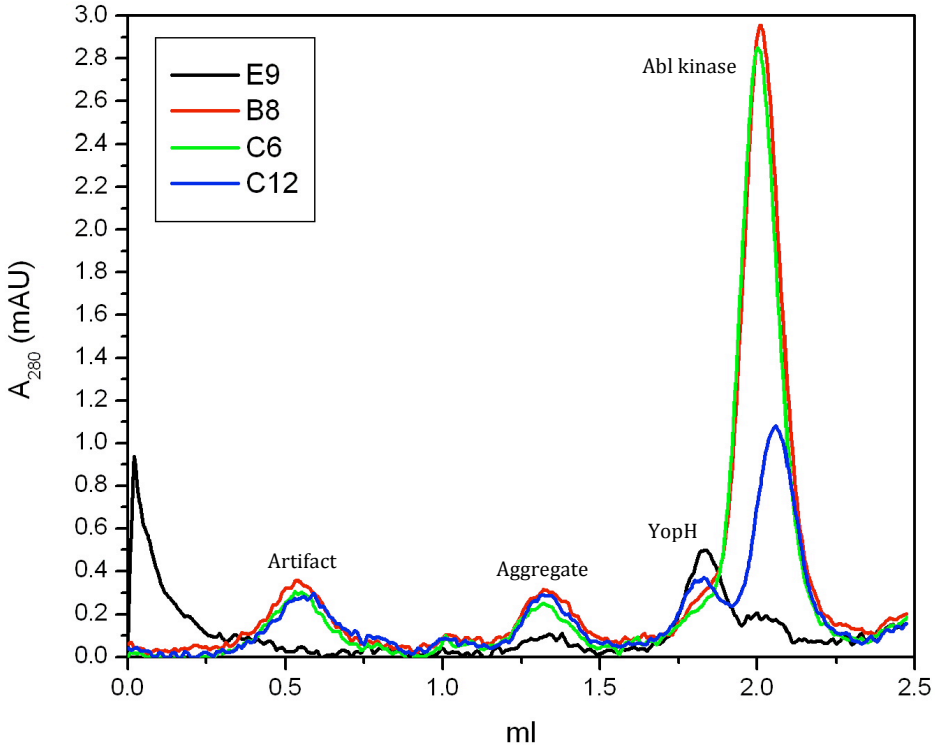
Colored by start residue: black = 228; red = 229; green = 230; blue = 241

229 or 230 to 512 or 513 are the best expressed, although the concentrations are not significantly higher than many of the other truncations.

Estimating the yield from a large scale purification is difficult based on this data. Typically we have found that a target protein should be detected by the Caliper chip at a concentration of at least 250 ng/µl in order to have confidence that a reasonable amount of protein will be purified on a large scale. We do not have any data to comfortably extrapolate from these low expression levels in 1 ml format to expression in 1 L flasks, but based upon some other experiments I performed, I would expect yields of 1 mg per L cells at best, and probably rather lower.

Size Exclusion Chromatography.

A 30 µl aliquot of each well expressed sample was run on a Superdex 200 5/150 GL column. Representative chromatograms are shown below. All Abl kinase domains were predominantly monomeric with small amounts of aggregate (of around 300 kDa). YopH was also detectable as a separate peak eluting a little earlier than the kinase. The peak at 0.5 ml is an artifact due to the presence of high imidazole concentrations in the samples.



E9 is YopH only, the Abl did not express. This was run as a control. Apparent molecular weight: 55 kDa.
B8 and C6 are two of the best expressed truncations. Apparent molecular weight: 32 kDa.
C12 showed heavy contamination with YopH (about 1:1 on Caliper). The YopH peak is smaller than that of the kinase domain as it has a much lower extinction coefficient.

Note that for B8, the peak represents about 0.8 µg protein. Pooling this peak would result in a protein concentration of 3 ng/µl. There would probably still be trace amounts of YopH present, as the peaks run very close together.

Recommendations

Repeating the expression testing using IPTG induction rather than autoinduction gave qualitatively similar results, but yields were even lower still. Since most of the expressed protein is insoluble, it is unlikely that we can improve on these yields.

A repeat expression of the D382N mutant showed that while this inactive mutant is indeed expressed better than the wild-type, the difference is not so dramatic as I had initially thought (in fact, the purifications I did back in 2011 may have been somewhat anomalous in that the yields were quite high).

Further purification to remove the YopH from the Ni-pulldowns is unlikely to be successful due to the very small amounts of kinase present. Even slight losses would dramatically reduce the yield, and protein concentrations would approach levels which will be difficult to detect.

Should you wish to have samples from either the Ni-pulldown or the size-exclusion peaks, it would be advisable for us to discuss this in detail first. That way I can be sure of what you need, and improve the chances of being able to provide you with useful samples. I have a duplicate 96-well expression block and can repeat the pulldown/size-exclusion chromatography on the best looking samples.

Because it is very hard to predict the yield upon scale-up of these samples, you may wish us to try a few large scale purifications first, to be sure that we can provide sufficient sample for your future needs. The anticipated yield of 1 mg per L cells is significantly (10x) lower than the reported expression of the wild-type kinase domain (see Seeliger et al. in *Protein Science* (2005) **14**, 3135-9).

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