iGEM Evry 2012 presents:

Xenopus FOR DUMMIES®

1st edition !

An iGEM guideline for Xenopus projects

iGEM Évry - 2012

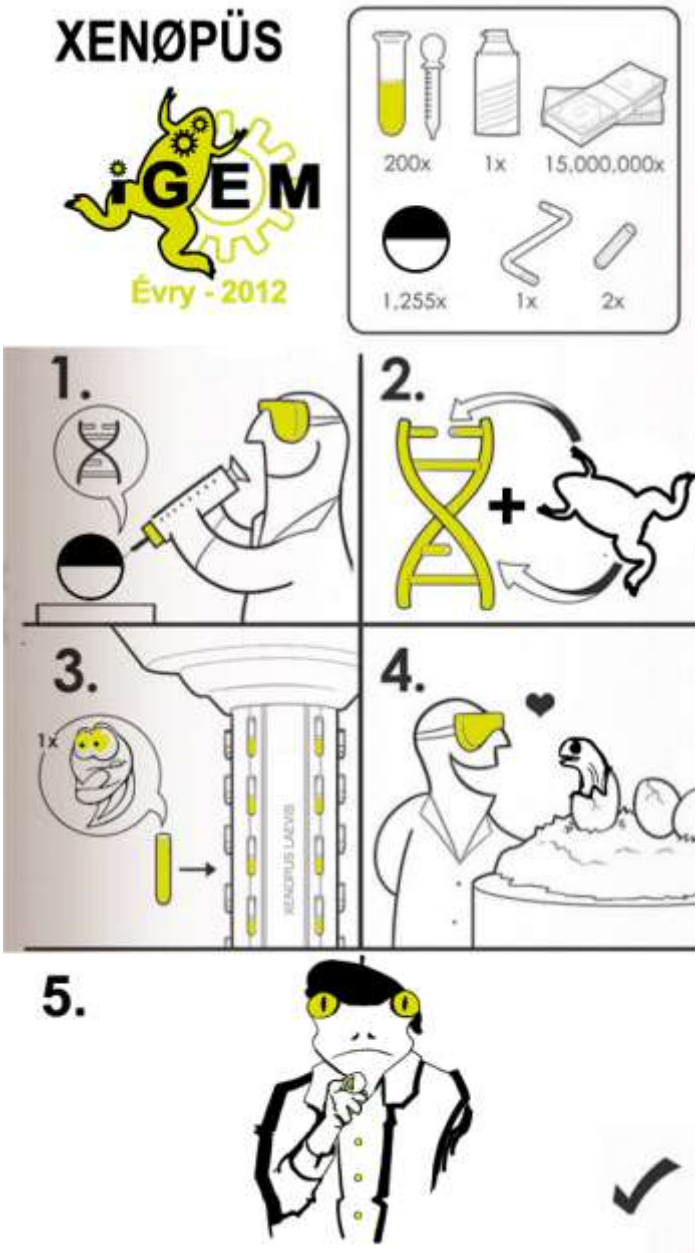
The French Froggies project !

OUTLINE

INTRODUCTION

Hi iGEMer! Are you brainstorming on a new project? Do you plan on working with Xenopus? You are reading the right thing; this book is dedicated for you!

We are the 2012 Evry iGEM team and, this year, we made the first iGEM project on *Xenopus* and created the first biobricks to work with. In this small book, we wanted to share with you our experience with this animal, try to give you guidelines for designing your own project and give you references to the resources you can use.

