CAENORHABDITIS ELEGANS BIOGRAPHY

During our adventure through space and time we landed on the **Elegans** planet where we met its inhabitants the *C. elegans*. This cute crawling creatures really caught our attention. Their singularity, biological and physiological caracteristics pushed us to create a *C. elegans* guide.

In this guide we propose a thorough description of *C. elegans*. To allow the use of our data, we will present the role of *C. elegans* both within research and iGEM competition. We are also proposing you all the data we could harvest about promoters, genes and protocols associated with the *C. elegans* manipulation into a laboratory.

I) WHO IS C. ELEGANS

Our iGEM team has decided to work on *C. elegans*. on the grounds of the numerous advantages it presents regarding biological research.

This little worm is indeed easy to handle and suitable for microscope observation as it is 1 millimiter tall and transparent. Moreover this organism presents a short development cycle (approximatively 3 days) and a lifetime of three weeks. This is a great advantage that enables us to obtain a lot of individuals in a short time and assure the reproducibility of our experiments.

Despite its short size and its shape, this worm has many common points with our species. Actually, over 40% of its genes (19 099) would be similar to ours which makes it a great model to understand many human diseases. It notably made possible studies about apoptosis, an important process which helps the understanding of human cancer but also cellular aging.

http://wormclassroom.org/about-c-elegans
http://wormclassroom.org/short-history-c-elegans-research
http://www.people.ku.edu/~erikl/Lundquist_Lab/Why_study_C._elegans.html
http://hobertlab.org/wp-content/uploads/2013/03/Ankeny_2001.pdf

II) WHY IS *C. ELEGANS* SO IMPORTANT IN PUSHING BACK FRONTIERS OF SCIENCE ?

C.elegans has been used in a wide variety of domains like:

- Determining the effects of genetics versus environment on lifespan. [Klass MR Mech Ageing Dev. 1977 Nov-Dec; 6(6):413-29.]
- Evaluating which genes can individually impact lifespan, and how their mutations affect lifespan [Friedman DB, Johnson TE Genetics. 1988 Jan; 118(1):75-86.]
- Numerous studies on lifespan regulating genes in C.elegans has led to the theory that the FOXO3 gene in humans might impact human lifespan too.

Of course, these examples are only a small part of all the research on C.elegans but are the most accurate ones that were produced in the past few years.

https://scholar.google.fr/scholar?q=c+elegans
http://modencode.sciencemag.org/worm/introduction
https://www.yourgenome.org/facts/why-use-the-worm-in-research
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4464094/

III) And what is its importance for the iGEM adventure ?

Team Valencia 2013 : creation of an artificial symbiosis between *C.elegans* and bacteria. This symbiosis relies on the moving ability of *C.elegans* but also on the biotechnological possibilities of bacteria :

- *E.coli* : Interfering RNA synthesis to induce the social feeding behaviour of *C.elegans* (biobrick Bba_K1112000 dans la souche *E.coli*, XL1-Blue)

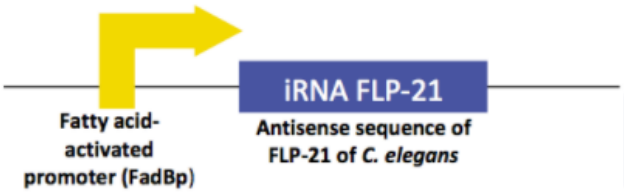

Fig. 2. Biobrick designed for *E. coli*. The antisense sequence of FLP-21 of *C. Elegans* is up-regulated by a promoter activated by fatty acids.

- *P.putida* : KT2440 strain will be transported to point of interest (hot spots) and produce bioplastics, PHA
- *C.elegans* : OP50 strain feeds on XL1-Blue strain of *E.coli*. *C.elegans* uses chemotaxis. There are two pathogens (*Y.pestis* and *X.nematophila*) capable of producing biofilms on *C.elegans*. This relies on the operon encoding the **hmsHFRS** proteins. When a bacterium expresses this operon it can stick to the worm surface

Fig.1. Biobrick for inducing the formation of the biofilm design.

