

TARPOL
CONSORTIUM

Targeting environmental pollution with
engineered microbial systems à la carte



FINAL REPORT

Grant no.: 212894 FP7

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PROJECT FINAL REPORT



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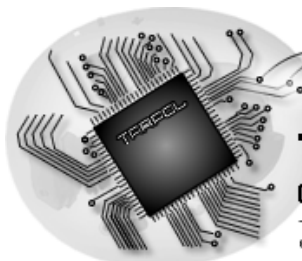
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1 Final publishable summary report

1.1 Executive summary

The development of Synthetic Biology (SB) in Europe faces three major obstacles that have, up to date, hampered the development of an SB-based framework in our continent: [i] The new field is still missing a comprehensive language and a shared conceptual framework for the description of minimally functional biological; [ii] scientists and technicians, particularly in Europe, have so far failed to recognize their latent capacity to shape a brand-new discipline within their very scope; and [iii] promotion of SB might touch upon social sensitivities related to recreating “life-in-the-test-tube”, which threatens a re-enactment of the controversy stirred up by GMOs. TARPOL led by the Universitat de València and composed of a total of 18 partners (<http://www.sb-tarpol.eu/>), has developed a dynamic two-year program of activities, run by a large collection of European stakeholders in the field and aimed at coordinating the so-far fragmented efforts to channel this emerging discipline into the most industrially beneficial and socially viable directions. TARPOL has been successful in recruiting the necessary environmental competences from neighbouring disciplines and developing numerous material and computational resources for advanced refactoring of biological systems. Furthermore, TARPOL has progressed in laying the foundations of SB, particularly on genome reduction strategies including a novel view of living organisms as information traps and contributed with dozens of basic, conceptual and applied publications to the development of this new field. The TARPOL consortium has paid particular attention to education, and a range of conferences, dissemination and training activities have been organized, including the support to a successful iGEM team and the organization of two intensive SB Summer Schools in Valencia (Spain) and Basel (Switzerland) in Spring and Summer 2010, respectively.

Mobilization, interdisciplinary collaboration and training: the objectives of TARPOL concerning SB converge into a single goal: to set up this brand-new discipline which may have revolutionary effects on our lives, in Europe. TARPOL has significantly contributed to such objectives as a key step to implementing a powerful, sustainable knowledge-based bio-economy in our old continent.

1.2 Summary description of project context and objectives

The basic premise of SB is that methods commonly used to design and construct non-biological systems, such as those employed in the computational sciences and the engineering disciplines, could also be used to model and program novel synthetic biological systems. SB is thus intrinsically transdisciplinary and draws expertise from Biology, Chemistry, Physics, Computer Science, Mathematics and Engineering. Synthetic biologists are attempting to develop 'artificial life', for both its tremendous applications in biotechnology and as a proxy for shedding light into the question of the origins of life. This is attempted by following two separate and competing routes: the 'top-down' and 'bottom-up' approaches to minimal cells. In the former, a primordial or minimal cell is generated by systematically reducing a biological cell's genome until it no longer functions (Glass et al., 2006; Lartigue et al., 2007). The bottom-up methodology, on the other hand, seeks to assemble from scratch components or information units until an aspect of life emerges (Bedau, 2003). The overall intellectual and experimental challenges of implementing artificial life remain relatively long-term goals. However, along the way, guiding principles, experimental methodologies and theoretical insights from Biomimetic Chemistry and SB can be adopted in new ways for practical applications on a realistic, yet not necessarily immediate, time-frame.

Based on system biology (SB) achievements, there is a possibility of building a living being, or a part of it, which either already exists or is a non-natural entity. This particular view on SB represents a very promising applied field to areas as different as biomedicine, bioenergy, environment, etc. This type of program requires a serious reflection on the acquired responsibilities, as a consequence of the new natures that we, humans, may create. In addition, synthetic biology demands a philosophical thinking on the panoply of futures that, more than ever, are close to man-made reality.

The main objective of TARPOL is to catalyze the shaping of the European SB Community for translating this new conceptual and technical field into relevant environmental applications. This general goal comprises several specific objectives, including:

- To foster the transfer of SB conceptual fields into technical applications directed to solve environmental problems.
- To brand the conceptual and material interfaces between the various disciplines required for the surfacing and establishment of a European community on SB

focused on tackling, monitoring and preventing environmental problems.

- To empower the SB-Environmental Biotechnology field with material, technical and web based resources.
- To ensure that all research areas of SB are coordinated at the European level, so there is a maximum exchange of knowledge.
- To enable the development of a conceptual frame in which ethical and safety aspects of the novel SB-related technologies, especially those related with environmental applications, can be optimally integrated into an emerging community.
- To promote the scientific training and specialization on SB, especially among scientists in early stages of their careers.
- To identify European R&D needs and priorities on SB and to make recommendations for future cooperation areas and innovative research activities to be launched in the EU.
- To create awareness at the academic, industry, social, policy and decision-makers level on the economic and scientific potential of SB for environmental applications.

1.3 Description of the main S&T results/foregrounds

We strongly believe that TARPOL project has led to important advances in the conceptual field of SB. For instance, the state of the art of cell engineering in the context of genome research has been reviewed by **UVEG** during the first year, paying particular attention to what has been learned on naturally reduced genomes from either symbiotic or free living bacteria. Also by **UVEG**, the identification of a genomic core for a cyanobacterium (*Synechococcus elongatus*) was accomplished. This analysis will set the basis to designing a minimal photoautotrophic system suitable for a plethora of biotechnological applications. During the whole project, as the leading partner, **UVEG** focused on the general coordination of the project, management, dissemination activities, and conceptual frame and consensual language definitions. The latter task has been broadly covered with a range of peer-reviewed contributions on the definition of the minimal genetic array for life to exist; the evolution of prokaryote-animal symbiosis; and theoretical limitations of synthetic life. Examples of this production are a review paper dealing with the milestones and challenges of synthetic biologists, coauthored by several TARPOL partners and

coordinated by UVEG, which is currently under revision, several book chapters on minimal cells, and a myriad of publications on the origin of life, ethics of teaching evolution and a range of conceptual aspects of SB (see publications).

Dissemination and training activities organized by UVEG were key points of the project, with activities including a Summer Course on SB held in Valencia on April 2010 and the international Meeting on Synthetic and Streamlined Genomes. Thinking of Synthetic Biology (SB) as a new field of technology in the intersection of biology and engineering, the importance of attracting and training new researchers in this discipline becomes evident. With this objective, the Cavanilles Institute (UVEG) and the Intertech Group (UPVLC), co-organized the above-mentioned Summer Course on Synthetic Biology, with several TARPOL partners as speakers, as a pioneer international SB course with a clear focus on young researchers. This activity was developed from two sides: training and motivational. For this reason, the program offered the combined expertise of the organizers to train scientists from an interdisciplinary perspective, in order to offer a range of activities for young researchers from a wide range of fields. The Summer Course on Synthetic Biology also included lectures by leading scientists of this new discipline on the constitutive principles underlying SB, which allowed attendees to see and understand the great possibilities of this new scientific area.

Regarding the Workshop on Streamlined and Synthetic Genomes, it was organized as part of the TARPOL and EMERGENCE Programs to promote Synthetic Biology in Europe, and was held in Valencia, Spain, in November 16-17, 2009. The workshop joined world-wide recognized experts, including Nobel laureate Hamilton Smith or Luis Serrano. The objectives of this two-day workshop were to assess the status, identify constraints, and discuss the potential of research on minimal/streamlined genomes from different perspectives. This included the minimal and sufficient features of life, the study of naturally evolved reduced genomes, the engineering of minimal cells from bottom-up and top-down approaches, as well as various practical applications derived from research on minimal living systems. This event was supported, among others, by the Cavanilles Institute on Biodiversity and Evolutionary Biology (University of València, www.uv.es/~biodiver/e/index.htm), and Centre for Public Health Research CSISP (València, www.csisp.gva.es/web/csisp/home). The workshop consisted of four sessions:

Synthetic and Digital Biology, chaired by V. Martins dos Santos; Streamlining Genomes, chaired by I. Economidis; Building Genomes, chaired by G. Posfai; and Circuit Design and Evolution, chaired by V. de Lorenzo.

A Satellite Meeting on Insect Symbiosis in the Era of Systems and Synthetic Biology was held on November 18, 2009, also in Valencia, and allowed many of the workshop attendants to join the community of synthetic biologists and discuss topics at the very interphase between these closely related disciplines.

Besides meetings, formation and training efforts, UVEG-led initiatives also served as a forum on which TARPOL partners had the opportunity to discuss and prepare further research projects. For example, members of UVEG and CSIC attended the BBSRC meeting of the UK networks on Synthetic Biology at Costwold (UK). We also participated in a course held at Spetses (Greece) on September 2010 focusing on how Bioscience will generate major advances in our understanding of how molecular systems can support all of the properties associated with living organisms. The key to progress here was concluded to be to increase the number of significant inputs from the physical sciences, engineering, computation and mathematics, leading to powerful new quantitative and precise methods of analysis and far deeper insight into the fundamental principles of living systems. In the introductory parts of the course, it was explored how one can build up a comprehensive picture of a living system starting with principles of macromolecular structure and function, how molecules in living cells self-organise, how macromolecules assemble to form complexes, pathways and subcellular structures, how they function in pathways, how these are all networked together, and how they are controlled and regulated. Emphasis was placed on the full range of quantitative techniques required to study biosystems. Different approaches to capturing the kinetics of pathways using mathematical representations such as biochemical systems theory and metabolic control analysis were presented, and the analysis of rate control and regulation discussed. Particular consideration was given to the challenges for modelling posed by gene expression systems and macromolecular assembly pathways. Following on from this, in the sessions on synthetic biology, it was shown how the design, modelling, construction and testing of man-made biomolecular systems can be developed from a thorough understanding of naturally evolved biomolecular systems. This unique course was of value to talented young PhD students and postdocs who are keen to engage with the

exciting opportunities provided by these burgeoning interdisciplinary areas of research. We like to think that the course was informative, challenging, exciting and thought-provoking. More information is available here: <http://www.mib.ac.uk/spetses2010/index.html>.

In addition to courses and meetings, the interaction between TARPOL members also resulted in the germ of -hopefully- new European Research Projects. Organizational meetings took place in Kolymbari (Greece) and Vienna in order to set-up a promising SB-based engineering of endosymbionts with medical applications.

And finally, in the intersection among research, collaboration, training and dissemination, a TARPOL-linked iGEM team (supervised by UVEG and UPVLC members), the Valencia team, attended during 2009 and 2010 the international Genetic Engineered Machine competition and recently got, in 2010, a Gold medal with an innovative project on the implementation of a prion-based control circuit for tuning the Martian climate. The project was entitled "Mad yeasts on Mars?" and under this curious title it is hidden the ambitious Project of the Valencia iGEM team in which the students presented an intermediate scenario in the pathway towards the terraforming of Mars (i. e., modifying the atmosphere and temperature of Mars in order to get the appropriate conditions to make it habitable for Terran living organisms). The idea is that, after preliminary changes devoted to make Mars conditions more suitable for life, it can be colonized by microorganisms that will accelerate some changes to make the planet conditions acceptable for plant life which, then, will be able to generate enough oxygen to eventually allow the colonization by animals, including humans. The success of the Valencia team in 2009 was even more important, with Best New Application, Best experimental Measurement and Second-Runner Up awards. The project also included a Human Practices report with the largest inquiry on SB ever made, with more than 1000 interviews (http://2009.igem.org/wiki/images/0/0d/Sins_Ethics_and_Biology.pdf). The iGEM 2009 research project, describing the first biological lightning display with aequorin-expressing yeasts as living pixels reported (<http://2009.igem.org/Team:Valencia>; <http://en.wikipedia.org/wiki/ILCD>) had, besides the recognition of the iGEM organization Committee, an unprecedented impact on international media, including many broadcast and TV news, and a plethora of articles on general journals, among which a mention in The New York Times Magazine as "a real breakthrough" (<http://www.nytimes.com/2010/02/14/magazine/14Biology-t.html?ref=global-home>).

Besides the Valencia team contribution, it has to be noted the overwhelming success of European teams in the competition (5 out of six finalists and 6/6 in 2010 and 2009, respectively, were European), which demonstrates that the combined effort of European Universities and Research Centres may be able to change the fate of the geographic location of the excellence core on Synthetic Biology.

iGEM might be called THE major educational success in the area of synthetic biology. In our estimate, the following elements in iGEM together provide an essentially “irresistible” attraction to students (as can be witnessed by the appr. 130 teams which participated in iGEM2010, again a substantial increase over the number of teams of the year before. Appr. one third of the teams were from Europe). Given the fact that iGEM projects are a rather expensive form of education (lab work, overseas travel and accommodation for entire teams) that does not (yet) mix well with more traditional educational programs in the life sciences, the TARPOL consortium suggests that the EU commission takes the funding of iGEM projects under consideration under the auspices of its research or education funding schemes.

