Edinburgh, 20 May 2010

Dear Kevin,

here are a few plasmids and strains that I've generated over the last years, that I think might be useful to other people. I've tried to put all the relevant information into the deposition forms and double checked each of them. It should all be correct, but please have a careful look through and let me know if something is missing or in case it is not self-explanatory.

The plasmids:

pAL1 is pBARGRG1 expressing sGFP from pMF272. I've included this as it was the basic host vector for all the other ones I've made. I also got it completely sequenced at the beginning of the project, as until that date no full sequence of pBARGRG1 was available. Thus it might be a good idea to add a link from pBARGRG1 to pAL1 to access it.

pAL2-Lifeact contains tdTomato as fluorescent protein. Although we had problems with artefacts using that probe – included this comment on the deposition form – I've included it as there is no tdTomato vector in the plasmid list yet, and it still might be useful for somebody.

pAL3-Lifeact and pAL5-Lifeact contain stabilized versions of TagRFP and TagRFP-T (10x more photostable than TagRFP). I've send them out to 13 different labs already and requests still keep coming in. TagRFP-T is the 'best' monomeric RFP at the moment.

pAL4-Lifeact is like pAL3-Lifeact in terms of FP construct expression, but instead of the *bar* gene (Ignite) uses the *nat1* gene from pD-Nat1 (Nourseothricin) as selection marker. Works like a treat in *Neurospora*.

The strains:

Obviously *N. crassa* strains transformed with the above plasmids. All are randomly integrated in the genome and not targeted. I used this approach to transform a number of KO mutants in the quickest way possible. I've send you the strains that I've been routinely working with and so far performed well and as expected. All of the Lifeact strains I've also send out to numerous labs and got no complaints so far. However, I've got more clones of each and can send alternative ones in case people experience problems. Only the strains published in our recent EukCell paper have been verified by Southern blotting (NCAL004-4, NCAL005-2 and NCAL006-5); the is data available in the supplementary material.