Works?	Name	Type	Description	Designer	Length	Fav
✓	BBa_K1112000	Regulatory	fadB promoter + FLP-21 iRNA	Pedro Luis Dorado Morales	344	❤
✓	BBa_K1112001	Coding	pGlnA + hmsHFRS operon	Alba Iglesias Vilches	6342	
✓	BBa_K1112002	Coding	Cluster PHA	Alba Iglesias Vilches	4978	

Team Hong-Kong UCCKE 2016 : the idea was to control an organism behavior. They tried to modify bacteria to make them produce chemicals attracting or repelling *C.elegans*. Cinnamaldehyde repelling : part K1889021.

Phenylpyruvic acid attirant : part K1889006.

Team Bordeaux 2016 : That year, iGEM Bordeaux aimed at studying DSIP, a sleep-inducing peptide. In order to understand in which mechanisms this peptide is involved, it was introduced in the nematode *Caenorhabditis elegans* to see the effects on its sleep. Another aspect was to control the nematode's sleep pattern. They wanted to make a photo-inducible system that could be used on sleep gene promoters. On the other hand, they aimed to create a new tool to modify the genes involved in sleep using epigenetics: EpiCRISPR. Based on the CRISPR-CAS9 concept, they wanted to design it according to many strategies, see more details in the Description part.

Team Bordeaux 2017 : This year, IGEM bordeaux aimed at studying the alternative splicing of the unc-60 gene in *C.elegans*. In order to control the alternative splicing of this gene, it is important to control the synthesis of proteins involved in it's splicing. We wanted to create a photo-inducible system that would use two promoters of regulatory proteins ASD2 and SUP12. On the other hand, we aimed to use another system called the Q system allowing more precise control of the regulatory proteins of the unc-60 gene.

IV) PROMOTERS AND GENES

In order to investigate the *C.elegans*' promoters we used the Promoterome database which regroups around 6000 promoters that can be used to visualise (fluorescence) specific tissues or fusion proteins. This database also gives an access to many primers for each promoter.

Name	***Size on Promoterome***	***Location***	***Ref Promoterome***
Myo-2	*976pb*	*-Pharyngeal muscle cells -Intestine -.....*	*http://worfdb.dfci.harvard.edu/promoteromedb/searchPromoterome.pl?by=name&sid=Myo-2*
Myo-3	*2001pb*	*-Body wall musculature -Intestinal muscle -Vulval muscle -.....*	*http://worfdb.dfci.harvard.edu/promoteromedb/searchPromoterome.pl?by=name&sid=Myo-3*
Unc-119	*2000pb*	*-Neurons -Nerve ring -Dorsal nerve cord*	*http://worfdb.dfci.harvard.edu/promoteromedb/searchPromoterome.pl?by=name&sid=unc-119*
Unc-17	*2000pb*	*-Cholinergic neurons*	*http://worfdb.dfci.harvard.edu/promoteromedb/searchPromoterome.pl?by=name&sid=unc-17*
Unc-47	*1444pb*	*-GABAergic neurons*	*http://worfdb.dfci.harvard.edu/promoteromedb/searchPromoterome.pl?by=name&sid=unc-47*
mec-8	*2000pb*	*-nervous system -vulva -.....*	*http://worfdb.dfci.harvard.edu/promoteromedb/searchPromoterome.pl?by=name&sid=mec-8*
…..			
Ref Promoteurs :	*http://www.wormbase.org/#012-34-5*		

To access the gene sequences of *C.elegans* we can use the wormbase database which regroups approximatively 20 000 coding genes and 16 000 non coding genes. Moreover this database allows to work on several nematodes such as *C. briggsae*, *C.remanei*, …

Three interesting housekeeping genes for the 3'UTR region can be important for transcriptional regulation :

Name	Fonction	WormBase ID
rps-0	Coding for a ribosomal sub-unit	WBGene00004469
tbb-2	Coding for Beta-tubuline	WBGene00006537
unc-54	Coding for an havy chain of class II myosin	WBGene00006789

V) How to use *C.elegans* for laboratory experiments ?