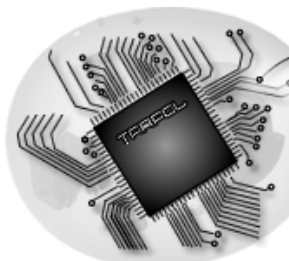
As a partner, **UVEG** has focused on training and theoretical development. But it is obvious that, in addition to the theoretical framework aiming at defining how a basic artificial cell should be, a real chassis is needed. In order to achieve this goal, **BCR-HAS** has carried out an applied work by developing core-genome *E. coli* strains to serve as host cells (“chassis and engine”) for various synthetic biology applications. Reduced complexity of the cells allows for more precise control/programming of cellular processes and for increased stability of engineered, synthetic genetic circuits. A set of bacterial strains is now available for use. In order to construct streamlined genome *E. coli* strains, up to 75 deletions were combined in a single genome, representing all together a 25% genome reduction. This reduced genome approaches the core genome size predicted by comparative genomics for intestinal *E. coli* strains. The strain series was characterized for growth, transformation efficiency, mutation rate and other practical parameters. Milestone strains with best phenotypic characteristics were further modified for specific tasks. These include stable maintenance of normally unstable DNA constructs, and inducible, high efficiency recombinant protein expression. Strains with enhanced mutagenic capability, serving as host for in vivo mutagenesis, are also available. Specific attention was paid to reduced-evolvability variants. Evolvability is an intrinsic feature of all living cells. However, newly emerging, evolved features can be undesirable when genetic circuits,

designed and fabricated by rational, synthetic biological approaches, are installed in the cell. **BCR-HAS** has shown that delayed genetic adaptation of reduced-genome host cells, devoid of all mutation-generating mobile genetic elements, improve maintenance of unstable genetic constructs. To further reduce the mutability of the host, point-mutation rates were significantly reduced by specific gene deletions. It was shown that various stress conditions, including recombinant protein expression, induce mutation-generating mechanisms to a high level in regular *E. coli* hosts. In contrast, the modified multi-deletional strains display low mutation rates, even under stress conditions. These minimalized, genetically stabilized strains are suggested to be beneficial hosts (SB chassis/engine) in both laboratory and industrial settings.

With the aim of defining the limits of construction of a cell factory, through re-sequencing and re-annotating *Bacillus subtilis* a better comprehension of the limits between the paleome and the cenome has been achieved by **IP**. A genomic database for *B. subtilis* has been created by **IP** partner (BacilluScope), and another one is in process (SubtiliCyc).

Regarding advances in molecular assets, **CSIC** partner, has develop, in the first year, a fully synthetic mini-Tn5 delivery vector (Figure 1), and has also developed an application of one *Pseudomonas putida* strain for detection of 2,4 DNT in soil. During the second year, **CSCIC** has focused on synthetic genetic and molecular tools. Indeed, the functioning of complex regulatory networks, or even a single gene, is revealed only when perturbations are entered in the corresponding dynamic systems and the outcome monitored. These endeavours rely on the availability of genetic tools to successfully modify *à la carte* the chromosome of target bacteria. Key aspects to this end include the removal of undesired genomic segments, systems for production of directed mutants and allelic replacements, random mutant libraries to discover new functions, and means to stably implant larger genetic networks into the genome of specific hosts. The list of Gram-negative species that are appealing for such genetic refactoring operations is growingly expanding. However, the repertoire of available molecular techniques to do so is very limited beyond *Escherichia coli*. In this Report, utilization of novel tools is described (exemplified in two plasmids systems: pBAM1 and pEMG) tailored for facilitating chromosomal engineering procedures in a wide variety of Gram-negative microorganisms. The way that goes from Genetically Engineered Microorganisms to synthetic, or at least heavily refactored counterparts involves the stepwise replacement of growing portions or their naturally occurring genomes by rationally designed and

chemically manufactured DNA. Although the current ability to synthesize long genomic segments is now in the range of 1 Mb, the contemporary level of knowledge does not allow assembling new activities or genetic circuits involving more than 20-30 kb of engineered DNA. It is thus likely that still for some time most Synthetic Biology endeavors of this sort will focus on handling a relatively short range of DNA sizes, whether for deletions from existing chromosomes, for genomic replacements of alleles by designed variants, or by straight implantation of new sequences. Numerous genetic tools exist in *E. coli* to this end but the state of affairs for other biotechnologically relevant Gram-negative bacteria such as *Pseudomonas putida* is far less satisfactory. In this Chapter two strategies are described for implementing a large number of genetic manipulations in the chromosome of a large variety of Gram-negative microorganisms. For this purpose, *P. putida* was used as the target bacterium, and the constructs named pBAM1 and pEMG (**Fig. 1**) adopted to give details of the underlying concepts and their practical application. As explained below, these plasmids are tailored for either implantation/insertion of heterologous DNA segments in the genome of the targeted strain as well as for directed mutagenesis or deletion or pre-specified chromosomal regions. Even though the procedures are different for each plasmid system, their utilization share a good deal of the biological materials listed in the corresponding section below. The organization and properties of pBAM1 (**Fig. 1A**) make it suitable to be used either for creating saturated random transposon mutant libraries or for stably introducing gene networks or functional cassettes into the genome of a specific bacterial host. Both of these properties are due to the special characteristics of mini-transposons. The pBAM1 plasmid is composed of 4 blocks (**Fig. 1A**).



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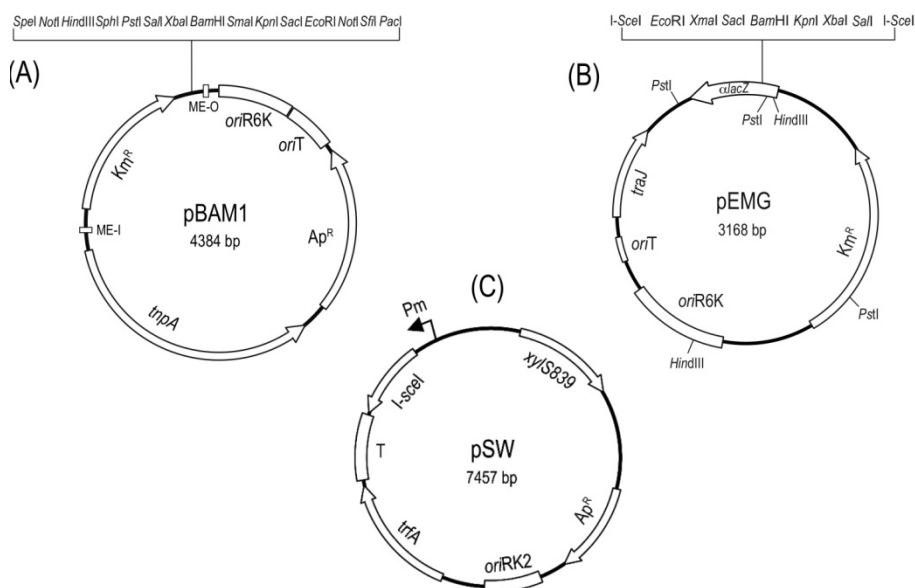


Fig. 1. Plasmids maps. (A) pBAM1. Functional elements of the plasmid include relevant restriction sites, antibiotic markers (Ap, ampicillin, Km, kanamycin), transposase (*tnpA*), origin of replication (R6K), the origin of transfer region (*oriT*), mosaic element O (ME-O), and mosaic element I (ME-I), as shown. (B) pEMG. Note functional elements, in particular multiple cloning site (MCS) flanked by I-SceI sites and merged in an α -*lac* sequence. (C) pSW. Broad host range plasmid for conditional expression of I-SceI nuclease. The *Pm* promoter is activated by 3-methylbenzoate because of the action of a variant of the XylS regulator, which tightly controls transcription.

The first segment correspond to the plasmid selectable marker, ampicillin. Next, an R6K origin of replication that makes its maintenance dependent of the trans supply of the p protein (*pir* gene). Thus, pBAM1 must be replicated in specialized *E. coli* strains, which expresses the p protein from a lysogenic phage, such as *E. coli* CC118/*pir*. A Tn5 transposase borne by the same plasmid (*tnpA*) recognizes the end sequences of the mini-Tn5 transposon module (ME-I and ME-O) and catalyzes the random motion of the mini-Tn5 cassette the target genome. All of these features have been individually optimized by CSIC, cured of the most common restriction sites present within its sequence, and then assembled and chemically synthesized *de novo*. The pBAM1 frequencies of transposon insertions when applied to *P. putida* are in the range of 10^{-3} when the plasmid is delivered to the recipient by mating (see procedure below), or 10^{-7} when electroporation is used as an alternative method of suicide donation. On the other hand, the pEMG plasmid (Fig. 1B) is used to generate directed scar-less deletions, as well as allelic replacements in the genome (Martínez-García and de Lorenzo *in preparation*). This genetic system is a recreation of the method developed before for the same purpose in *E. coli*. The procedure is based on the homologous recombination forced by the appearance of double strand breaks (DSB) in the genome of the target bacterium upon cleavage in vivo by I-SceI, a

homing endonuclease from *Saccharomyces cerevisiae* that recognizes an 18-bp DNA sequence.

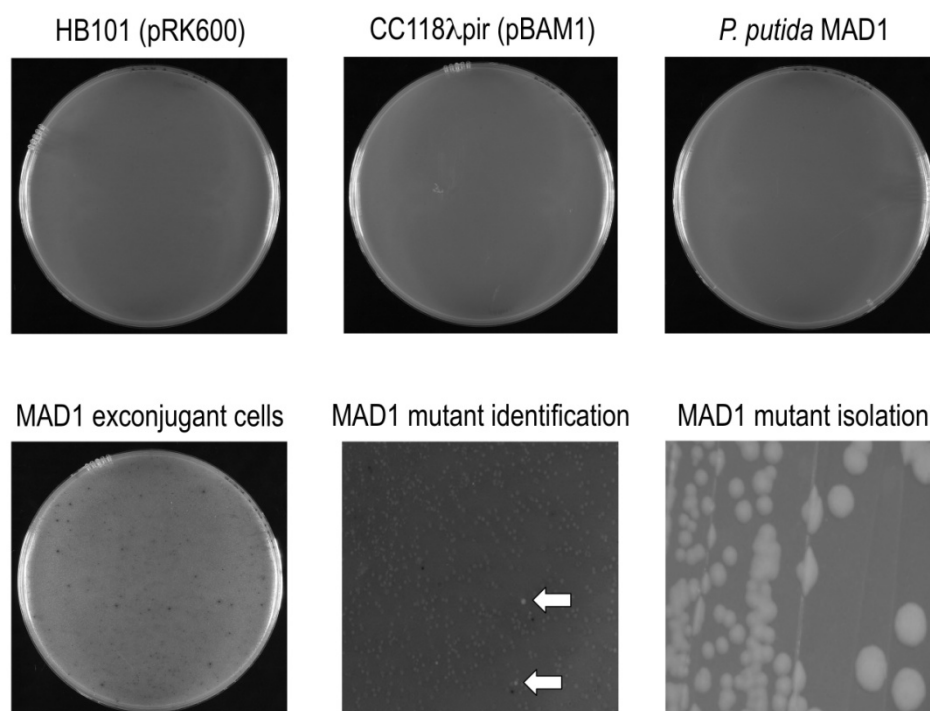


Fig. 2. Representative stages of a pBAM1-based transposition experiment. This picture corresponds to a pBAM1 transposition into *P. putida* MAD1 strain {Fernandez, 1995 #22}. The MAD1 strain bears in its genome a *xyIRIPu-lacZ* fusion that makes the strain to respond to *m*-xylene, thereby turning blue in M9 citrate + Km 50 $\mu\text{g ml}^{-1}$ + Xgal medium. Thus, transposition of the mobile element into any of these genes will render white cells. This setup pictures the typical output of a phenotypical transposition screening. In the upper part the negative controls of the experiment are shown. These include *E. coli* HB101 (pRK600), *E. coli* CC118 λ .pir (pBAM1) and *P. putida* MAD1 cells plated onto M9 citrate + Km 50 $\mu\text{g ml}^{-1}$ + Xgal. The first picture on the lower part pictured a plate onto which 200 μl from the mating mix was spread. The second lower picture is a zoom in of the exconjugant plate, where 2 white clones can be observed. After several isolation passes, white clones are picked (third lower picture) which are to be later subjected to arbitrary PCR for determining the specific transposon insertion point.

The I-SceI recognition sequence is not present in any of the microbial genomes sequenced so far, as revealed by blastn search against the 1379 completed (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). In this way, integration of pEMG into the chromosome of choice endows flanking I-SceI target sequences to the extremes of a *lacZ*-alpha pUC19-based polylinker. Intracellular expression of the I-SceI enzyme in live bacteria is brought about in vivo by the cognate pSW plasmid (Fig. 1C). Transient expression of the nuclease is tightly controlled in pSW by means of the 3-methylbenzoate-inducible promoter *Pm*. The steps of the deletion strategy process include [i] cloning regions homologous to those flanking the desired deletion/replacement into pEMG, [ii] cointegrating the resulting

plasmid into the genome of the target host, [iii] introduction of pSW into cells bearing the co-integrate, [iv] induction of the DSBs, [v] selection of the deleted/replaced strain and [vi] pSW curation.