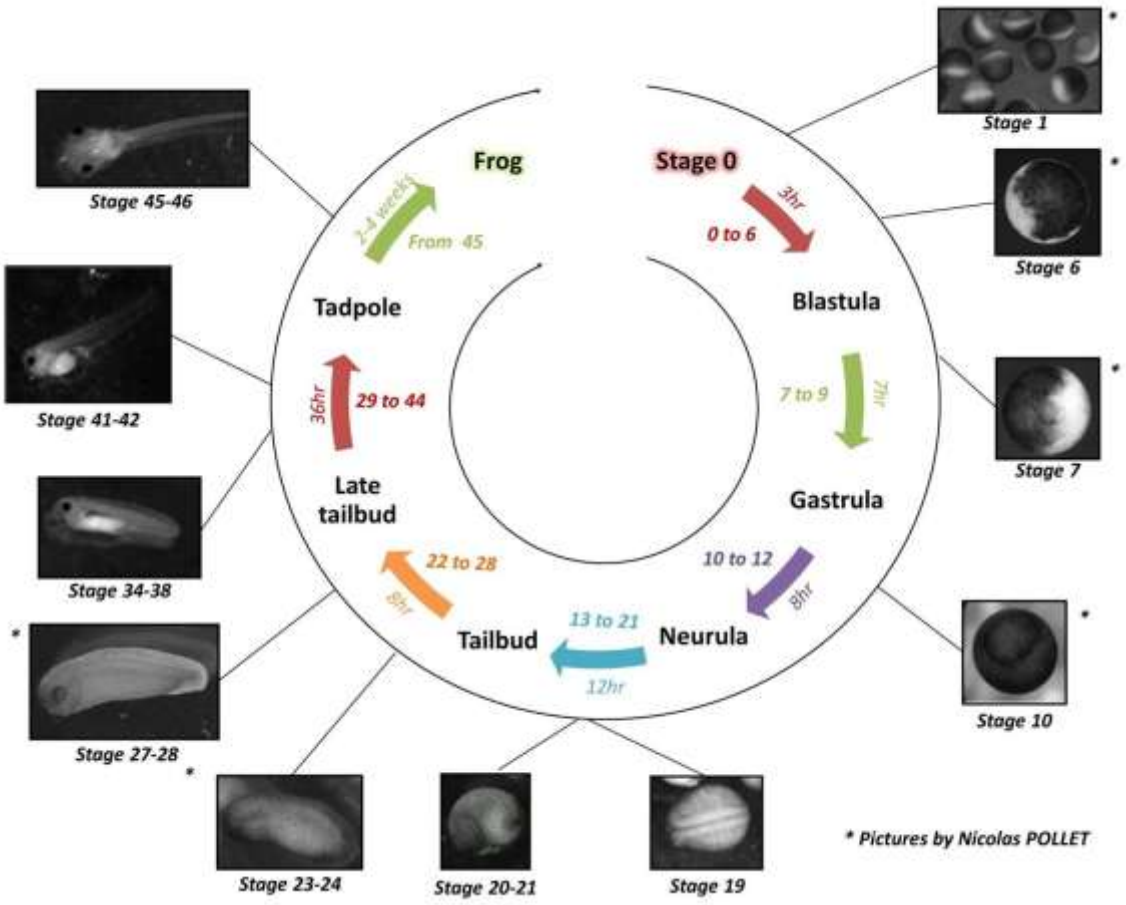


This book is not meant to be an exhaustive protocol book. We advise you to have a careful look at our wiki for this purpose. It is more of a short instruction manual for quick references, as illustrated by the drawing below!

1.WHY IS *XENOPUS* INTERESTING FOR IGEM?

Xenopus laevis or *Xenopus tropicalis* find their origins in Africa and are well-known anuran amphibian species as they are commonly studied as a model organism. *Xenopus laevis* is a more robust animal, however only t*ropicalis* is diploid, which makes the genetic studies easier.

Regarding the bench work, *Xenopus* is really convenient to work with. Gene expression is visible by fluorescence one day after microinjection. Most of the major organs are developed in 3 days after the *in vitro* fecundation. The figure below shows the different stages of *Xenopus tropicalis*.



According to European law, *Xenopus larvae* are not considered as animal until the stage 45, because they do not eat. It makes experimentation easier, because we are not constrained by legal certifications. However, it doesn't mean that you have right to do anything you want with it!

