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**EastBio IBB training meeting 1**

**26th of October 2015**

**Tutorial summary**

Advantages of using E.coli- growth, high cell density easily achieved, media not expensive, transformation is easy

Host choice- E.coli

Plasmid choice- *note:* replicon, promoter, selection marker, affinity tags + tag removal

**Exercise:**

Form two groups (2-3 students per group)

***Based on the tutorial paper and the two afternoon talks:***

Group one - ***Suggest strategies for overexpression of a protein that forms inclusion bodies***

Group two- ***Suggest strategies for overexpression of a protein which is toxic to the host cell***

Have a joint discussion explaining decided strategies

**Group one: Paul, Dainius, Efrain:**

Proposed a system (host choice E.coli) where they would use a generic plasmid, under the control of inducible promoter, with an antibiotic resistance marker. For protein purification they prosed the use of a SUMO- tag.

**Group two: Ara and Sally**

Proposed two systems:

1. If the target of interest is toxic before induction: High copy number system T7/lac in a pLys/LysE plasmid in an E. coli strain (C41 bearing strain). With a plan for tight control over the plasmids promoter in low glucose media with added tryptone/or peptone.
2. If the target of interest is toxic after induction: Low copy number system with again tunable promoter in a E.coli host.
3. In both cases for protein purification they proposed the usage of a peptite tag, bond c-terminal to methionine to be removed with Cyanogen bromide.

In the discussion afterward it was suggested that:

*For group 1*: Their plan was good and achievable, but the usage of Sumo tag restricts the protein of interest to be a secreted protein.

*For group 2*: Their plan was also reasonable, good and achievable, but perhaps it is safer to use low copy number systems in both cases. They also would have to make sure that their protein of interest does not have any other methionine apart from the one that they target in their tag removal protein purification process.