For this deletion/replacement process, the I-SceI expressing plasmid (pSW) can be introduced before (preferred option if multiple deletions within the same strain are desired) or after obtaining the co-integrate (if one plans a single deletion). Only the second option is described here. To facilitate a specific example of the deletion protocol one example of the procedure is detailed below. To this end a chromosomal region of *P. putida* KT2440 between the genome coordinates 5680657-5690333 was chosen. This region comprises 7 genes that resemble a type IV pili operon.

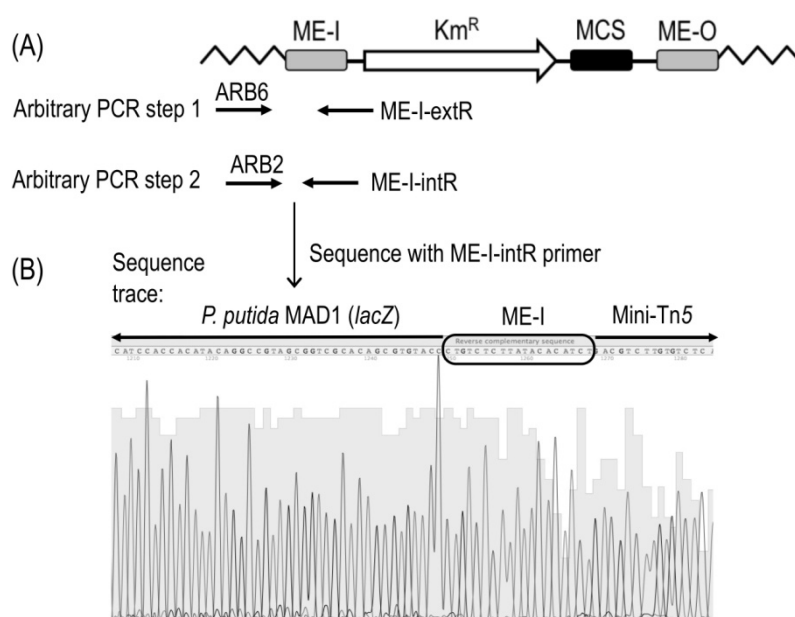


Fig. 3. Example of the principal steps involved in identification of the mini-transposon landing spot. (A) A picture of the mini-Tn5 module of pBAM1 with the position of the ME-I primers together with the arbitrary primers used in the process. (B) A portion of a characteristic sequence chromatogram that included marked the position of ME-I, the mini-Tn5 segment and the sequence portion corresponding to the *lacZ* gene of the *P. putida* MAD1 white clone obtained from the transposition experiment in this strain.

The delivery of the pEMG plasmid could be done either by mating or electroporation. Here, only the electroporation technique is discussed (for mating just follow the same procedure described before but using *E. coli* DH5 α *pir* harboring pEMG-Ts and finally plate onto M9 citrate Km 50 μ g ml⁻¹). To prepare electrocompetent cells of *P. putida* KT2440 (hereafter KT2440) follow the same steps described earlier.

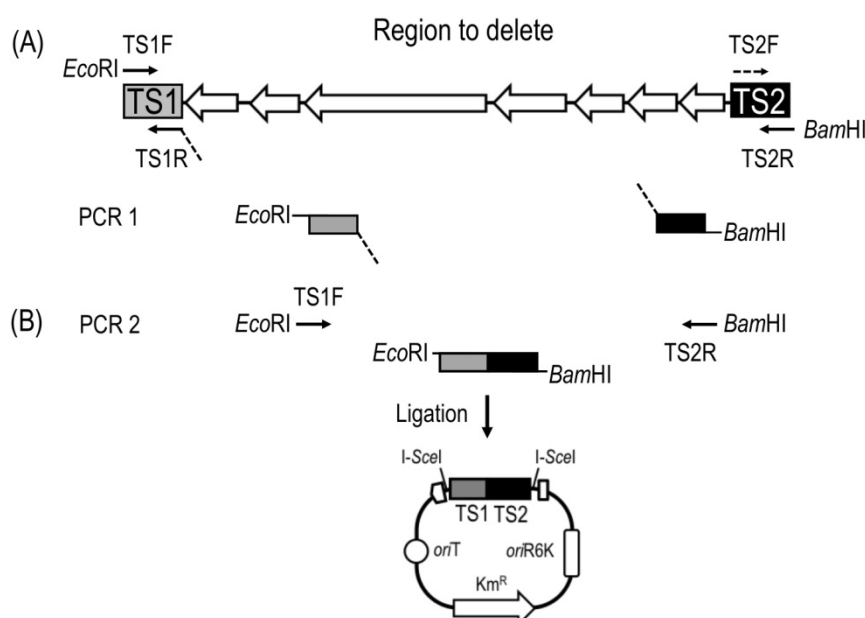


Fig. 4. (A) SOEing PCR scheme, with the two PCR rounds and the corresponding primers used. (B) A drawn of the TS1-TS2 cloning into pEMG to generate pEMG-TSs, where the position of the two I-SceI sites flanking the TS1-TS2 can be observed.

To summarize the **CSIC**'s work, a synthetic plasmid composed of multiple formatted and optimized functional parts that behave as predicted -both individually and as an integrated system- has been created. To the best of our knowledge, since the 90s this is the first report that describes a fully edited genetic tool optimized and streamlined for its final applications -rather than relying on cutting and pasting naturally occurring sequences⁵². In a nutshell, non-functional DNA sequences were trimmed-off, common restriction sites present outside the multiple cloning site inside the mobile element were eliminated and the plasmid was designed following a modular pattern in which each business sequence was flanked by non-frequent restriction sites. In this respect, the key features of pBAM1 include not only the removal of many bottlenecks that flaw utilization of many of its predecessors, but also the incorporation of a fixed standard for physical assembly and exchange, where required, of new DNA pieces while maintaining its overall layout. The modularity of the design and the origin of the parts (in mobile elements which considerable orthogonality) enables pBAM1 for two specific applications. The first is the exploitation of the cargo site (Fig. 1 and 2) to place a whole collection of extra genetic *gadgets* for expression of heterologous genes, reporter systems and environmental markers at user's will. The second is the possibility of cloning large DNA fragments inside the

mobile element for a final implantation of new traits into the chromosome of the target strain. Given the randomness and the high frequencies of such insertions, one can then select the insertion out of a large collection, which adjusts expression of the desired feature to the right level under the desired operation conditions^{53,54}. Furthermore, the ease of replacement of the antibiotic resistance marker (or any other functional part) allows the same transposition/delivery system to be reused for subsequent insertions. In sum, this work shows the value of DNA synthesis and standardization of functional modules for combining in a single genetic tool many valuable properties that are otherwise scattered in various vectors and rendered useless for the lack of fixed assembly formats. We anticipate pBAM1 to become one frame of reference for the construction of a large number of vectors aimed at deployment of heavily engineered genetic and metabolic circuits

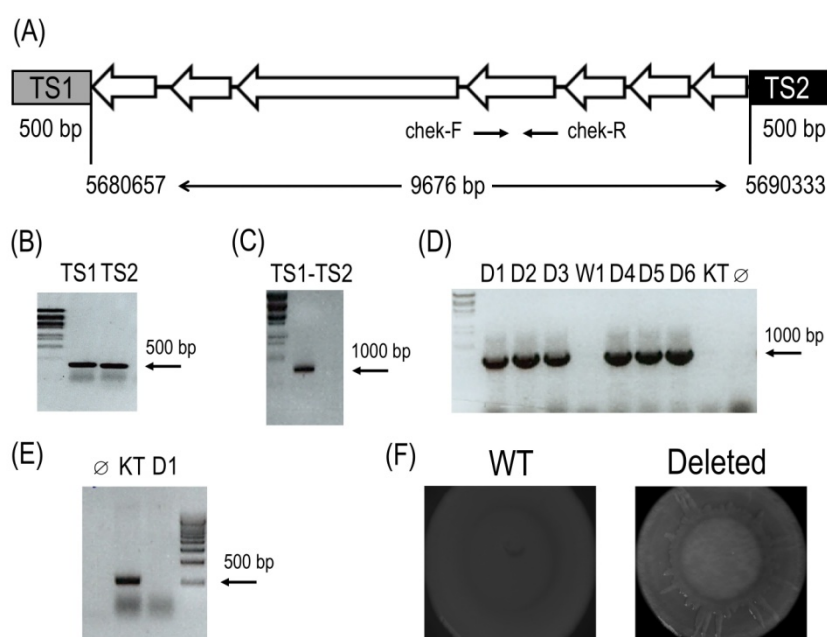


Fig. 5. Example of key steps in the deletion of a genomic region in *P. putida* KT2440. (A) Chromosomal organization of the deleted operon, including the upstream (TS1) and downstream (TS2) regions of homology, and the *P. putida* K2440 genomic coordinates of the region are shown. (B) Electrophoresis result of the SOEing PCR round 1, showing the purified DNA fragments of TS1 and TS2 (500 bp each). (C) Electrophoresis outcome of the SOEing PCR round 2, presenting the combined TS1-TS2 piece (a 1000 bp product). (D) Electrophoresis showing the PCR products, using TS1F and TS2R as primer combination, to check whether the recombination process yielded deleted or wild-type cells. (D) corresponds to a deleted clone (amplification of a 1000 bp band), W: wild-type, KT: *P. putida* KT2440 (wild-type control) and ∅: negative control (no DNA template). In this procedure 6 out of 7 clones analyzed were deleted strains and only 1 revert to a wild-type cell. (E) Confirmative result of the deletion, visualized after a PCR amplification using primers that hybridize within a gene inside the operon (primers check-F and check-R in figure). The wild type strain produces a PCR-

band while not the deleted strain, fact that confirms the removal of the operon. (F) Phenotypic characterization of the mutant strain. 2 µl of overnight cultures were spotted onto LB agar plates containing 40 mg l⁻¹ of Congo red and 15 mg L⁻¹ of Coomassie Brilliant Blue and let them grow at room temperature for several days. Note that the lack of the fimbrial genes endows the colony with a different morphology.

UMIL partner also focused on *P. putida*, particularly on optimizing the genetic background of these bacteria through the abolishment of physiological bottlenecks to the expression of the desired phenotypes. Moreover, **UNIL** is developing a site-specific integration system that will enable to insert large DNA fragments into the genomes of re-engineered bacterial strains with synthetic constructs. **UNIL** has already been successful in producing artificial integration sites, which are targeted with a higher efficiency than the natural ones. Nowadays, these target sites carry a conditional switch that let the cell produce green fluorescent protein when the DNA is integrated in the correct site.

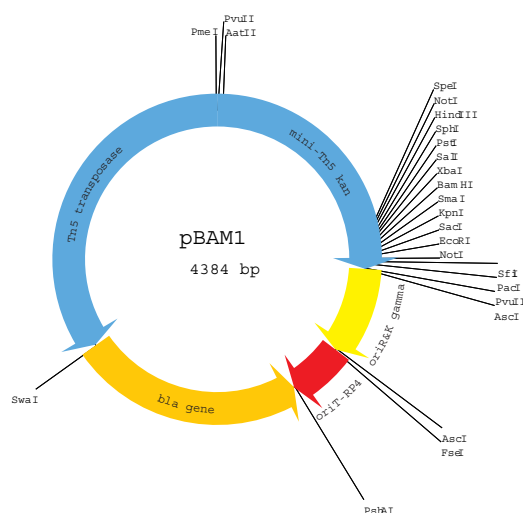


Figure 1. Synthetic mini-Tn5 delivery vector.

It is remarkable the substantial increase of knowledge obtained during the last years about the genetics, regulatory processes and metabolism of microbial forms of life. Such information obtained by means of different technologies of increasing power and efficiency is being included in large databases, many of them of free access. Once

combined with the vast number of scientific publications a huge volume of data is deposited in the hands of researchers. Synthetic biology aims the partial design of organisms with specific functionalities by a rational usage of such information. It is well known that there is a large room for improvement concerning the available computational tools and the limitation in storage capacity, coherence and interoperability of the existing databases. This is a main target of the international efforts in bringing S.B to a mature state capable of producing highly reliable organism capable of performing all kinds of useful tasks. The work carried out during the duration of this project by our group has the potential of modifying substantially the state of the art in this realm.

One of the fundamental principles of synthetic biology is the construction of biological standardized parts and devices, which are interchangeable. A proper characterization of these parts and devices appears as a key issue in order to make them reusable in a predictive way. In the recent past scientists have witnessed several initiatives towards the design and fabrication of synthetic biological components and systems as a promising way to explore, understand and obtain beneficial applications from live. For instance, in the post genomic era one of the most fascinating challenges scientists are facing is to understand how the phenotypic behaviour of living cells arise out of the properties of their complex network of signalling proteins. While the interacting biomolecules perform many essential functions in these systems, the underlying design principles behind the functioning of such intracellular networks still remain poorly understood. Several initiatives have been reported in this line of thought to uncover some key working principles of such genetic regulatory networks via quantitative analysis of some relatively simple, experimentally well characterized, artificial genetic circuits.