Widely used in laboratories, *Xenopus* might be one of the most convenient vertebrate to obtain for any iGEM team. There are two *Xenopus* stock centers in Europe and USA. *Xenopus* allow for high-throughput *in vivo* analyses of gene function and biochemistry. It is used for its powerful combination of experimental tractability and close evolutionary relationship with humans. Additionally, its entire genome has been sequenced and an extensive database for *Xenopus* can also be found online to support your research.

Working with vertebrate in synthetic biology is very challenging but opens to new perspectives. iGEM teams can come up with new ideas and new applications that would not be possible with bacteria. Hopefully this guide can help and inspire you into the path of synthetic physiology.

2. REQUIREMENTS BEFORE PLANNING TO WORK ON *XENOPUS*

Xenopus is an animal that requires a great care and a lot of expertise to handle. Moreover, it requires special injection equipment, thermoregulated and secured rooms. Finally, you will need a special stereo-microscope and light-source. It is unlikely that one can start working with *Xenopus* without making any association with a laboratory already working on this organism.

We don't recommend you to start working with *Xenopus* on your own for the well-being of your frogs and the sake of your iGEM project.

3. GUIDELINES TO DEFINE AN IGEM PROJECT WITH *XENOPUS*

First of all, we have to say that we really enjoyed brainstorming for projects on *Xenopus*, although we found the process very hard. *Xenopus* is a very interesting organism for the purpose of synthetic biology. It is a multicellular organism, which contains almost the same tissues and organs than human. But it also has some drawbacks as far as iGEM is concerned: It is longer to work with than bacteria, and more difficult to test and characterize.

Since it is a living animal, you should also restrain your ideas to system that doesn't hurt the animal or use it for fun, and not for science.

When brainstorming for your project:

- Take advantage of the organs and tissues of the tadpole for your device
- Use the fact that you can track your system over several days during the development of the animal
- Take advantage of the water environment to communicate with the tadpole

- Use its high sensitivity to compound present in the water to detect their effect on the tadpole physiology and report potential toxicity for other organism
- Place genetically modified bacteria in the tadpole's gut and communicate with the tadpole through the blood
- You can use the development of the animal as a variable
- Tadpole behavior is complex; this can be exploited in a certain extent

But there are things you should take care of!

- First of all, you should discard any project that doesn't require the use of living animal. Bacteria or cell lines are easier to work with.
- You should take care of the animal well-being when it is carrying your system. The system should not hurt or cripple the animal uselessly.
- You should work with transient genetic systems and avoid the use of stable genetic modification to avoid the propagation of genes in nature if the animal escapes.

From our experience as iGEMers, there are a couple of details you might like to take care of:

- Be aware of the time it takes to develop constructs and micro-inject them. It takes a week to characterize the system
- Your DNA will not diffuse in the entire egg. It will stay close to the injection point in the tissues and stay in the cells that derive from this part of the cytoplasm where the injection has been made.
- We recommend you to limit to two or three persons the work with the animal. It is important for the care of the animal, the quality of your micro-injection and for your microscope.

We hope you will enjoy working with *Xenopus* as we enjoyed it all this summer!

