# Promoter and UTR screening in *Escherichia coli*

Adrià Sogues Castrejon, Degree in Genetics. Universitat Autònoma de Barcelona (UAB)

UAB Universitat Autònoma de Barcelona

## Antecedents

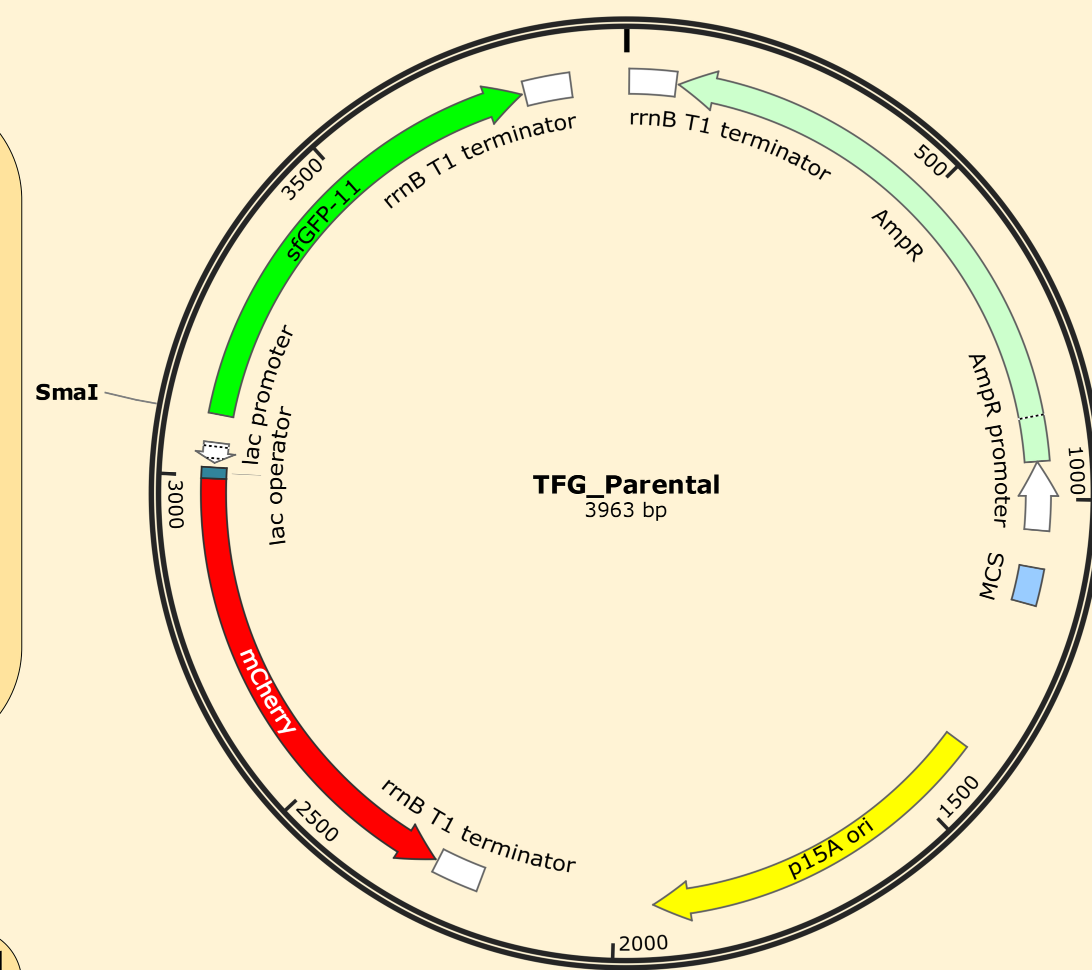
**Several studies use the florescence expression strategy in order to evaluate regulatory elements:**
02/**2013** *M. Church*; construction library combining endogenous *E. coli* promoters with endogenous Ribosome Binding Sites (RBS). Synthesized 12,563 combinations [1].
04/**2013** *Ki Jung J*eeong; in *Corynebacterium glutamicum* the 70 bp promoter region and 5' UTR were fully randomized except the RBS[2].
07/**2014** *L. Wang*; randomized 6 bases upstream ATG and 2 bases downstream of GFP gene in eukaryote cells. This strategy was employed to determine the efficiency of start codon recognition for all possible translation initiation sites (TIS) utilizing AUG start codons[3].

## General aims

The principal aim is **the construction of two libraries**: First to evaluate the promoter region and second to evaluate the UTR region.