The desired performance of these synthetic networks and in turn the resultant phenotype is strongly dependent on the expression level of the corresponding genes, which is further controlled by several factors such as promoter strength, cis- and trans-acting factors, cell growth stage, the expression level of various RNA polymerase-associated factors and other gene-level regulation characteristics. Thus, one important ingredient to elucidate gene function and genetic control on phenotype would be to have access to well-characterized promoter libraries. These promoter libraries could be in turn useful for the design and construction of novel biological systems.

Recently a methodology (<http://partsregistry.org/Measurement>) has been reported to characterize the activity of promoters in the Registry of Standard Biological Parts

(<http://partsregistry.org>) by using two different cell strains. As a part of our work **UPVLC** proposed the use of a synthetic gene regulatory network as a framework to characterize different promoter specifications by using a single-cell strategy. In this context characterization stands for evaluating the parameters of a query promoter as compared to a standard promoter acting as a “scale”.

A proper promoter characterization is an essential step towards a realistic standardization. Once this step is accomplished S.B will arrive to a new stage where simplicity will allow the massive design of organisms. This desired transition will provide society a powerful tools that could be employed to address several important goals such as the massive production of biofuels or the reduction of atmospheric pollution.

HZI developed ToBiN, which is a collection of computational tools for the genome-wide study of microbial physiology. Currently the platform ToBiN contains several modules articulated in a way that allows them to go far beyond the exposure of annotation flaws and to reach a transversal view of the interaction’s hierarchy, from regulatory circuits to host-pathogen relationships. Among the several components supplied by the platform, the one most-likely to be used by the highest number of modules is a Visualization Engine able to render a representation of the genome-wide physiological organization of the cell. This visual component can be panned and zoomed in a Google-Maps™ fashion and, whenever connected to modules generating data-sets with values mappable to a particular compound, reaction or gene, is able to overlay graphical representations (e.g. heatmaps) of selected quantitative and/or qualitative data-sets. An example of the utility of the Visualization Engine would be on visually-aiding the perception of the correspondence between a metabolic flux distribution and transcription levels for the various pathways.

Developing automatic design tools was the goal of **CNRS** partner, who have to aid in the engineering of gene and RNA circuits from modular components. Their modules will be an important component in the development of a language for SB. They have further developed computational tools to be added as part of the SynBio Toolbox (design gene networks: Genetdes software; design gene networks from assemblies of SBML parts: Asmparts software). **CNRS** also set a Working Group and a Workshop Series on Computational Frameworks and Tools for SB, and contributed to the SynBio Toolbox, a dynamic repository for modelling and design tools in Synthetic Biology, with the PROTDES, Genetdes and Asmparts tools. Finally, they contributed with the *ad-hoc*

developed tool Desharky to automatically design biodegradation pathways using a database of enzymes. The Kegg database has been used as a proof-of-concept, but the tool could be adapted to any database.

The **Imperial College** group participates in terms of setting up a repository of modelling frameworks for SB and developing an open-source system for collaborative tool development and problem solving in SB. Their study shows that the genetic circuit model effectively describes accurately the function of the system and its dynamics providing a solid basis for a systems understanding of the metabolism of important pollutants, such as toluene and xylenes. Also, in the side of modeling, **GA** contributed with the development of foundational tools and concepts in accordance with its firm technical expertise in DNA synthesis methodology. Within this task a number of straight forward developments and applications have been initiated, and existing processes and tools have been expanded and adjusted to the requirements of environmental and general SB (methods, vectors, computer programs, etc.), which have immediately been implemented into the company's technology platform to expand the service portfolio. Further, partner **GA's** tightened strategic gene synthesis market position provided access to many industrial, academic and governmental SB stakeholders, opening opportunities for a continuous dialogue with different experts from diverse disciplines. As illustrated by the comprehensive list of dissemination activities, addressing the scientific community, the industry sectors, civil society, policy makers, and students, this dialog has been maintained and is further continued in order to address and influence social, ethical and regulatory issues, as well as to trace potential market opportunities in environmental SB. The dissemination activities were mainly related to inform scientific and industrial researchers and developers about technological opportunities and applications (large scale gene synthesis, gene assembly, directed evolution, enzyme evolution, etc.), to discuss regulatory (e.g. Biosecurity/Biosafety) and financial (funding) issues and for teaching purposes. In addition, the engagement in synthetic biology resulted in participation in and even foundation of interest groups (IGSC, SBIA, BioM-WB, DECHEMA work group) involved

in regulation, cooperation, information, funding and other related activities on national and international levels. The topics addressed within this project and the activities initiated and concluded represent an integral part of the ongoing development of SB in Europe that is far from being finalized. The development of SB not only provides new and exciting opportunities and research potential for science but it also represents a very promising economical prospect. The results of this project directly contribute to the technological basis required to take advantage of this prospect, although the actual demand and market for commercial SB projects is limited.

Databases are an integral part SB. CNIO has developed Bionemo database (Figure 2), which stores manually curated information about proteins and genes directly implicated in the Biodegradation metabolism. When possible, the database also includes information on sequence, domains and structures for proteins, as well as regulatory elements and transcription units for genes.

Figure 2. Example of information retrieval using the Bionemo web site.

CNIO accomplished the proposed objectives on WP4, leading to an important advance on the development of SB-related databases. First, the creation of a corpus of document relevant to Biodegradation metabolism and regulation. It will be necessary to

generate a set of documents enriched in the desired information. This task, which was completed in the previous reporting period, was extended for the generation of a bibliome (literature collection) consisting in all the articles relevant for any given bacterium. This methodology will be tested in the contexts of the MICROME project. Second, a database containing all the knowledge on biodegradation reactions was created and the Bionemo database created. Bionemo can be accessed via its web site (<http://bionemo.bioinfo.cnio.es>). The web server implements a simple search interface that allows simultaneously querying all the biological entities described above. The results are shown categorized by tabs representing classes containing the entity types (reactions, complexes, etc.). From the results page, the user can easily access entity-specific pages, in which all information available is summarized. Links to external databases including the original UM-BBD metabolic information, GenBank and Uniprot, the NCBI Taxonomy database for microbial species, and the PubMed references to the original information sources, are provided. In addition to the Web site access described above, currently Bionemo can be downloaded as a SQL dump and installed locally. A Perl API (application program interface) is also provided (<http://bionemo.bioinfo.cnio.es/api.html>). A REST service (a key design idiom that embraces a stateless client-server architecture in which the web services are viewed as resources and can be identified by their URLs) is currently integrated in the Biological Web service Proxy (<http://code.google.com/p/bwsproxy/>). This is a free resource developed by CNIO which main goal is to speedup the responses from different web services related with biology, bioinformatics and synthetic biology. The proxy catches several operations that highly demand computational resources. Finally, a MaDAS system was implemented. The main goal of MaDAS (<http://madas2.bioinfo.cnio.es>) is to allow users to add their own annotations. A project is a unit that typically stores different annotations related to one genome or stores annotations related to a particular issue across several genomes. This is the case of the TARPOL project where biodegradation related annotations were collected in several microbial species. A Bionemo plug-in that connect MaDAS with the Bionemo database was created. Through this plug-in the annotations stored in Bionemo are now also available in MaDAS and can be retrieved in DAS format using the embed MaDAS DAS server.

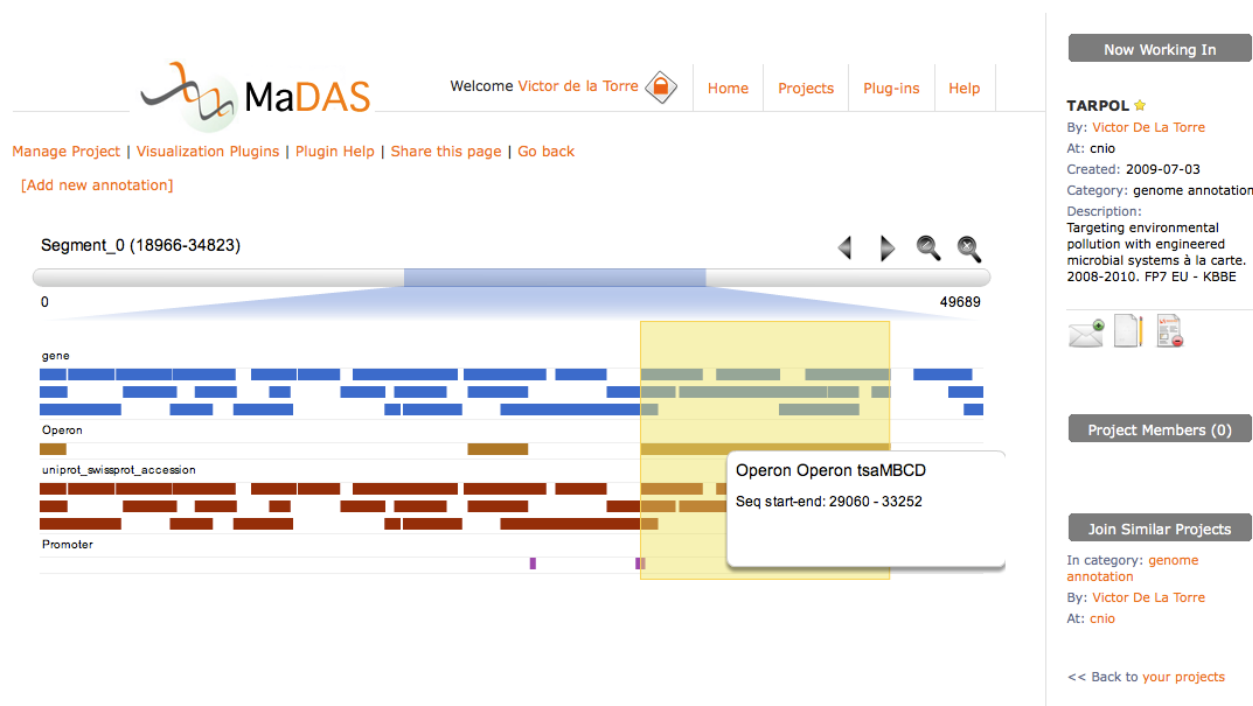


Figure 4. TARPOL project in MaDAS, organism: *Pseudomonas putida* F1. The operon tsaMBCD is represented in the MaDASmap plug-in

The design of metabolic pathways for “Biochemical Building Blocks” has been identified as foreseen applications in SB by IDC. Instead of searching for particular biomaterials or compounds, IDC has suggested to look for useful biochemical “building blocks” and their potential to serve as a starting material for a larger group of derivatives. Many of those building blocks have excellent potential to compete with petrochemical equivalents, as many new products are possible with novel functionality or new applications.

TARPOL is also contributing to the exchange of knowledge among European researchers working on SB through the organization of several workshops and conferences. Among these activities CNRS-IHPST organised a two-day workshop in Paris, in the Ecole Normale Supérieure, gathering historians, philosophers and biologists to evaluate the place of SB among other biological disciplines, and its novelty; HZI organized a special session on the topic Computational Design Tool for Synthetic Biology within the BioPathways meeting in Stockholm; and UPVLC organized a symposium on SB

titled “III Jornadas Internacionales de Biología Sintética”; least but not last, **UVEG** in coordination with **HZI** is organizing a workshop to foster the discussion on minimal cells and its applications to take place next November. **CSIC** will organize the 5th Meeting of the Spanish Network of Systems Biology in December 2009 on the general topic “Fostering Systems and Synthetic Biology in Southern Europe.”

1.4 Potential impact and the main dissemination activities and exploitation of results

Training and dissemination activities on SB are also an important subject of TARPOL project. In this vein, **UMIL** has scheduled a course entitled “Evolution and Design of Biomolecular Systems: Concepts and strategies for systems and synthetic biology”, which took place in Illetes-Mallorca (Spain) with an outstanding panel of speakers. Many dissemination activities have been carried on by the diverse partners of TARPOL. These include participation in scientific meetings, peer-reviewed articles, conferences to the general public, and science popularization articles, among others (Figure 3). A web site for the TARPOL Project (<http://www.sb-tarpol.eu/>) has been designed, and is under continuous update. It contains public and private sections.

UPVLC has also participated intensively in the organization of dissemination activities. Institute Cavanilles (**UVEG**) and Intertech Group of **UPVLC** co-organized the Summer Course on Synthetic Biology. This activity was developed from two sides : training and motivational. For this reason, the program combined the expertise of the organizers to train scientists from an interdisciplinary perspective, in order to offer a range of activities for young researchers from a wide range of fields. The Summer Course on Synthetic Biology conference also included lectures by the leading scientists of this new discipline on the constitutive principles underlying SB, which allowed attendees to see and understand the great possibilities of this new scientific area. The laboratory practice of SB - "wetlab" - aimed at researchers from technological branches who had no expertise, with the working principles of a molecular biology laboratory. Practices of "drylab" aimed at students from Life Sciences, with the intent to convey the basics of computer design in SB. The result of such and initiative will hopefully be a motivated group of young researchers in the field leading to its desired expansion. The participation in dissemination activities not directly supported by TARPOL consisted on the preparation of two courses

on synthetic Biology (2008 and 2009) and the organization of two days conferences on the same topic during the three years of the project.

