Electronic Supplementary Information for CC-COM-06-2013-044647

Amplification of phage stock and isolation of DNA

Liquid LB (5 ml) was inoculated with an overnight stock of BLT5615 *E.coli* (100 μl) and incubated at 37 ^{o}C with shaking at 120 rpm for 90 min to reach exponential growth phase. IPTG (100 μl of 100 μM solution) was added and the solution incubated for a further 45 min. A sample of the purified phage clone (2 μl) was added to the bacteria and the culture returned to incubate at 37 ^{o}C with shaking at 120 rpm for a further 3 h. The culture was subjected to centrifuge at 12000 rpm for 12 min, and the supernatant collected. A sample of supernatant was subjected to PCR and analysed by gel chromatography to confirm the presence of the correct phage clone by the identification of a DNA band approximately 800 base pairs long. The PCR product was cleaned up using the Promega Wizard SV PCR Clean-up system under the published protocol. UV analysis of the purified product at 260 and 280 nm revealed 47 ng/μl DNA to be present.

Isolation of DNA insert from phage vector and addition of restriction sites

Primers used:

DownStream: CGCTGTTTAAACTTATTTATTTAGATATCGCGTATGCATATCGTAGGCATCATC

UpStream Frame 1: TCTCGCGATCGCCGTCTTCTCTGTGTTCTCCCAG

UpStream Frame 2: TCTCGCGATCGCCTCTTCTCTGTGTTCTCCCAG

UpStream Frame 3: TCTCGCGATCGCCCTTCTCTGTGTTCTCCCAG

Restriction sites were added to the DNA with SgfI added by the UpStream primers, and Pme1, NSiI and EcoRV being added by the DownStream primer. PCR of the purified DNA with a combination of the DownStream primer combined individually with each of the UpStream primers produced three DNA samples labelled Frame 1, Frame 2 and Frame 3, which each contained the desired restriction sites and the DNA insert from the phage in each available expression frame.

The success of the PCR reaction was confirmed by the presence of a 400 base pair DNA band in a gel electrophoresis, and the PCR products clean-up using the Promega Wizard SV PCR Clean up kit as above. UV analysis at 260 and 280 nm demonstrated the clean PCR products to contain 61 ng/μl, 65 ng/μl and 52 ng/μl for Frame 1, Frame 2 and Frame 3 samples respectively.

Plasmid formation and insertion

The Promega pFN2A(GST) Flexi Vector Kit was used to generate plasmids containing the desired DNA fused to a GST tag, following the standard procedures, such that after expression the GST fusion protein will be attached to the protein of interest in each frame of expression.

JM109 competent cells were transfected with the plasmid by heat shocking to 42 ^{o}C for 45 s, and then placing in an ice bath. SOC media was added to each sample and the samples were incubated

at 37 °C with shaking at 120 rpm for 90 min. A streak plate from each sample was prepared on individual LB agar plates and incubated overnight. From each plate one colony was selected and inoculated into an individual flask of 50 ml LB media and incubated overnight at 37 °C with shaking at 120 rpm. 1 ml of overnight stock was used immediately in the expression of fusion proteins step below, and with the remainder, multiple 1 ml glycerol stocks of each sample were prepared by mixing 250 µl 80% glycerol solution with 750 ul JM109 culture and stored at -80 °C.

Colonies were analysed to confirm the presence of the correct plasmid by PCR using the corresponding earlier primers and DNA bands of 400 base pairs were detected by Gel Electrophoresis.

Expression of Fusion Proteins

For each frame of expression the corresponding JM109 overnight stock (1 ml) was diluted with fresh LB media (500 ml) and incubated at 37 °C with shaking at 120 rpm for 3 h. Magnesium sulfate solution (2.5M, 0.4 ml) was added and the flasks swirled briefly to mix. CE6 bacteriophage (10 ml at 3 x 10^8 pfu/ml) was added to promote the protein expression. The flasks were incubated at 37 °C without shaking for 5 min and then shaking at 120 rpm for a further 3 h. After centrifugation at 4000 rpm for 10 min the supernatant was discarded and the pellet collected. Protease inhibitor cocktail (10 µl) was added to each pellet and the pellets flicked gently to mix. The cells were then lysed by 5 x 30 s pulses of sonication with 30 s rest periods between pulses. The lysates were centrifuged at 12000 rpm for 15 min, the pellets were discarded and the supernatant retained. Presence of protein at the correct size to correspond to the protein of interest fused to the GST tag was confimed by SDS PAGE gel with silver staining of the crude lysates. Frame 1 demonstrated the presence of increased protein at 41 kDa, Frame 2 at 34 kDa and Frame 3 at 36 kDa corresponding to the desired proteins (sequences shown below).

