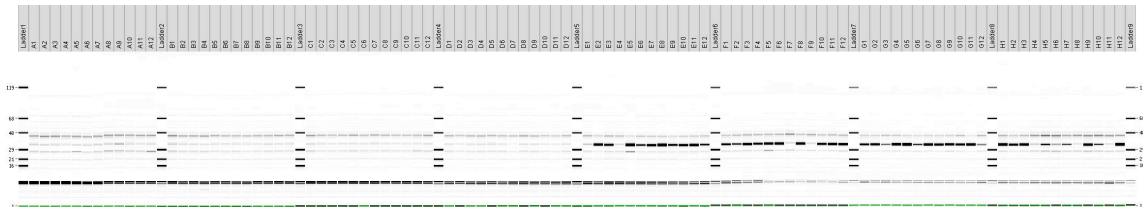


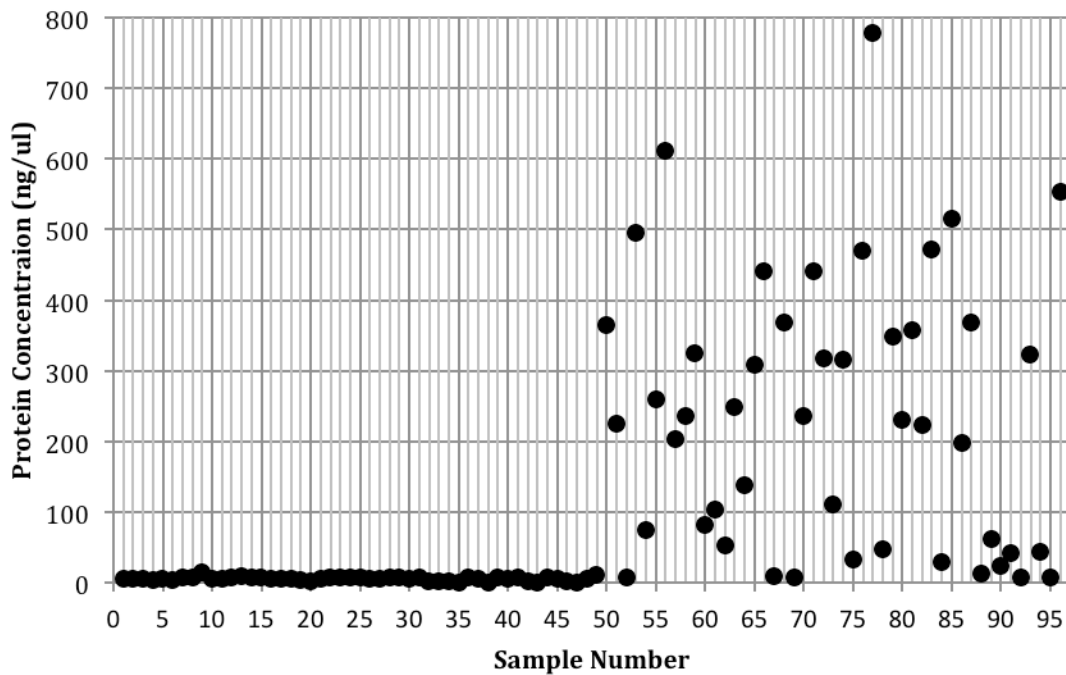
Rosetta2(DE3)pLysS cells were cotransformed with the kinase QC mutant and the truncated YopH phosphatase. Expression was in Magic Media at 16°C for 40 h, using 900 µl media per clone. Cells were pelleted and frozen at -80°C before performing the Ni pulldown. Cell pellets were thawed and lysed with BugBuster + lysonase mix, and clarified lysate was added to Ni-NTA resin. After incubation for 30 mins with mixing, the resin was washed three times with 25 mM HEPES pH 7.5, 400 mM NaCl, 5% glycerol, 20 mM imidazole, 1 mM BME. Bound protein was eluted with 80 µl 25 mM HEPES pH 7.5, 400 mM NaCl, 5% glycerol, 400 mM imidazole, 1 mM BME. A small aliquot of the eluted samples was analyzed on a Caliper microfluidic chip and the remainder frozen at -20°C.

## Results

*Caliper gel image: see file p614 pulldown\_Caliper.jpg*



*Target protein concentrations from Caliper analysis*

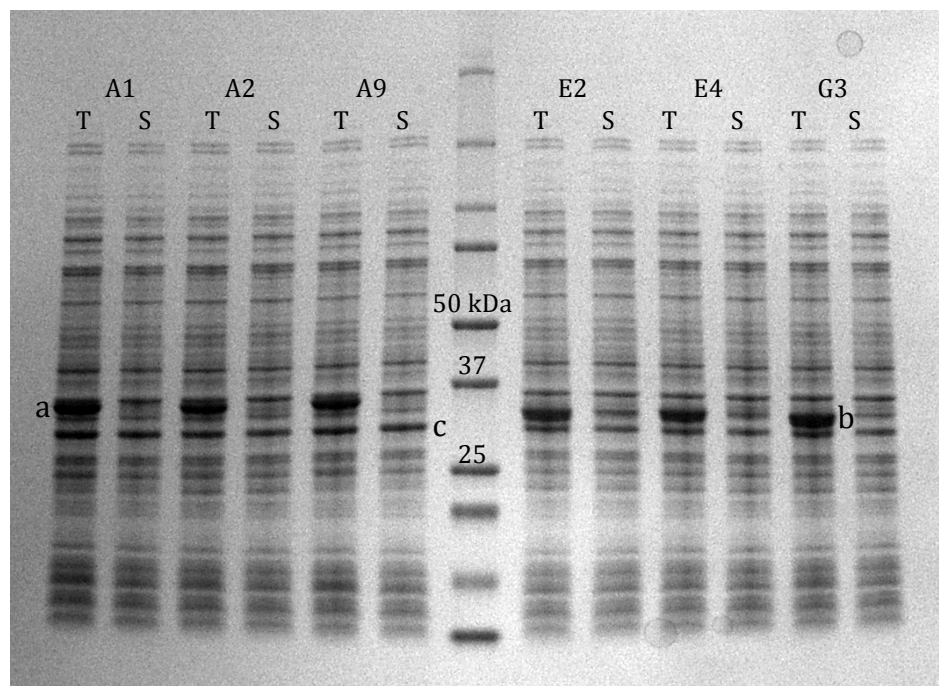


Samples 1-49 are Abl, 50-96 are Src. Samples 1 and 50 are the 'wild-type' versions of those proteins as used in previous work (p470).

Both the Abl and Src proteins were expected to be fairly low yield, based on the results of p470. It was therefore decided to use imidazole to elute the bound protein (rather than TEV protease as was used in p470) as this would maximize the yield. Nevertheless, all of the Abl proteins are basically undetectable. The Src proteins are generally well expressed, many of them at higher levels than the 'wild-type'.

Samples of the total and soluble cell lysates were run on SDS-PAGE to check for solubility. As expected, most of the expressed protein is insoluble. However, it is unclear why the Abl protein behaved so poorly here when soluble protein (albeit an extremely low level) was detected in previous work (p470).

*SDS-PAGE of selected cell lysates (T = total, S = soluble)*



A1 is 'wild-type' Abl; A2 showed nothing in elute; A9 may have Abl present at very low concentration in elute.

E2 is 'wild-type' Src; E4 showed nothing in elute; G3 showed low concentration in elute.

Labeled bands:

a = Abl

b = Src

c = YopH164