Finally, the ethical, human practices and safety issues have been approached in an unprecedented way. The US Presidential Commission for the Study of Bioethical Issues held its first meeting on July 8-9 in Washington, DC. The primary topic was synthetic biology. Speakers included Craig Venter, Drew Endy, George Church, and other leaders in synthetic biology, as well as experts in ethics, policy, regulation and government. TARPOL partner Markus Schmidt from IDC was the only European speaker at this meeting, giving an overview of completed, ongoing and planned activities about societal implications of synthetic biology in Europe. (See agenda: <http://www.bioethics.gov/meetings/070810/>). The work performed on economic, environmental and ethical implications of synthetic biology applications in environmental biotechnology has been reviewed in a comprehensive report, which is one of the most complete reviews on the societal and environmental implications of a novel technology. The report deserves the following exhaustive summary:

The report led by IDC and prepared in collaboration with a group of partners (BU, UVEG, UNIVE, CNRS-ENS, IP and CEA), tries to give a glimpse into the future of synthetic biology (SB) and its potential applications in the area of environmental biotechnology. There are a number of applications where SB could well make a difference in order to transfer our society to become more economical and environmental sustainable. In this report we have highlighted 4 major areas (biofuels, bioremediation, biomaterials and novel developments in SB) with a total of 20 specific applications where SB has a great likelihood to improve currently available technologies. Each of the 20 applications has been assessed in detail in order to find out (1) to what extent SB could improve current technologies; (2) what the economic impact of SB could be; (3) what the environmental benefits and downsides could be and (4) whether any social or ethical problems would be created, exacerbated or improved. This assessment is intended to support national and international funding agencies in their decisions to allocate resources to SB-based biotech applications while taking into account any foreseeable economic, environmental and social/ethical issues. Our outlook is based on the current scientific state-of-the-art, however, there is of course a notable degree of uncertainty about future development paths which we have to acknowledge when giving recommendations for what we see as the most promising directions for SB in environmental biotech.

Biofuels

We are convinced that synthetic biology can help to produce state-of-the-art and next generation biofuels. Current efforts are mainly targeted towards an improved production of bio-ethanol from agricultural products, although we see significant problems with this approach as ethanol exhibits some technical problems (miscible with water, limited use in existing engines). Other non-ethanol biofuels such as bio-butanol or biodiesel are much better suited to replace petroleum-based gasoline, as their chemical properties resemble it much closer. Synthetic biology could help to overcome current impasses in the production of butanol and other non-ethanol fuels, namely poor fermentation yield and toxicity to butanol-producing microorganisms. One problem that is faced by most biofuels produced from plant material is the limitation of the use of hemi- and lignocellulosic material. Any improvement in that area would definitely increase economic feasibility of biofuel production. One important problem will arise, should synthetic biology be able to provide a solution to the technical problems just mentioned, namely that more and more agricultural land will be devoted to plant energy-crops instead of food crops. In order to avoid this competition for food, we suggest to use also non-food-competing biological resources such as perennial plants grown on degraded lands abandoned from agricultural use, crop residues, sustainably harvested wood and forest residues, double crops and mixed cropping systems, municipal and industrial wastes.

In contrast to agricultural based ethanol, biodiesel and butanol, there is also algae based biofuels and biohydrogen. Current concepts foresee a significant advantage of algae-based biofuels over agriculture-based biofuels, because of higher yield per area and the independence of arable land, and clean water. First calculations predict, however, that future algae production systems will only be economically feasible if the price for one barrel oil is constantly above 70U\$ and if the production systems entails at least an area of 200ha. The capital costs of such large production facilities will probably lead to an exclusion of SMEs and play in favour of "big oil". Still, algae production systems could be a highly promising avenue of future fuel production, once major obstacles are solved dealing with algae genomics, metabolism and harvesting. Although bio-hydrogen has been praised as an extremely promising fuel by many scientists, our assessment is more cautious. Hydrogen is only useful as fuel if large changes in infrastructure take place (distribution and storage system, new fuel cell engines), and point to a more distant future

beyond 2050, also termed as the hydrogen economy. Although synthetic biology could well contribute to improve yield of hydrogen producing cyanobacteria, the actual impact of hydrogen in society and economy depends much more on other areas such as infrastructure. Finally we analysed the prospects of microbial fuel cells (MFC), as energy converter. Although we see MFCs as extremely promising and an area where synthetic biology could contribute a lot, it will most likely be applied in some niche markets and areas of application, rather than large scale deployment due to the limited energy production.

Bioremediation

Bioremediation is an area with a great potential of benefits provided by Synthetic Biology. Bioremediation is usually applied on materials with a massive occurrence such as solid (organic) wastes, sewage, industrial waste water, contaminated soil or contaminated ground water, any of them measured in millions of tons or cubic meters. We believe that SB has the potential to create tools to improve the treatment methods, saving costs and environmental resources. Moreover, it can provide methods to produce energy or valuable goods from waste or wastewater. It can also provide tools for making up fresh or drinking water either from contaminated water or seawater. Another possible field of application is the production of biosensors to monitor environmental goods and hazards. At a differentiated evaluation, we have concluded that biosensors provided by SB-tools would have a great positive effect on the environment since they will help to survey environmental hazards more precisely and effectively. However, their economic and social impact is rather low because they can be considered as niche products.

Synthetic Biology based approaches may provide a way of capturing, storing and recycling carbon dioxide. This may be through the re-engineering of existing organisms or the creation of novel carbon processes especially using bottom up approaches where inorganic chemistry is linked to living processes through agents such as the emerging protocell technology.

Synthetic Biology based carbon capture may not be able to sink carbon dioxide to completely remediate the current escalating levels that are being released through fossil fuel consumption as geoengineering scale approaches are necessary but they can offer the possibility of carbon capture and recycling which current industrial scale processes cannot do.

It is recommended that because of the scale of the problem with carbon dioxide emissions and the urgent need for remediation that Synthetic Biology approaches are supported in order to develop the next generations of carbon capture technologies which will do more than store the carbon dioxide but recycling it into fuels and biopolymers with positive environmental impact.

Another positive impact, particularly to the environment, can be expected for soil and ground water remediation, especially with regard to the enhancement of the clean-up efficiency and to the development of new methods. On this field, the economical and social impacts are rather moderate, since it is a specific field with a limited scope of time.

The strongest impact we expect is for solid waste and wastewater treatment and for water desalination. The importance of the latter cannot be overstated in a world, where billions of people have no access to clean drinking water or to fresh water for agricultural use. Solid waste and wastewater treatment also bear a great potential for improvement by Synthetic Biology due to their sheer amount and to their considerable organic content. We therefore strongly recommend to support the development of these 3 issues. However, a possible constraint should be mentioned: solid waste and waste water can not be treated in sealed vessels or rooms, simply due to their huge amount. They have to be treated openly in piles or basins. Therefore, the use of engineered cells may create a problem of interaction with the environment, which has to be kept in mind. But we expect no limitations for the use of non-proliferative systems like enzymes or protocells created with the aid of Synthetic Biology.

Biomaterials

Synthetic Biology will have a significant impact on the biomaterials market particularly in the areas of fine chemicals and bioplastics. A tool box of products that will act as biodegradable materials is recommended. The bulk chemicals industry will also be significantly affected by the Synthetic Biology based technology but uptake and therefore environmental impacts will be slower although when new practices are adopted changes will last longer and take place on a much larger scale. In fine chemicals industry the incentives for investment relate to the economic potential of the end product (in contrast to bulk chemical manufacturing). The payoffs could predominantly have environmental impacts although these may be significantly limited to more efficient use of energy since the core manufacturing practice relies on petrochemicals. There is also limited potential

for Synthetic Biology based techniques to have an impact on avoiding recalcitrant molecules in the production process. Nonetheless investment in Synthetic Biology based processes in bulk chemicals is likely to have a positive overall impact on the manufacturing systems used in the running of the plant such as, use of biodiesel and less overall chemical waste and because of the scale at which these processes take place, small changes may have significant positive environmental impacts. For both fine and bulk chemical production we recommend the deployment of the „Chemical Building Block System“ as designed (or similar to) the USDoE.

The field of biopolymers and bioplastics most urgently needs revisiting in terms of its current labeling for recycling purposes since categorization of the various products is extremely complex with negative economic consequences because of this (bioplastics are not necessarily biodegradable). We recommend application of a method through which those bioplastics that need recycling and those that can be composted are clearly recognizable, before large scale use of Synthetic Biology for production of bioplastics take place. An urgent need to develop completely biodegradable plastics exists which would benefit from focused Synthetic Biology research and development into this area. Additionally there is also a pressing need for high performance structural bioplastics for manufacturing coupled with completely biodegradable additives. Both of these significant growth areas in the bioplastics industry could be greatly improved by Synthetic Biology based research.

Investment is particularly needed in research and development for new methods and products that will expand and develop tools and manufacturing processes with reduced environmental impact compared with the current manufacturing approaches although adequate biosafety issues on large scale manufacturing units need to be established. Cellulosomes (complex molecules that degrade hemi- and lignocellulosic material) possess high economic potential for biofuels, paper and waste processing. Synthetic Biology has the potential to design more efficient and completely new cellulosome complexes to make new, efficient cellulose digesting proteins. Open sourcing of the cellulosome technology is recommended owing to the justice of distribution issues involved in the technology.

Novel developments in SB

Protocell technology represents a bottom up approach to Synthetic Biology bridging inorganic and organic processes. Protocell technology enables better understanding of Synthetic Biology as a whole to develop new technologies. Although the research is in an

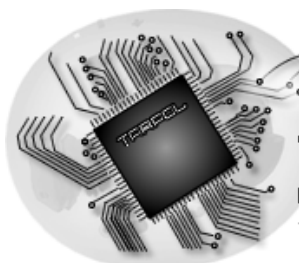
early stage, the development of potentially radically novel and significant environmental interventions e.g. for remediation of carbon emissions and alternative biofuels technology seem feasible. Investment in basic science to underpin the research and support it whilst imminent private investment is happening is strongly recommended.

Protocell technology has a huge potential to offer radically different tools and methods than previously encountered with Synthetic Biology based approaches because of its bottom up nature and because of its overlaps with basic chemistry. A toolbox of potential products and investigation of issues related to open sourcing the technology should also be looked into. Xenobiology (also known as chemical synthetic biology) is another bottom up approach to design and construct radically new biological systems with properties not found in nature. Using e.g. non-canonical amino acids, alternative base pairs to enlarge the genetic alphabet, or different chemical backbones in a xenonucleic acid, these chemically modified organisms and systems will enable a much higher level of biosafety when using engineered biosystems for or in the environment. For example novel enzymes (such as amylase) with non-canonical amino acids can be used to reduce the optimal temperature for breaking starch into glucose, thus saving enormous amounts of energy, contributing to a decrease in green house gas emissions. Organisms with an enlarged genetic alphabet or a DNA with a different chemical backbone could be designed by Synthetic Biology in order to impede horizontal gene transfer and genetic pollution between engineered and natural organisms. Similar to protocells, xenobiology is in a very early stage of development and requires increased support for basic research in order to be able to achieve radically new concepts and applications.

Table A summarises our assessment of each of the 20 applications.

Table A: Overview of assessment of potential synthetic biology applications in environmental biotechnology for the next 10-15 years.

	Opportunity for SB contribution	Economic benefit	Environmental impact	Ethics and social impact	Overall assessment
Biofuels					
Ethanol	○	○	○	○	○
Non-Ethanol	●	○	○	○	○
Algae-based fuels	○	●	○	○	○
Bio-hydrogen	○	○	●	○	○
Microbial fuel cell	○	○	○	○	○



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Bioremediation					
Biosensors	●	○	●	○	●
Soil and ground water remediation	○	○	●	○	○
Water desalination	○	●	○	●	●
Soil remediation	○	○	○	○	○
Solid waste	○	○	○	○	○
CO2 recapturing	○	○	○	○	○
Biomaterials					
Bioplastics	●	●	○	○	●
Bulk chemicals	○	○	○	○	○
Fine chemicals	●	●	○	○	●
Cellulosomes	○	●	○	○	○
Novel developments					
Protocells	●	○	○	○	○
Xenobiology	●	○	○	○	○

Key:

○ Low or even negative impact, no or hardly any improvement

○ Rather low impact, some improvement

○ Positive impact, notable improvement

● Excellent, significant improvement

1.5 Address of the project public website

<http://sb-tarpol.org>

2 Use and dissemination of foreground¹

The Tarpol results of the TARPOL project, from experimental procedures, to databases, websites, meetings and conferences, students training including iGEM and others can be summarized in the 161-references list of TARPOL-related dissemination activities shown below.

¹ A plan for use and dissemination of foreground (including socio-economic impact and target groups for the results of the research) shall be established at the end of the project. It should, where appropriate, be an update of the initial plan in Annex I for use and dissemination of foreground and be consistent with the report on societal implications on the use and dissemination of foreground (section 4.3 – H).