Figure S1. Protein sequences (GST tag shaded in grey, approximate weight indicated in brackets)

Frame 1:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADK
HNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFM
LYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSGGGGGENLYF
QAIAVFSVFSQKQIAEFKEAFQLMDADKDGIIGKNDLRAAFDSVGKIANDKELDAMLGEASGPINFTQLLTLFANR
MATSGANDEDEVVIAAFKTFDNDGLIDGDKFREMLMNFGDKFTMKEVDDAYDMHTRYLN (41.5 kDa)

Frame 2:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADK
HNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFM
LYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSGGGGGENLYF
QAIAVFSCSPRSRSPSSRRPSNSWMPTRTVLLARTICALPSTPSARSPTTRSWTPCWARPRVRSTSPSC (34 kDa)

Frame 3:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADK HNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFM LYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSGGGGGENLYF QAIALLCVLPEADRRVQGGLPTHGCRQGRYYWQERSARCLRLRRQDRQRQGVGRHAGRGLGSDQLHPVADPVR QPHGHLRCQR (36 kDa)

Binding Assessment by ELISA

Flecainide acetate was immobilized on 5 x Magic Tag "MT1" strips in wells A – D with wells E – H receiving buffer solution only during the immobilization process as 'no drug' control wells.

100 µl of Frame 1 lysate was added to each well of strip 1, 100 µl Frame 2 lysate to strip 2, 100 µl Frame 3 lysate to strip 3, and 100 µl PBS was added to each well in strips 4 and 5 and the plate incubated at room temperature for 1 hour.

The plate was washed 6 times with 0.05% Tween 20 in PBS, and then strips 1 – 4 were treated with Rabbit Anti-GST peroxidase. Strip 5 was treated with PBS to provide a 'no antibody' control. The plate was incubated at room temperature for 1 hour and then washed 8 times with 0.05% Tween 20 in PBS.

100 µl TMB ELISA substrate solution was added to all wells, and the plate incubated at room temperature for 10 min. The ELISA reaction was stopped by the addition of sulfuric acid (0.5M, 100 µl) to each well and the absorbance read at 450 nm, to give the readings below. Using the one-tailed Mann-Whitney statistical test, there is a statistically significant increase in the amount of antibody bound in the wells representing the Frame 2 expression fusion protein exposed to flecainide compared to no drug. This corresponds to the phage displaying the protein: VFSCSPRSRSPSSRRPSNSWMPTRTVLLARTICALPSTPSARSPTTRSWTPCWARPRVRSTSPSC during the binding event seen in the screening experiment.

There is no statistical difference between the flecainide and 'no drug' wells when exposed to either Frame 1 or Frame 2 expression proteins.

Table S1. Absorbance data (450 nm, arbitrary units)

	Strip 1 Frame 1		**Strip 2 Frame 2**		**Strip 3 Frame 3**		**Strip 4 Control 1**		**Strip 5 Control 2**	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
	0.394	0.441	0.818	0.425	0.346	0.344	2.815	2.713	0.052	0.069
	0.486	0.422	0.644	0.425	0.298	0.335	2.952	2.83	0.054	0.052
	0.456	0.496	0.596	0.417	0.314	0.291	2.866	2.867	0.054	0.049
	0.464	0.395	0.603	0.476	0.346	0.252	2.629	2.401	0.061	0.054
mean	0.450	0.439	0.665	0.436	0.326	0.306	2.816	2.703	0.055	0.056
Standard Error	0.020	0.021	0.052	0.014	0.012	0.021	0.068	0.106	0.002	0.004
CV	8.76	9.74	15.63	6.22	7.36	13.92	4.85	7.83	7.14	15.91
Mann-Whitney (one tailed)	0.44		0.015		0.15		0.33		0.38	

Figure S2. Competitive elution of clones using free flecainide in buffer vs. drug free buffer, according to our published procedure.[12]