4. THE MICRO-INJECTION PROCEDURE

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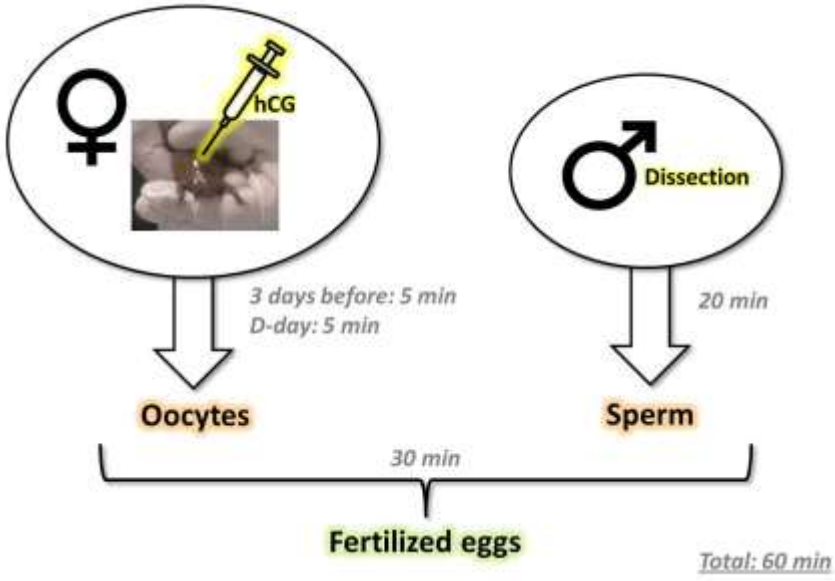
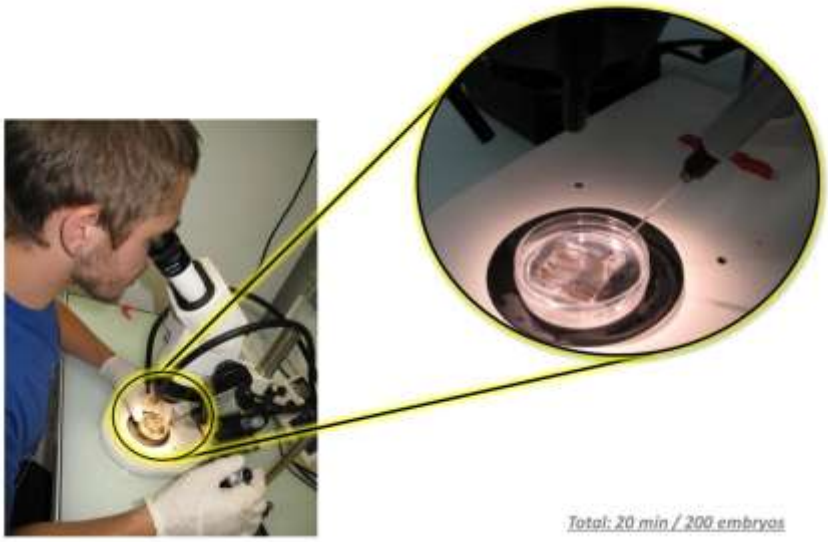
a. Step 1: Fertilized eggs