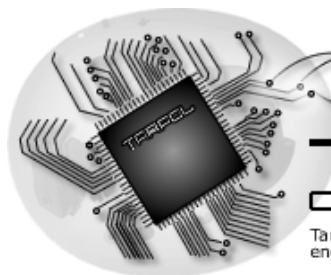
The plan should consist of:

☐ Section A

This section should describe the dissemination measures, including any scientific publications relating to foreground. Its content will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Union.

☐ Section B

This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Union



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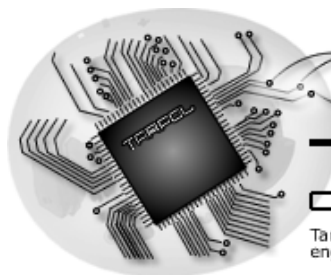
2.1 Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Perman identifier
1	From a consortium sequence to a unified sequence: The <i>Bacillus subtilis</i> 168 reference genome a decade later	V Barbe, S. Cruveiller, F. Kunst, P. Lenoble, G. Meurice, A. Sekowska, D. Vallencet, TZ Wang, I. Moszer, C. Médigue, A. Danchin	Microbiology	N° 155			2009	1758-1775	
2	Novel auto-inducing expression systems for the development of whole-cell biocatalysts	Di Gennaro P, Ferrara S, Bestetti G, Sello G, Solera D, Galli E, Renzi F, Bertoni G	Appl. Microbiol Biotechnol.	79			2008	617-625	
3	Modular Model-based Design for heterologous bioproduction in Bacteria.	Landrain TE, Carrera J, Kirov B, Rodrigo G, Jaramillo A	Current Opinion in Biotechnology	20			2009	272-279	
4	Towards the automated engineering of a synthetic genome	Carrera J, Rodrigo G, Jaramillo A	Mol. Biosyst.	5			2009	733-743	
5	Challenges in the computational design of proteins	Suarez M, Jaramillo A	J R soc Interface	Suppl 4			2009	5477-5491	

² A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

³ Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.



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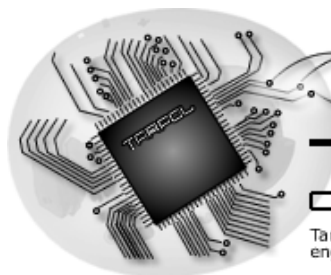
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Permissible availability
6	Global Sensitivity Analysis Challenges in Biological Systems Modeling.	Kiparissides A, Kucherenko, SS, Mantalaris A, Pistikopoulos EN	Industrial and Engineering Chemistry Research				7 July 2009		DOI: 10.10139
7	Dynamic Modeling of the Pr-Ps node of the TOL Plasmid Regulatory Network for m-Xylene Biodegradation.	Koutinas M, Lam M-C, Kiparissides A, Silva-Rocha R, Godinho M, Livingston AG, de Lorenzo V, Pistikopoulos EN, Martins dos Santos VAP, Mantalaris A,	Molecular Systems Biology						
8	Bridging the Gap between Genetic Circuit and Microbial Growth Kinetic Models.	Koutinas M, Kiparissides A, Lam M-C, Silva-Rocha R, Godinho M, de Lorenzo V, Livingston AG, Martins dos Santos VAP, Pistikopoulos EN, Mantalaris A,	Biotechnology and Bioengineering						
9	A modular synthetic device to calibrate promoters.	JF Urchueguía et al.	Bioinformatics						
10	Metabolic flux analysis of the hydrogen production potential in synechocystis sp. PCC6803.	JF Urchueguía et al.	International Journal of Hydrogen Energy						
11	Yeast cultures with UCP1 uncoupling activity as a heating device.	JF Urchueguía et al.	New Biotechnology						
12	Bionemo molecular information on biodegradation metabolism	Carbajosa G, Trigo A, Valencia A, Cases I	Nucleic Acids Res.	37			January 2009	D598-602	
13	Systemic approaches to biodegradation	Trigo A, Valencia A, Cases I	FEMS Microbiology Rev.	33			2009	98-108	
14	A critical perspective on synthetic biology.	Michel Morange	HYLE: International Journal for Philosophy of chemistry	Vol. 15 n° 1			2009	21-30	
15	A new revolution? The place of systems biology and synthetic biology in the history of biology.	Michel Morange	EMBO reports	10, S1			2009	S60-S53	°



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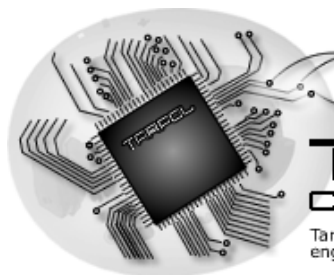
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Permissible identifier available
16	Recombinant bacteria for environmental release: what went wrong and what we have learnt from it	de Lorenzo, V.	Clinical Microbiology and Infection	1			2009	63-65	
17	Microbial responses to environmental arsenic	Páez-Espino, D; Tamames, J.; de Lorenzo, V., Canovas, D.	BioMetals	22			2009	117-130	
18	Microbial systems biology: bottom up and top down.	de Lorenzo, V; Galperin, M.	FEMS Microbial Rev.	33			2009	1-2	
19	Systems Biology approaches to Bioremediation	de Lorenzo, V.	Curr Op Biotech	19			2008	579-89	
20	Synthetic biology: discovering new worlds and new words. The new and not so new aspects of this emerging research field	de Lorenzo, V. and Danchin, A.	EMBO Rep	9			2008	822-827	
21	Stable implantation of orthogonal sensor circuits in Gram-negative bacteria for environmental release.	de las Heras, A; Carreño, CA; de Lorenzo, V	Env. Microbiol	10			2008	3305-3316	
22	Transcriptional wiring of the TOL plasmid regulatory network to its host involves the submission of the -promoter Pu to the response regulator PprA.	Vitale, E.; Milani, A.; Renzi, F.; Galli, E.; Rescalli, E; de Lorenzo, V. Bertoni, G	Mol.Microbiol.	69			2008	698-713	
23	Evidence of in vivo cross-talk between the nitrogen-related and fructose-related branches of the carbohydrate phosphotransferase system of Pseudomonas putida	Pflüger, K; de Lorenzo, V.	J. Bacteriol.	190			2008	3374-3380	
24	Mining logic gates in prokaryotic transcriptional regulation networks	Silva-Rocha, R. and de Lorenzo, V	FEBS Lett	582			2008	1237-44	
25	Tracing explosives in soil with transcriptional regulators of Pseudomonas putida evolved for responding to nitrotoluenes.	Garmedia, J.; de las Heras, A.; Calcagno Galvão, T.; de Lorenzo, V	Microb. Biotech	1			2008	236-246	
26	Reduced evolvability of Escherichia coli MDS42, an IS-less cellular chassis for molecular and synthetic biology applications.	Umenhoffer K., ... Pósai Gy.	Microb Cell Fact.	9:38.	BioMed Central Ltd.		2010	pp. 1-12	http://microbialcellfactories.biomedcentral.com/content/9/1/38



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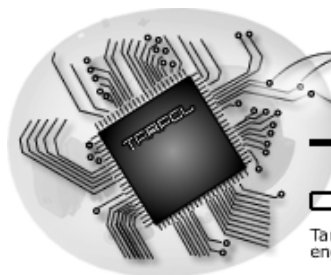
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27	Systematic Reduction of Microbial Genomes	Fehér T	Microbial Systems Biology - New Directions and New Opportunities.	Book chapter	John Wiley and Sons	Weinheim, Germany	2010		ISBN 978-3-527-32470-4
28	Challenges in the computational design of proteins.	Alfonso Jaramillo	Journal of the Royal Society Interface	doi: 10.1098/rsif.2008.0508.focus			2009		
29	Modular Model-based Design for Heterologous Bioproduction in Bacteria.	Alfonso Jaramillo	Current Opinion in Biotechnology	DOI:10.1016/j.copbio.2009.06.003.			2009		
30	Towards the automated engineering of a synthetic genome". Molecular BioSystems. 2009. DOI: 10.1039/B904400K.	Alfonso Jaramillo	Molecular BioSystems	DOI: 10.1039/B904400K			2009		
31	Optimal viral strategies for bypassing RNA silencing	S. Elena	J. R. Soc. Interface				2010		
32	Robust Dynamical Pattern Formation from a Multifunctional Minimal Genetic Circuit.	A. Jaramillo	BMC Systems Biology				2010		
33	Using multi-objective computational design to extend protein promiscuity	A. Jaramillo	Biophys Chem.				2010		
34	Reverse-engineering the Arabidopsis thaliana transcriptional network under changing environmental conditions	S. Elena	Genome Biol.				2009		
35	Genes that move the window of viability of life: lessons from bacteria thriving at the cold extreme.	de Lorenzo, V.	BioEssays	(In press)			2010		
36	Beware of metaphors: chasses and orthogonality in Synthetic Biology	de Lorenzo, V	BioEngineered Bugs	(in press)			2010		



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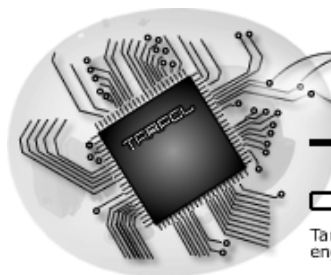
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37	Environmental biosafety in the age of Synthetic Biology: Do we really need a radical new approach?	de Lorenzo, V	BioEssays	(in press)			2010		
38	Regulatory exaptation of the catabolite repression protein (Crp)-cAMP system in <i>Pseudomonas putida</i> .	Milanesio, P., Arce, A., Muñoz, A., Calles, B. and de Lorenzo, V.	Environ Microbiol.	(in press)			2010		
39	An electronical device from a biofilm structure created by bacterial activity.	Castellón, E., Chavarría, M., de Lorenzo, V., Zayat, M., Levy, D.	Advanced Materials	(in press)			2010		
40	Engineering input/output nodes in prokaryotic regulatory circuits.	de las Heras, A, Carreño, CA, Martínez, E and de Lorenzo, V	FEMS Microbiology	34(5)			2010	842-65	
41	The regulatory logic of <i>m</i> -xylene biodegradation by <i>Pseudomonas putida</i> mt-2 exposed by dynamic modelling of the principal node <i>PsPr</i> of the TOL plasmid.	Koutinas, M.; Lam, M.C.; Kiparissides, A.; Silva-Rocha, R.; Godinho, M.; Livingston, A.G.; Pistikopoulos, E.N.; de Lorenzo, V.; Martins dos Santos, VAP; Mantalaris, A.	Env. Micro	12			2010	1705-1718	
42	EnvMine: A text-mining system for the automatic extraction of contextual information.	Tamames, J; de Lorenzo, V.	BMC Bioinformatics	11			2010	254	
43	Noise and robustness in prokaryotic regulatory Networks	Silva-Rocha, R.; de Lorenzo, V.	Ann Rev Microbiol	(in press)			2010		
44	Microbial bioremediation of chemical pollutants: How bacteria cope with multi-stress environmental scenarios.	de Lorenzo, V.; Loza-Tavera, H.	Bacterial Stress Responses (Gisela Storz and REgine Hengge, Eds) ADM Press, Washington DC				2010		
45	Sensing xenobiotic compounds: lessons from bacteria that face pollutants in the environment.	de Lorenzo, V.; Silva-Rocha, R.; Carbajosa, G.; Galvão, TC; and Cases I	Sensory Mechanisms in Bacteria (Ed. S. Spiro and R. Dixon) Horizon Scientific Press, Norwich				2010		



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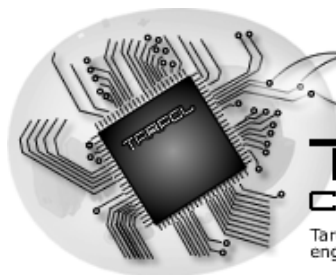
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Per identifier available
46	Xenobiology: a new form of life as the ultimate biosafety tool.:	Schmidt M	BioEssays	Vol.32(4)	wiley		2010-04	322-331	
47	Special issue: societal aspects of synthetic biology	Schmidt M	Systems and Synthetic Biology	Vol.3(1-4):	Springer		2009	1-2	
48	A Priority Paper for the Societal and Ethical Aspects of Synthetic Biology	Schmidt M et al.	Systems and Synthetic Biology	Vol.3(1-4):	Springer		2009	3-7	
49	Of Newtons and Heretics	Ganguli Mitra A, Schmidt M et al.	Nature Biotechnology	Vol 27(4):	Nature		2009	321 - 322	
50	Living Technology: 5 Questions	Schmidt M			Automatic Press/VIP		2010-08	155-166	
51	Global Sensitivity Analysis Challenges in Biological Systems Modeling	Alexandros Kiparissides	Industrial and Engineering Chemistry Research	No 48, August 2009	American Chemical Society	Washington	2009	pp. 7168-7180	
52	The regulatory logic of m-xylene biodegradation by <i>Pseudomonas putida</i> mt-2 exposed by dynamic modelling of the principal node Ps/Pr of the TOL plasmid	Michalis Koutinas	Environmental Microbiology	No 12, June 2010	John Wiley & Sons, Inc.	New Jersey	2010	pp. 1705-1718	
53	Identification of genes regulated by the MvaT-like paralogues TurA and TurB of <i>Pseudomonas putida</i> KT2440	Francesco Renzi, Emanuela Rescalli, Enrica Galli Giovanni Bertoni	Environmental Microbiology (2010),	No 12(1), January 2010	Wiley-Blackwell		2010	pp. 254-263	http://ary.wdoi/10.11462-2920.064.x
54	Intracellular excision and reintegration dynamics of the ICEclc genomic island of <i>Pseudomonas knackmussii</i> sp. strain B13	Sentchilo, V.	Molecular Microbiology	72(5),	Blackwell Publishing Ltd		2009	1293-1306	



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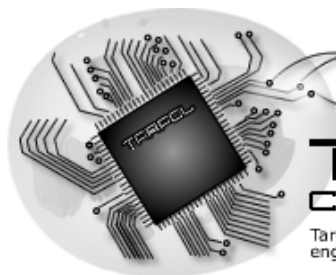
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55	Dual origin of transfer system of the ICE _{Ec} genomic island of <i>Pseudomonas knackmussii</i> B13	Miyazaki, R.	Molecular Microbiology	In revision	Blackwell Publishing Ltd		2011?		
56	Bacterial Sensors: Synthetic design and Application Principles	Van der Meer, J. R.	Synthesis Lectures on Synthetic Biology	In revision	Morgan & Claypool		2011?		