This work proposes a **methodology able to assess** the whole universe of promoter and UTR sequences without restriction enzyme site scar.

Finally, the size of the characterized library also provides a resource for researchers seeking to achieve **particular expression levels.**




**Backbone plasmid**: with SmaI restriction site for cloning process, two florescent proteins: sfGFP as a reporter and mCherry as a control. Ori p15A has a low-medium copy number (≈15). Ampicillin resistance. (AmpR) .

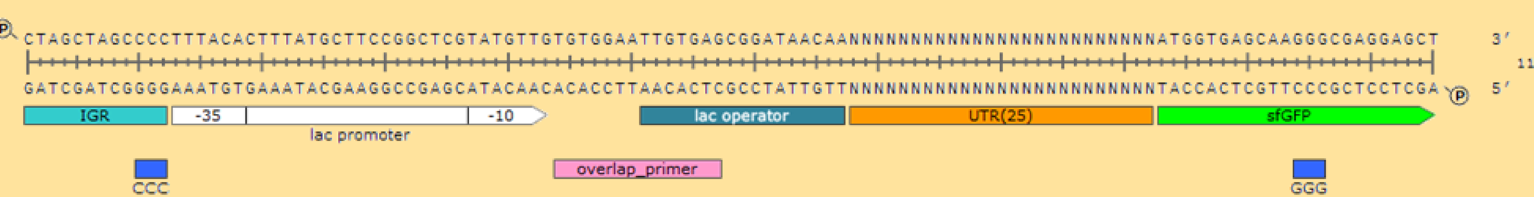
## Introduction

• *E. coli* plays an important role in modern biological engineering and industrial microbiology due to ease of manipulation and their physiological requirements. *E. coli* is considered **the prokaryote model organism**, for this reason E. coli was one of the first organisms to have its genome sequenced.
• Promoter and untranslated region (UTR) are relevant regulatory elements in prokaryotes. Promoter is involved in transcription whereas the UTR has a role in translation; however, in some genes UTR also has a control over transcription[4].

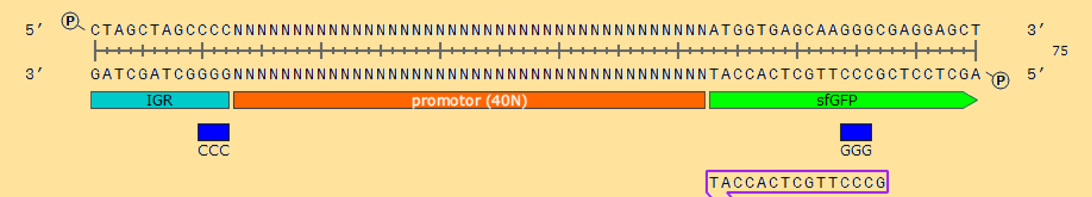
## Material and Methods

**Strain:** *NEB 5-alpha F'I[q] Competent E. coli (High Efficiency).*

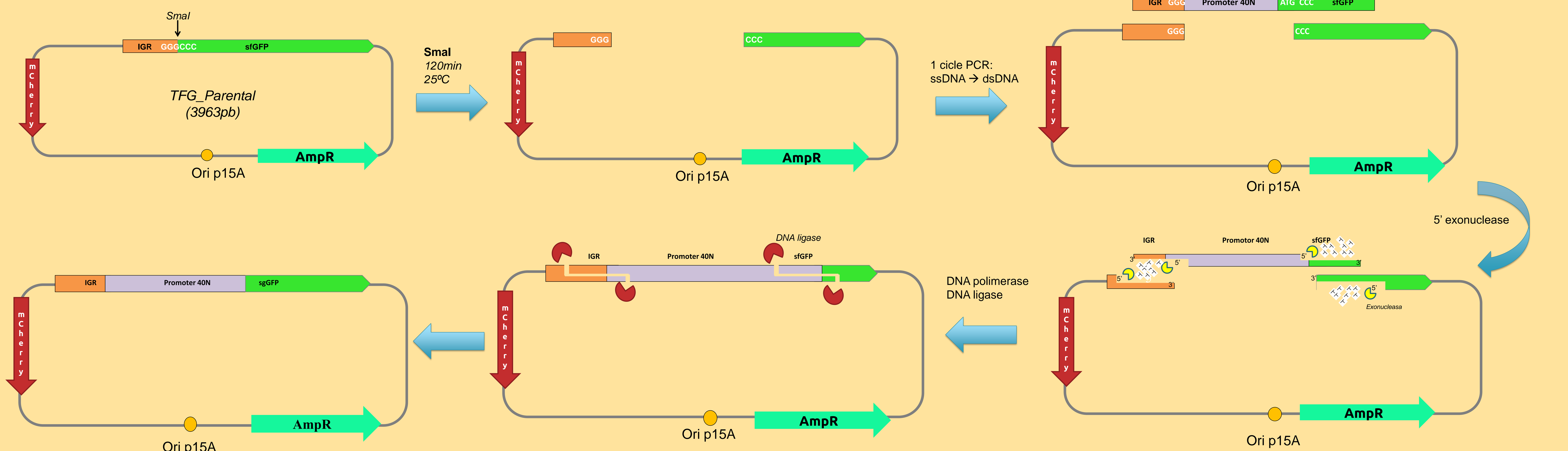
**Fragment** UTR:



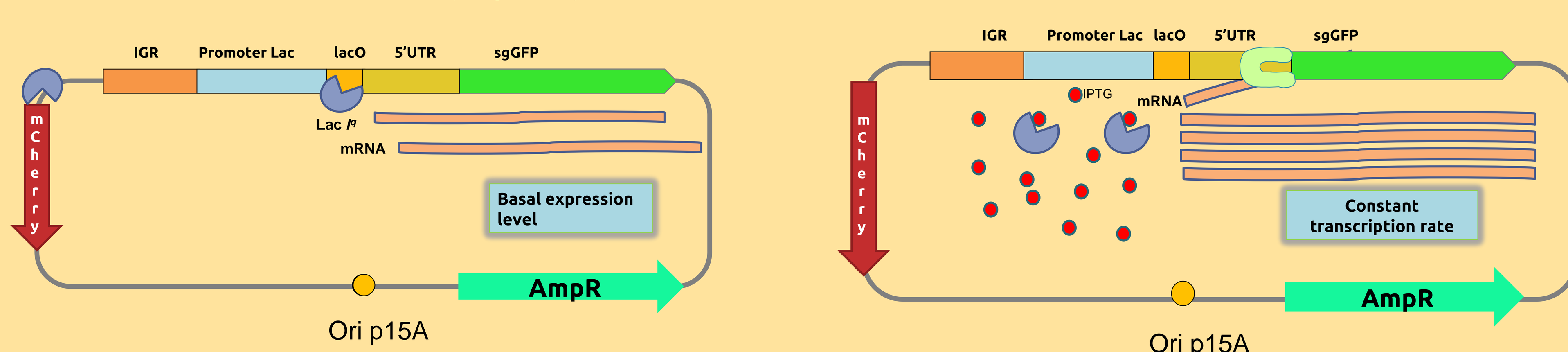

Promoter:



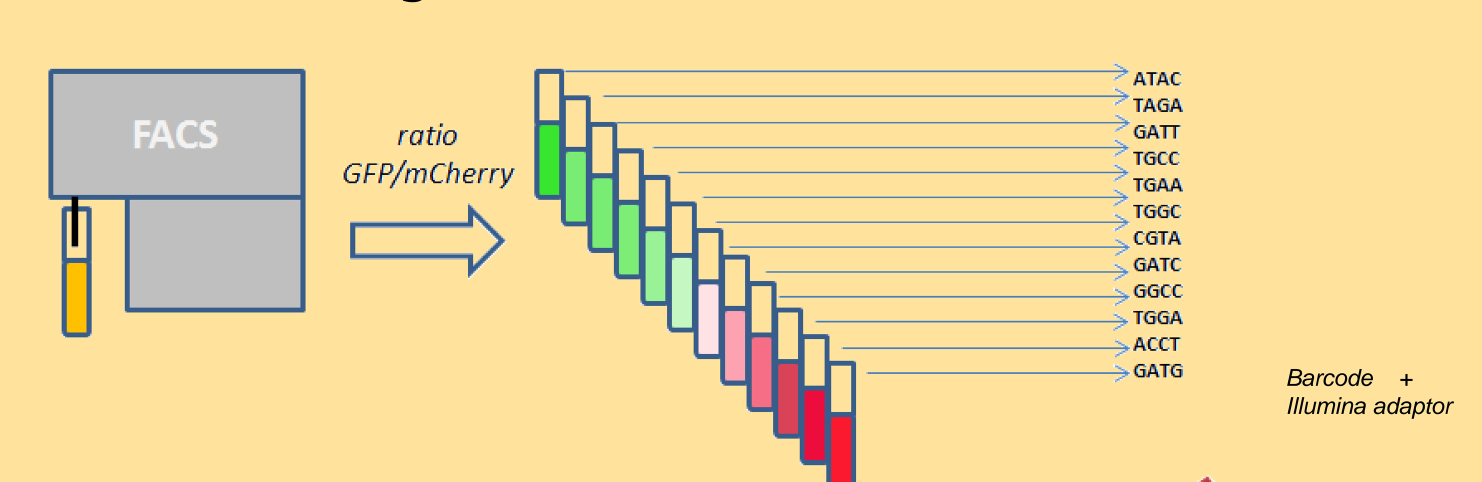

**Cloning process:** ***Gibson assembly***

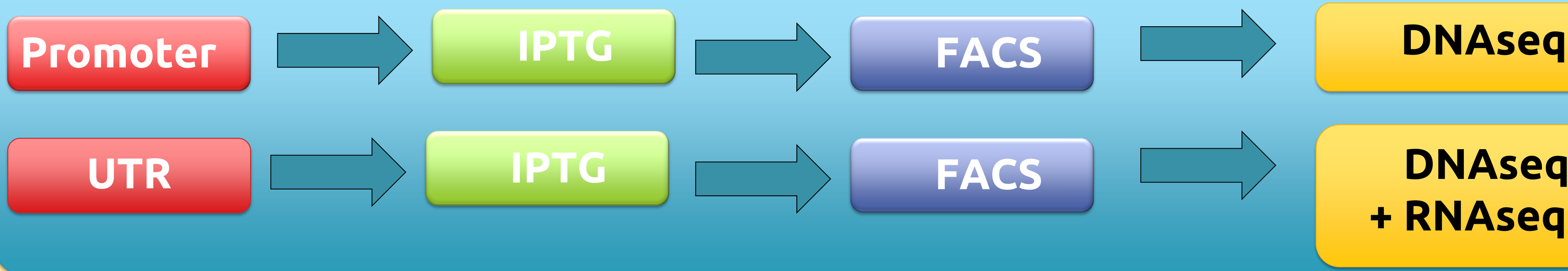



**Inducciton:** ***IPTG*** (in plate)




**FACS sorting:**




IGR | Promoter 40N | sgGFP

Sequencing by Illumina HiSeq®2500 illumina

## Expected results and validation

1. Alternative transcription start site (inside UTR)
2. High expression level: optimum -35 and -10 regions for promoter and *Shine Dalgarno* sequence in UTR
3. Secondary structure presented in 5' UTR could play a role in RNA stability, increasing its half-life
4. Delete from the analysis all the constructs with ATG out of frame, indel mutation in promoter or UTR region and nonsense mutation in sfGFP
5. Normalize RNAseq data with DNA copies →RNA copies / DNA copies

Western Blot and qRT-PCR from several constructs in order to validate some results
Ribosome profiling[5] and Structurome[6] in order to complement the analysis

## References

**Estimated budget: 14,780.05€**
**Estimated time: 14 months**

1. Kosuri, S. *et al.* Composability of regulatory sequences controlling transcription and translation in Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 14024–14029 (2013).
2. Yim, S. S., An, S. J., Kang, M., Lee, J. & Jeong, K. J. Isolation of fully synthetic promoters for high-level gene expression in Corynebacterium glutamicum. *Biotechnol. Bioeng.* **110,** 2959–2969 (2013).
3. Noderer, W. L. *et al.* Quantitative analysis of mammalian translation initiation sites by FACS-seq. *Mol. Syst. Biol.* **10,** 748 (2014).
4. Holmqvist, E., Reimegård, J. & Wagner, E. G. H. Massive functional mapping of a 5'-UTR by saturation mutagenesis, phenotypic sorting and deep sequencing. *Nucleic Acids Res.* **41,** e122 (2013).
5. Ingolia, N. T., Ghaemmaghami, S., Newman, J. R. S. & Weissman, J. S. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* **324,** 218–223 (2009).
6. Loughrey, D., Watters, K. E., Settle, A. H. & Lucks, J. B. SHAPE-Seq 2.0: systematic optimization and extension of high-throughput chemical probing of RNA secondary structure with next generation sequencing. *Nucleic Acids Res.* **42,** (2014).