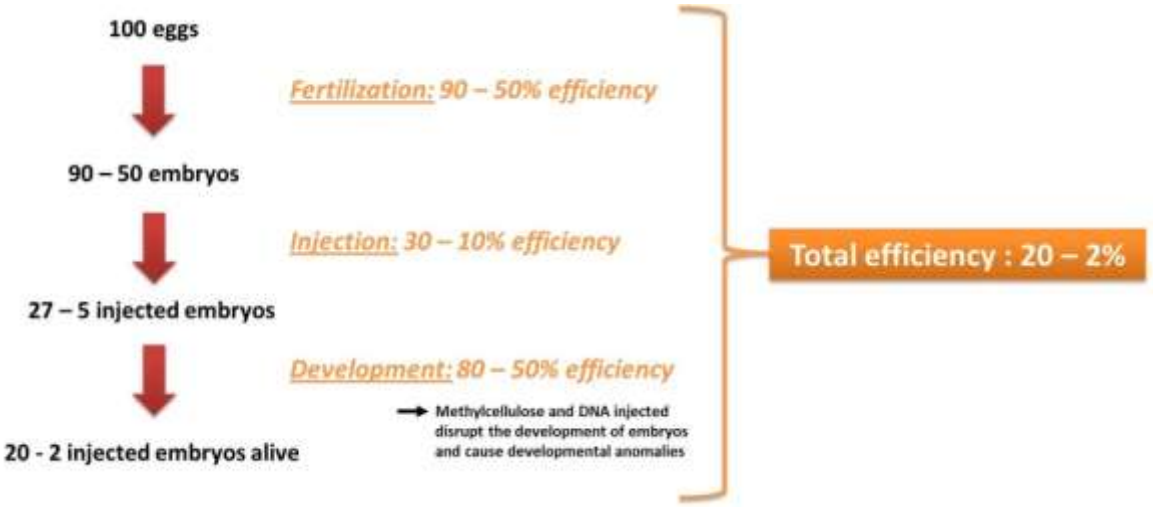
b. Step 2 : DNA injection



c. Place the injected embryos in incubator 21°C

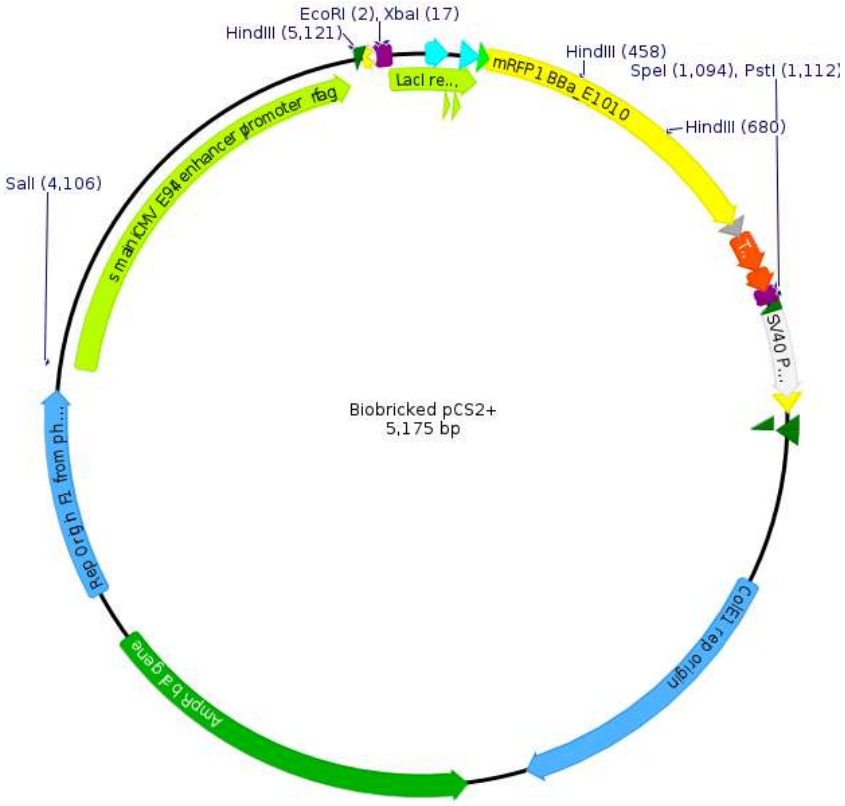
d. The following days

e. Embryos injection: efficiency



5. THE BIOBRICK PLASMIDS FOR *XENOPUS*

This summer we have created the first biobrick plasmid for rapid testing of constructs in *Xenopus*. This plasmid derives from pCS2+, a standard chicken and *Xenopus* plasmid. It contains a CMV promoter in between a SalI and HindIII restriction site, a β-globin 3' UTR before the biobrick prefix. The negative cloning control J00450 is inserted between the Biobrick prefix and suffix, and is to be substituted with your gene of interest before a kozak sequence. Finally, a SV40 polyA signal is added after the biobrick suffix for the stability of the mRNA and for a good overexpression in the cell.



This plasmid is an easy cloning device. In order to express a gene, you need to PCR it with the kozak sequence and the Biobrick prefix and suffix, digest it with EcoRI and PstI and ligate it into pCS2+. Once minipreped, you can directly micro-inject it in the oocyte, sort and observe them the day after.