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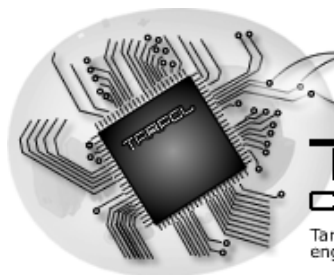
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57	<i>Metabolic flux analysis of the hydrogen production potential in Synechocystis sp. PCC6803</i>	E.Navarro	<i>International Journal of hydrogen energy</i>	<i>No 34, 2009</i>	<i>Elsevier</i>		<i>2009</i>	<i>pp. 8828-8838</i>	http://encece.com/science/article/pii/S0360591709000000



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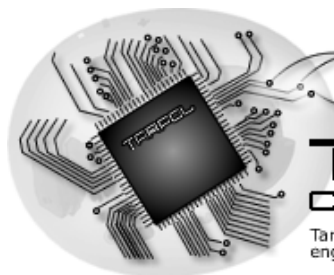
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Permanently available
58	Yeast cultures with UCP1 uncoupling activity as a heating device	J.Urchueguía	New Biotechnology	No 6 2009	Elsevier		2009	300-306	
59	Flux coupling and transcriptional regulation within genome-scale metabolic model of photosynthetic bacterium <i>Synechocystis</i>	A.Monteagud	To be published						
60	Rational Organism network painter: Una herramienta optimizada de visualización de redes metabólicas de fácil uso	Jorge Garrido	To be published						
61	Modelo metabólico de un organismo fotosintético, una fuente de energía a partir de radiación solar.	Julian Triana	To be published						
62	Reconstruction and analysis of genome-scale metabolic model of a photosynthetic bacterium.	Arnau Montagud	To be published						
63	A modular synthetic device to calibrate promoters	J.Urchueguía	To be published						
64	Life with a few genes: a survey on naturally evolved reduced genomes	Luis R. Delaye	The Open Evolution Journal	4, 2010	Bentham Open	online	2010	pp. 12-22	http://nathan.toev.es/VOL1/EVOL
65	Evolution of prokaryote-animal symbiosis from a genomics perspective. In <i>(Endo)symbiotic Methanogenic Archaea</i>	Rosario Gil	Microbiology Monographs	19	Springer-Verlag	Berlin	2011 (accepted)	pp. 207-234	



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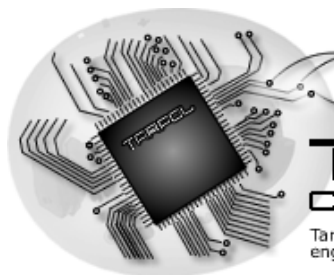
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Permitted identifier available
66	The Ethics of Protocells: Moral and Social Implications of Creating Life in the Laboratory. Edited by Mark A. Bedau and Emily C. Parke. Xii + 386 pp. Cambridge, MA: MIT Press. 2009.	Juli Pereto	American Journal of Human Biology	DOI: 10.1002/ajhb.21009		Published online 23 October 2009 in Wiley InterScience (www.interscience.wiley.com)	2009		
67	Should the Teaching of Biological Evolution Include the Origin of Life?	Antonio Lazcano, Juli Peretó	Evo Edu Outreach	3 DOI 10.1007/s12052-010-0285-2	Springer Science + Business Media, LLC 2010		Published online 8 October 2010	661-667	
68	Charles Darwin and the Origin of Life	Juli Peretó, Jeffrey L. Bada, Antonio Lazcano	Orig Life Evol Biosph	39 DOI 10.1007/s11084-009-9172-7			Published online 25 July 2009	395-406	
69	Defining Life or Bringing Biology to Life	Kepa Ruiz-Mirazo, Juli Peretó, Álvaro Moreno	Orig Life Evol Biosph	40 DOI 10.1007/s11084-010-9201-6	Springer Science + Business Media, B.V. 2010		Published online: 25 February 2010	203-213	



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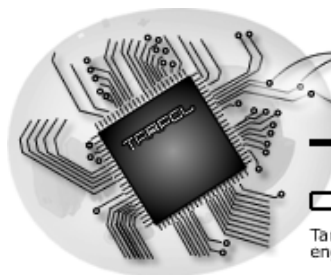
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Permissible availability
70	Effects of <i>Bacillus thuringiensis</i> δ -endotoxins on the pea aphid (<i>Acyrtosiphon pisum</i>).	Porcar, M	Applied and Environmental Microbiology.	75	Society for Applied Microbiology (USA)	USA	2009	4897-4900	
71	Yeast cultures with UCP1 uncoupling activity as a heating device.	Delás, M. Notari, J. Forés, J. Pechuan, M. Porcar, E. Navarro, A. Montagud, M. Baguena, J. Peretó, P. Fernández de Córdoba, M.M. González-Barroso, E. Rial, A. Moya and J. Urchueguía	New Biotechnology	26	Elsevier		2009	300-306	
72	Effects of <i>Bacillus thuringiensis</i> Cry1Ab and Cry3Aa endotoxins on predatory Coleoptera tested through artificial diet-incorporation bioassays.	Porcar, M, and Latorre, A.	Bulletin of Entomological Research	100	Cambridge journals online	OK	2010	297-302	
73	Beyond directed evolution: Darwinian selection as a tool for Synthetic Biology.	Porcar, M.	Systems and Synthetic Biology	4	Springer	USA	2010	1-6	
74	Paving the way for Synthetic Biology-based bioremediation in Europe.	Porcar, M. and Moya, A.	Microbial Biotechnology	3	Blackwell	Spain	2010	134-135	
75	Rice straw management: the big waste.	Porcar, M.	Biofuels, Bioproducts and Biorefining.	4	Society of Chemical Industry (SCI) and John Wiley & Sons Ltd.	USA	2010	154-159	
76	Craig Venter's synthetic bacteria: the dawn of a new era?	Porcar, M. and Moya, A.	Journal of Cosmology	8	Cosmology Science Publishers	USA	2010	article number 4	



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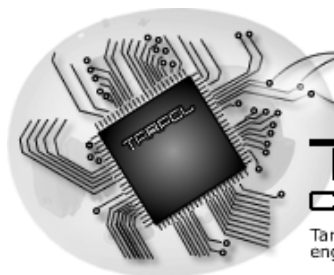
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Permissible identification available
77	On parts and organisms: implications for Synthetic Biology.	Porcar, M.	Symplectic Biology	N.A.	Fondation Fourmentin-Guilbert	France	2010		
78	Aequorin-expressing yeast emits light under electric control.	Porcar, M.	Journal of Biotechnology. In press	N.A.	Elsevier	USA	2010	N.A.	
79	Sins, ethics and Biology: a comprehensive approach.	Porcar, M,	Sins, ethics and Biology: a comprehensive approach	Complete book	Obrapropia editorial, Spain.	Spain	2009	Complete book	
80	La biología sintética frente al reto de la energía	L. Delaye, M. Porcar and A. Moya	XVII Foro Universitario Juan Luis Vives	Book chapter	Edited by J.L. Rubio	Spain	2009	Book chapter	
81	Genes that move the window of viability of life: lessons from bacteria thriving at the cold extreme.	V. de Lorenzo			<i>BioEssays</i> (In Press).		2010		
82	Beware of metaphors: chasses and orthogonality in Synthetic	V. de Lorenzo			<i>Biology. Bio Engineered Bugs</i> (In Press)		2010		
83	Environmental biosafety in the age of Synthetic Biology: Do we really need a radical new approach?	De Lorenzo, V.			<i>BioEssays</i> (In Press)				



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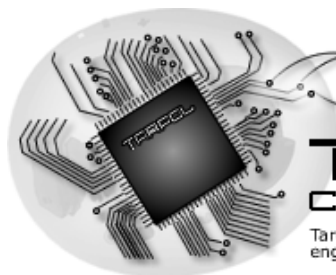
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84	Regulatory exaptation of the catabolite repression protein (Crp)-cAMP system in <i>Pseudomonas putida</i> . <i>Environ Microbiol</i> (In Press)	De Lorenz, V.			<i>Environ Microbiol</i> (In Press)		2010		
85	An electrooptical device from a biofilm structure created by bacterial activity.	De Lorenzo, V.			<i>Advanced Materials</i> (In Press).		2010		
86	Engineering input/output nodes in prokaryotic regulatory circuits.	V. de Lorenzo			<i>FEMS Microbiology Reviews</i>		2010	34(5):842-65.	
87	The regulatory logic of <i>m</i> -xylene biodegradation by <i>Pseudomonas putida</i> mt-2 exposed by dynamic modelling of the principal node <i>Ps/Pr</i> of the TOL plasmid. <i>Env Micro</i> 12: 1705-1718.	Mantalaris, A.			<i>Env Micro</i>		2010	12: 1705-1718.	
88	EnvMine: A text-mining system for the automatic extraction of contextual information.	V. de Lorenzo			<i>BMC Bioinformatics</i>		2010	11:294	
89	Noise and robustness in prokaryotic regulatory Networks.	V. de Lorenzo			<i>Ann Rev Microbiol</i> (In Press).		2010		
90	Microbial bioremediation of chemical pollutants: How bacteria cope with multi-stress environmental scenarios. In <i>Bacterial Stress Responses</i>	De Lorenzo, V.			ASM (Gisela Storz and Regine Hengge, Press, WashingtonDC.		2010		



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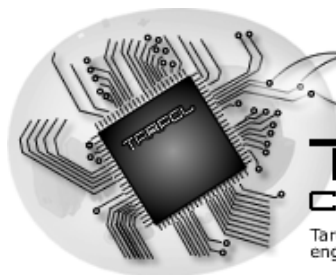
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91	Sensing xenobiotic compounds: lessons from bacteria that face pollutants in the environment In <i>Sensory Mechanisms in Bacteria</i>	De Lorenzo, V.			(Ed. S. Spiro and R. Dixon). Horizon Scientific Press, Norwich.			2010	
92	Extreme DNA bending: molecular basis of the regulatory breadth of IHF. In <i>Bacterial Chromatin</i>	De Lorenzo, V.			Ed. Charles Dorman Springer-Verlag Berlin Heidelberg (Chapter 16)			2009	



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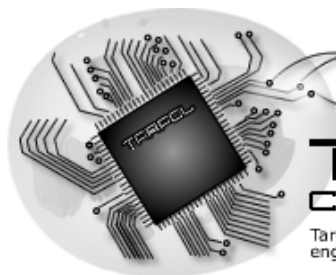
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93	Emerging Systems and Synthetic Biology Approaches to Hydrocarbon Biotechnology. In <i>Handbook of Hydrocarbon and Lipid Microbiology</i>			(Ed. K.N. Timmis) Springer-Verlag Berlin Heidelberg. Vol. 2. <i>Microbiology of utilization of hydrocarbons, oils and lipids</i> (Chapter 96).			2009		



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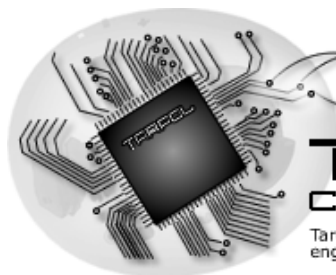
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94	Genetic constructs: molecular tools for the assembly of environmental bacterial biosensors. In <i>Handbook of Hydrocarbon and Lipid Microbiology</i>	De Lorenzo, V.		(Ed. K.N. Timmis) Springer-Verlag Berlin Heidelberg. Vol 3. <i>Consequences of microbial interactions with hydrocarbons, oils and lipids</i> (Chapter 19)			2009		



