Team meeting

Who: Wet Lab Team

Description:

- Interlab study
- Add validated new part and characterise it
- Calibration/human practices
- Prove system works
- improving previous part and coming up with new part.
- Experiments to prove it folds correctly and describe what it does.
- Look at other wikis of other teams etc. From get go plan what experiments we are going to do.
- Wiki – description of what you have done.
- Lab book !!! Particular format, after everyday you take the lab book and copy notes onto ur laptop. Then upload it. Results of that day online instantly.
- Part not been in the database before, characterise it.
- Same sequence but does something better – from things already on the database.
- igem.org – database full of protein sequences. Varying levels of characterisation. Characterisation terrible (according to Mog).
- Catalogue –> browse by type –> coding sequences (variety of protein). Decide how you describe what you are doing (enzyme eg).
- Reporters (YFP, 10 000 variants of each) – don't want new part as reporter.
- Improvement of reporter is a good shout.
- Antibody attached to GFP. Not in database already. Figure out how to characterise it. (structural study would b cool).
- Post-translational modification enzymes. Don't have glycosyl transferase (unlikely to work – fun to ask tracy to help us synthesise glycosyl transferase).
- Maggi – researched Pfizer. (look at application programs). Ask for sponsorship (give us ex lab supplies eg.).
- Emailing about various categories of parts. Protein domain (most logical for crystallisable fragment of the antibody). Someone's done an antibody fragment before (from 2008) but didn't publish it.
- Antibody fragment – under miscellaneous protein domain. Not the domain we would use but someone's done it so tells us we are on the right track.
- Wild type antibody fragment as our new part. Way to characterise it, conjugate it to a GFP, see that it folds properly. Structural study? Cost ? Shows us someone else has done it before which is excellent.
- Then we can try for a glycosyl transferase to do something fancy. Massive QUESTION MARK.

- Catalogue – improve a part. Doesn't have to be a protein. It can be a terminator, promoter, ribosome binding site. Easiest to improve a protein as easiest to measure.
- Protein coding sequences -> Reports (biggest category + most likely to use) -> post translational modifier -> selection marker (maybe? – would be an improvement part).
- Another sequence but does it better than the original sequence.
- Lastly year – mOrange fluorescent protein ?? and morange 2?
- Potentially could do the morange thing as no one ended up doing it.
- Move forward – improve
- Two groups working in parallel. One working with characterising each part that we make and other, which carries out the experiment that is determined by modellers.
- Use enzyme to catalyse isopeptide bond formation. (transglutamase (?) - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4307611/)
- How much there is – a curve.
- mOrange at the beginning this time. Relate to project – mOrange as reporter.
- Get antibody fragment into database and second part, transferase.
- Biochemical society (Cam is a member). – undergraduate student bursary. Money from BBRSC can go to science. RSC pay for 8 weeks and not 10, only give money if they are the only one giving the money. – One person works in modelling for a week and the rest of the time work in wet lab.
- Biochemical society – this Friday due.
- Marburg (in Germany) – find wiki. Overgraduate team that won.
- UC San Diego (undergrad team that did well)
- Two basic constructs and two improved. If they don't like one, they might like the other.
- Start of one of everything and then move into more advanced things, the further along we are.
- Made ladder and made sure the digestions were the right size (?). Recombinant protein expression. Fast liquid protein chromatography.
- Protein chromatography, nickel column, fluorescence assay, standard curve to make sure that fluorescence matches to an actual quantity. Lots of money – NMR.
- Backbone (easy) but side chains (need a pHd student – or ask mog's bio guy ).
- Standard curve for a reporter and tag with our fragment. Come up with quantity that we have produced. Folded correctly – glowing. Do a nickel column, histag (?? to antibody and not the folded part (glowing part). Know they haven't separated.
- Getting things to transform, express and characterise.
- Backbone that didn't transform – don't use it.

- Their backbone is rubbish, all of our testing+analysis, another backbone. Ligate our construct into their backbone (without RBS, no promoter, terminator). Do when we have finished characterising it.
- Take next week to familiarise ourselves with database (parts registry). Look at it, under reporters and other categories. Pick your favourite (the winners from last few years and what they did to characterise their improved+new parts). How that can be applied to our parts.