If you want to substitute the CMV promoter by another promoter, you can digest the plasmid in SalI and HindIII clone your own promoter, as long as your inserted brick does not contains any SalI and HindIII site. In the registry, you will find two other version of the plasmid with the HSP70 and the Elastase promoter, submitted as the K812200 and K812300 in the registry.

6. *XENOPUS* DEBUGGING TOOL

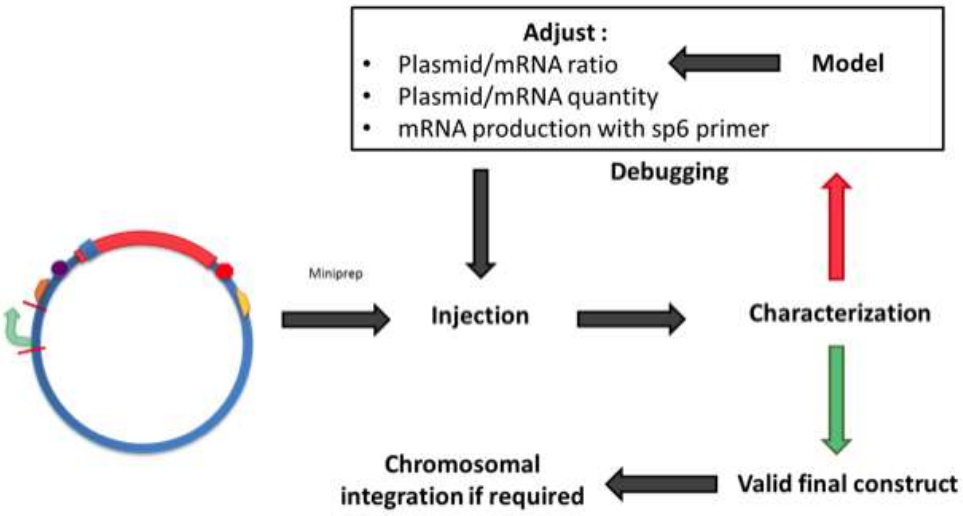
Since plasmids can be directly injected into the *Xenopus* embryo, we can play around with what we inject, how much we inject and when we do it.

The different components of a genetic system can be assembled on separate plasmids, and then co-injected. By omitting to inject certain parts of a system, we can test the various components individually or in small groups, before testing the whole device. Another advantage is that we can inject plasmids at different concentrations: By varying the quantities or ratios of the injected plasmids, we can test different configurations and debug the system. Finally, we can also inject at the 2 cell stage, which will result in half the tadpole containing the system, while the other half can be used as a control.

a. How?

The relative quantities of a gene product in the cell can be critical for its correct function.

Imagine a simple system where one protein A produces green light, whereas another protein B blocks protein A. At first, we would imagine that if only A is present, the system will be green, but if A and B are present, it will switch off. But maybe A degrades at half the rate as B. As a result, if both genes are behind the same promoter, we will end up with twice as much A as B, and even if both proteins are expressed we will still have green light. But by injecting 2 plasmids, one containing B and one containing A, we can fix our system by injecting the plasmids at different concentrations. This way, we can apply the lessons we get from our modelers and directly test them *in vivo*, simply by diluting the DNA to the correct concentration, avoiding long weeks of cloning. When the working system is characterized, we can reassemble the parts with a promoter of the desired strength.



b. mRNA, probes, etc.

Our plasmid also contains other debugging tools. A **sp6 primer** is present upstream of the 5' UTR, allowing *in-vitro* transcription of mRNA which can be directly injected. This will allow eliminating the possibility that a system is broken due to the absence of transcription, and also allows a finer control of protein levels. The plasmid also contains a reverse **T7 promoter**, allowing rapid probe synthesis for any gene inserted in our frog biobrick plasmid to make Southern or northern blots.

7. MODELING IN *XENOPUS*

a. General advice

As any vertebrate, *Xenopus* is a far more complex animal than the usual synthetic biology chassis such as *E.Coli* or yeast. Working with a vertebrate rather than in a unicellular organism means your project must use somehow this multicellular organization.
Modeling extremely precisely the entire organism is obviously not feasible (even in unicellular organism).