Many protocols can be found in Jonhatan Millet's thesis (pages 125-144).

	M9 medium	Freezing solution	NGM medium	LB medium
Specifications	This culture medium allows to maintain *C.elegans* alive and is widely used to manipulate populations (genomic DNA preparation, post injection regeneration, …). Solution filtered through filtration units.	allows long term conservation at -80°C.	used to keep alive the worms	liquid or solid medium used for bacteria growth.
Composition	3 g KH_2PO_4 6 g Na_2HPO_4 5 g NaCl 1 mL $MgSO_4$ (1M) H_2O qsp 1 L	0,58g NaCl 0,68g KH_2PO_4 30g glycerol H_2O qsp 50mL	3 g NaCl 2.5 g peptone 12 g agar 5 mg cholesterol Autoclaving 1 mL $CaCl_2$ (1M filtered) 1 mL $MgSO_4$ (1M filtered) 0.40 g/l G418 (selection)	10 g tryptone 5 g yeast extract 10 g NaCl H_2O qsp 1 L For solid medium add 12 g agar for 1 L Autoclaving before use Add antiobiotic to make the medium selective (100 µg/mL) For iRNA this medium is complemented with ampicillin (100 µg/mL) 1 mM IPTG

V.1) Growing conditions

The worms are grown onto NGM medium (or NGM + G418) covered by a *E.coli* carpet (OP50 or OP50-neoR). Incubation at 20°C or 15°C. Worms are then transferred into another box every 3 to 4 days.

V.2) Microinjection

Injection mix has to be centrifuged (10 000g, 30 min) before each injection to pellet particles that could obstruct the needle.

5 µL of injection mix is loaded into a micro-injection capillary which is plugged into an injector.

The micro-injection is visualised through microscopy.

The injection is performed into syncytial area of one of the gonads for individuals in a young adult stage immobilized into an oil drop on an agarose (5%) lamina. Five individuals are then dropped onto boxes NGM + 418. The medium is checked by dropping five non injected worms.

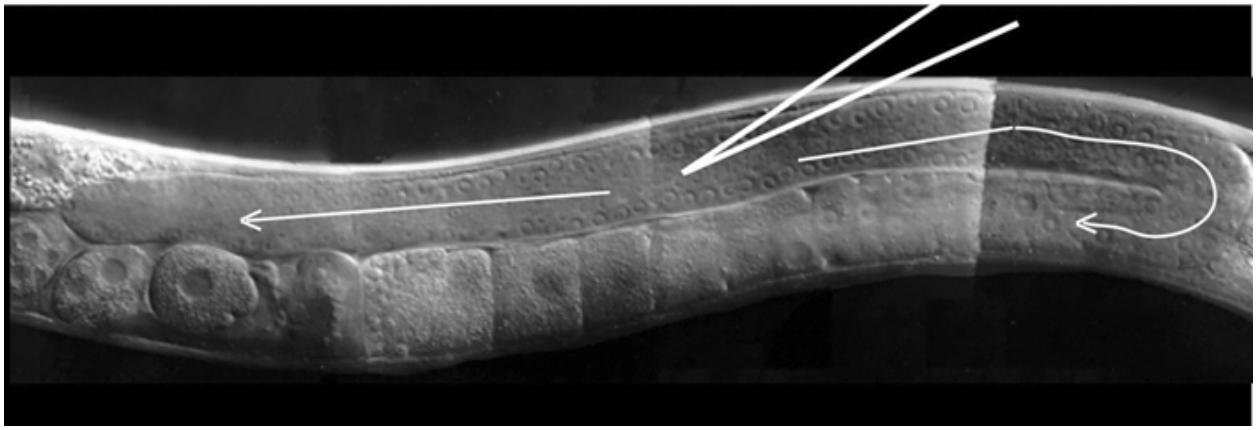

Illustration 1: C. elegans pachytene region from wormbook

V.3) Microscope observation

Worms are dropped onto lamina covered with 5% agarose into a drop of sodium azoture (10 mM) or levamisole (1 mM) to anesthetize the worms. Then a coverslip is used and the sample can be observed with a microscope.