**PROJECT PROPOSITION - Lab1** (M1, second semester)

Supervisor(s): Guillaume Cambray and Luca Ciandrini

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Hosting lab: CBS

Period of proposed project (put **x** instead of ロ) :

ロ Only 1st slot ロ Only 2nd slot

ロ One slot, but I have no preference on which X Both slots (with different groups)

1st slot: thursdays and fridays, from 3/2/2021 to 18/3/2021

2nd slot: thursdays and fridays (except for the last 2 weeks), from 31/3/2021 to 6/5/2021

Impact of ribosome recycling on translation

Subject (5 lines max for the description)

Ribosomes are costly and their efficient use conditions protein production levels and cell fitness. We will investigate the impact of favoring remobilization of ribosomes on a reporter transcript by two innovative approaches: 1) by leveraging an engineered system that enable transcript circularization; 2) by recursively cloning extra segments in a coding sequence to study potential impact of gene length on transcript looping.

Technical tools to be used:

* Molecular cloning to construct reporter variants
* RT-qPCR to determine transcript stability
* plate reader and flow cytometer to determine fluorescence levels.

Objectives:

* Understanding (and eventually adapting/extending) available models for ribosome recycling: [Fernandes et al. (2017)](https://www.nature.com/articles/s41598-017-17618-1); [Fernandes et al. (2019)](https://journals.aps.org/pre/abstract/10.1103/PhysRevE.99.052409)
* Determine experimentally whether physically bringing the end of a coding sequence close to the start of that same coding sequencing favors local re-utilization of ribosome, improves protein production and differentially impact cell growth.

*Molecular constructions:*

* Clone different reporter constructs in which Permuted Intron-Exon system flank a GFP coding sequence to catalyze the formation of circular reporter transcripts. Constructs will be designed to vary the proximity of the start and end of the reporter gene in the circularized molecule. In one version the start and end of translation will be made to overlap to favor translational coupling.
* Repeatedly clone the same chunk of coding sequence between two reporter genes to increase the length of the produced transcript and favor RNA looping.

*Upon successful cloning:*

* Perform RT-qPCR to quantify the efficiency of circularization and transcript stability.
* Measure fluorescence level and growth rate for all constructs produced.
* Analyze data to determine the impact of the different design on translation.