The first advice is to clearly state from start what the perimeter of your analysis is. What your model should account for. This must absolutely be discussed with members of your team who are performing experiments in order to know what they expect from your model and what kind of result and measures are possible.

Based on this, you want to divide all the aspects you want to represent into different modules. These modules should ideally follow natural separations. The most obvious choices are different tissues or different cells. But many other natural separations are possible, like time scale separation or orthogonality.

Then you may start thinking about the interactions between the modules before designing them. What output from a module will be an input for another? What mathematical representation this linking element could have (Simple constant value, function of time, qualitative insight to impact the design of other modules etc.)

Finally, you can design the modules, choosing the more convenient and adapted representation. If some modules have feedback loops one to another, you may implement these in a same framework (programming language, toolbox, software).

From our experience, working on several communicating modules in parallel proved more efficient.

In the course of development, you could need to change some aspects of a module. Having the others already well defined and under construction allows you to clearly define the range of modification and the impact on other modules. Sure, this could be thought to be counterproductive but on an iGEM project time scale, it allows you not to drift too much from your initial plan and therefore make sure you achieve a complete integration.

b. What you can use from the french froggies project

Our project shows a way of building a multiscale and modular model, fusing various modeling techniques. It first gives you an example, but also provides you with useful ModelBricks you may want to reuse for your own project. These are easily adaptable for detection or reporting units, transportation by blood or diffusion in tissues.

The provided blocks are summarized in the following table:

Name	Time scale	Space scale	Modeling technique	Inputs form models	Outputs
Global model	min - hour	mm (tadpole)	ODE	Concentration in emitter tissue over time	Concentration in blood, in receiver tissue.
Precise Diffusion model	min - hour	mm (tadpole)	PDE	Concentration in emitter tissue over time and space	Concentration in blood, in receiver tissue.
Auxin creation model	min - hour	µm (cell)	ODE	Number of plasmids	Concentration in cell
Degradation model	min - hour	µm (cell)	ODE	Number of plasmids, Incoming concentration	Exiting concentration in cell
Plasmids repartition model	hours - days	nm - µm (plasmid)	Agent based, stochastic		Plasmid distribution among cells

8. FURTHER INVESTIGATIONS INTO THE USE OF VERTEBRATES IN SYNTHETIC PHYSIOLOGY

We believe it is important for synthetic biology to develop self-critic human practices involving humanities. Our research this summer dealt with the question of concepts, metaphors and ethics, what does it mean to call a tadpole a chassis? Doesn't this term reduce our perception, masking the specificity of the animal? This question could be extended to the whole notion of chassis in synthetic biology, this imply a very particular conception of the living.

Yet many issues raised by the use of vertebrates in (synthetic) biology were not dealt in our human practices... Deeper reflections concerning animal patenting or the limits of the 3R principle guiding would be welcomed...

9. PERSPECTIVES

Hey iGEMer! We hope you know a little more about *Xenopus* now, and that this document helped your to see more clearly how to work with *Xenopus*. If you want to learn more about the project we did in 2012, the protocols we used and the other ethical aspect regarding synthetic biology on animals, we kindly invite you to have a closer look at our wiki.

If you choose to work with *Xenopus*, you will discover how interesting and how tricky is to work with this animal. The perspective *Xenopus* opens for synthetic biology are numerous: biosensing, synthetic endocrinology, multi-tissue systems and everything that your imagination can devise and create! This is the real beauty and craziness of iGEM.

As we did with the iGEM Slovenia 2012 team, you could test the constructions in Xenopus, allowing result in 5 days in a complete organism!

We hope that you are going to have fun working, or rather collaborating, with *Xenopus* tadpoles for the fun of an iGEM project, for the sake of Synthetic Biology and in the respect of the animal.

The iGEM Evry 2012 team

Visit our wiki: http://2012.igem.org/Team:Evry