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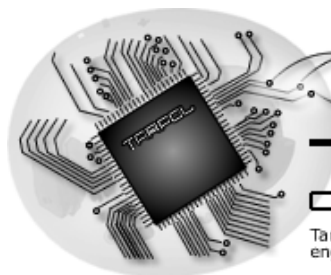
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#	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publ.	Year of publ.	Relevant pages	Per identifier available
95	Exploiting Microbial diversity: the challenges and the means. In <i>Handbook of Hydrocarbon and Lipid Microbiology</i>	De Lorenzo, V.		Ed. K.N. Timmis) Springer-Verlag Berlin Heidelberg. Vol 3. <i>Consequences of Microbial interactions with hydrocarbons, oils and lipids.</i> (Chapter 1)			2009		

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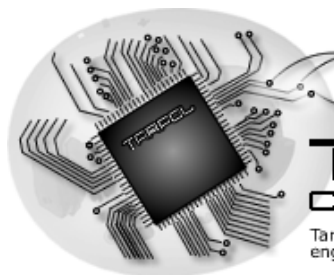
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TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

#	Type of activities ⁴	Main leader	Title	Date	
1	Conference at the XXIInd Meeting of the European Group on Ethics and New Technologies (EGE)	UVEG (Andrés Moya)	Ethics of Synthetic biology	17-18 February, 2009	Br Bel
2	Workshop: KBBE-Net. Collaborative Working group on Synthetic Biology	UVEG (Andrés Moya)	Potentials of transnational research cooperation and perspective for a pilot funding measure under KBBE-NET	8 May, 2009	Br Bel
3	Conference: Ciencia y cultura: un acercamiento desde la Innovación	UVEG (Andrés Moya)	Biología sintética: algunas consideraciones filosóficas	30 September, 2008	Bar Spa
4	Invited seminar	UVEG (Andrés Moya)	Células Mínimas: de la biología sintética natural a la artificial	5 October, 2009	Má
5	Conference: VI Encuentros con la Ciencia	UVEG (Amparo Latorre)	Simbiosis: aprendiendo a vivir juntos	6 October, 2008	Má
6	Conference: III Jornadas sobre Biología Sintética	UVEG (Andrés Moya)	Biología Sintética: el sueño de Goethe	11-12 December, 2008	Val
7	Conference: XVII Edición del Foro Luis Vives. Medio Ambiente: un medio de oportunidades	UVEG (Andrés Moya)	La biología sintética frente al reto de la energía	9 March, 2009	Val

⁴ A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

⁵ A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias ('multiple choices' is possible).



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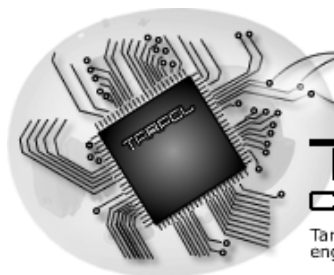
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#	Type of activities ⁴	Main leader	Title	Date	
8	Conference: XVI Jornadas sobre Derecho y Genoma Humano	UVEG (Andrés Moya)	Biología Sintética: el sueño de Goethe	4-5 May, 2009	Bilb
9	Conference: International Organization for Mycoplasmaology IOM	UVEG (Andrés Moya, Rosario Gil, Juli Peretó, Amparo Latorre)	Approaching minimal cells	6-11 July, 2008	Tia
10	Conference: International Organization for Mycoplasmaology IOM	UVEG (Amparo Latorre)	Reduced genomes: lessons from insect endosymbionts	6-11 July, 2008	Tia
11	Conference: Theoretical Biology of Systems Group	UVEG (Juli Pereto)	Genomic and metabolic insights into bacteria-insects symbioses	10 October, 2008	CN Ma Fra
12	Conference: ESF-UB Second European Conference on Synthetic Biology	UVEG (Andrés Moya)	Learning from minimal natural cells	29 March-3 April, 2009	Sar Gu
13	Conference: Seminar at the Departament de Ciències Mèdiques Bàsiques	UVEG (Rosario Gil)	Cel·lules mínims: evolució i disseny	10 Juli, 2009	Uni Lén Spa
14	Conference: 4 th Meeting of the Spanish Systems biology Network (REBS): From Genomes to in silico and back	UVEG (Luis Delaye)	Engineering a minimum photoautotrophic cell: the case of <i>Synechococcus elongates</i> PCC7942	1-2 December, 2008	Val



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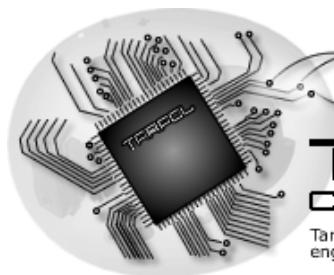
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#	Type of activities ⁴	Main leader	Title	Date	
15	Conference: Simposi sobre evolució	UVEG (Juli Peretó)	L'origen de la vida: el que a Darwin li hagués agradat saber	16 April, 2009	Fac Bio Uni Bar Spa
16	Conference: 9 th EMBO/EMBI. Joint conference on Science and Society: Systems and Synthetic Biology	UVEG (Andrés Moya)	Synthetic Biology: Goethe's Dream	7-8 November 2009	Hei Ge
17	Workshop: Historical and philosophical foundations of synthetic Biology	UVEG (Andrés Moya)	Synthetic Biology and the blind watchmaker	17-18 April 2009	Éco Sup Par
18	Workshop: Open questions on the origins of life	UVEG (Andrés Moya)	Godel, Biology and Emergent Properties	20-23 May 2009	Sar Spa
19	Workshop: Open questions on the origins of life	UVEG (Juli Peretó)	Heterotrophic or Autotrophic Origins?	20-23 May 2009	Sar Spa
20	Workshop: SynBioNT Workshop	UVEG (Luis Delaye)	From natural to synthetic minimal cells	13 March 2009	Uni Not Uni
21	Poster at scientific meeting: XIV International Conference on the Origin of Life, XII ISSOL meeting	UVEG (J. Peretó, M.J. López-Sánchez, A. Lamelas, A. Need, R. Gil, A. Moya, A. Latorre)	A genomic approach to the evolution of metabolism: convergence and complementation in insect endosymbionts	24-29 August 2008	Fir



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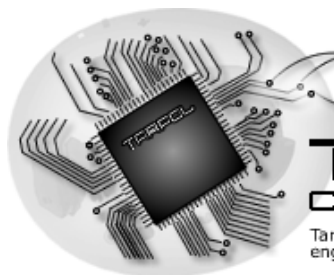
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#	Type of activities ⁴	Main leader	Title	Date	
22	Poster at scientific meeting: ECSB II: Design, programming and optimization of biological systems	UVEG (L. Delaye, M.P. Garcillán Barcia, D. Encinas, J. Peretó, F. de la Cruz, A. Moya)	Engineering the genome of <i>Synechococcus elongatus</i> : toward a minimum photoautotrophic cell	29 April 2009	Sal Gu
23	Science popularization articles: Treballs de la Societat Catalana de Biologia. 60, 45-55	UVEG (R. Gil)	Cèl·lules mínimes: evolució i diseny	2009	
24	Science popularization articles: Actualidad SEM 46:24-25	UVEG (A Moya)	A propósito de cómo aproximarnos a la biología Sintética para no llevarnos desilusiones	2008	
25	Science popularization articles: Pour la Science. Juillet-Septembre 48-52	UVEG (J. Peretó, J. Català, A. Moya)	La synthèse d'être vivants	2008	
26	Science popularization articles: Apuntes de Ciencia y Tecnología 27, 30-38	UVEG (J. Peretó)	Sobre la naturaleza y fabricación de seres vivos	2008	
27	Review articles: FEMS Microbiology Reviews 33:225-235	UVEG (A Moya, R. Gil, A. Latorre, J. Peretó, M.O. Garcillán Barcia, F. de la Cruz)	Towards minimal bacterial cells: evolution versus design	2009	



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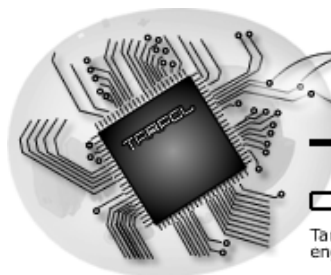
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#	Type of activities ⁴	Main leader	Title	Date	
28	Book chapter: Protocells: Bridging nonliving and living matter, pp. 347-366. Edited by S. Rasmussen, M.A. Bedau, L. Chen, D. Deamer, D.C. Krakauer, N.H. Packard and P.F. Stadler. The MIT Press, USA	UVEG (T. Gabaldón, R. Gil, J. Peretó, A. Latorre, A. Moya)	The core of a minimal gene set: insights from natural reduced genomes.	2009	
29	Interview: Interview in Science Careers by Elisabeth Pain. http://sciencecareers.sciencemag.org/career_magazine/previous_issues/articles/2008_10_17/caredita0800152	UVEG (Andrés Moya and others)	Getting Ready for Synthetic biology	17 October 2008	
30	Participation to the International Genetic Engineered Machine competition (iGEM)	UVEG, UPVLC	a SB project on thermogenesis entitled "Hot yeast Project". TARPOL was cited both in the wiki (http://2008.igem.org/Team:Valencia), the oral presentation, and the poster. The Hot Yeast Project was awarded a gold medal. Valencia iGEM team, under the supervision of UVEG and UPVLC, also prepared and presented a proposal for a code on SB ethical practices that received a mention of the Jury: (http://2008.igem.org/Team:Valencia/Project/Ethics).	November 2008	Car Ma US
31	Science popularization articles. Ludus vitalis 16:30 (Mexico)	IP (A. Danchin)	Les organismes vivants come pièges à information	2008	
32	Science Popularization Articles: Deliciouspaper 3:6 (France)	IP (A. Danchin)	Quelles cellules saurons-nous construire?	2009	
33	Conference (video recorder, public, available on the Internet)	IP (A. Danchin)	Saurons-nous synthétiser la vie?	8 January 2009	Auc cite Sci ville



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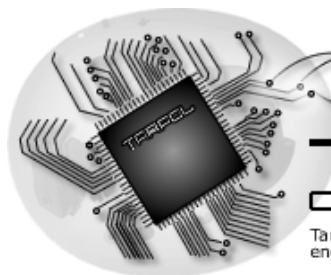
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#	Type of activities ⁴	Main leader	Title	Date	
34	Conference (video recorder, public, available on the Internet)	IP (A. Danchin)	La cellule et l'ordinateur: quels points communs?	15 January 2009	Auc cite Sci ville
35	Conference (video recorder, public, available on the Internet)	IP (A. Danchin)	La vie comme piège à information	22 January 2009	Auc cite Sci ville
36	A video for the new magazine BoOks (www.booksmag.fr)	IP (A. Danchin)	L'usine cellulaire synthétique: réalité ou fiction?	2009	
37	Conference: European group on ethics in science	IP (A. Danchin), UVEG (A. Moya)	Will we able to construct a synthetic cell?	17 th February 2009	Bru Bel
38	A. Danchin belong to the steering committee of the Vivagora group which discusses societal consequences of Synthetic Biology (www.vivagora.org/spip.php/rubrique70)	IP (A. Danchin)			
39	Teaching, lectures and scientific conferences with explicit acknowledgment of TARPOL support: Teaching at the Ecole Normale Supérieure	IP (A. Danchin)	Saurons-nous construire une cellule synthétique?	7 th January 2009	Fra
40	Teaching, lectures and scientific conferences with explicit acknowledgment of TARPOL support: Teaching at the Ecole Centrale des Arts et Manufactures	IP (A. Danchin)	Biotechnology course: Les organismes vivants comme pièges à information	10 th February 2009	Fra
41	Teaching, lectures and scientific conferences with explicit acknowledgment of TARPOL support: Teaching at the Ecole de Mines de Paris	IP (A. Danchin)	1 st year, Biotechnology. "L'usine cellulaire synthétique: réalité ou fiction?"	15 th June 2009	Fra
42	Conference for INRIA (French National _Agency for Computer Sciences)	IP (A. Danchin)	Les organismes vivants comme pièges à information	26 th January 2009	INS Fra



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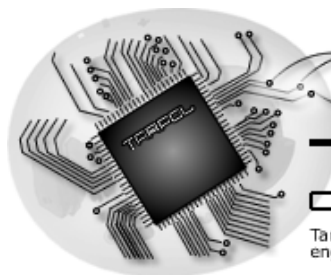
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43	Conference: European Science Fundation School TARPOL AGM	IP (A. Danchin)		31 st March 2009	Sar Gu Ge
44	Conference TARPOL at the Ecole Normale Supérieure	IP (A. Danchin)	Maxwell's demon's genes: towards a cell factory or towards a living synthetic cell?	18 th April 2009	Par
45	Conference 4 th Semmering Vaccine Conference	IP (A. Danchin)	Natural selection and immortality or Maxwell's demon's genes	24 th April 2009	Ba
46	Conference at the Institut Cochin	IP (A. Danchin)	Natural selection as a principle of physics, or Maxwell's demon's genes	28 th May 2009	Par
47	Conference: 69 th Meeting of the Swiss Microbiological Society	IP (A. Danchin)	Maxwell's demon's genes: towards a cell factory or towards a living synthetic cell?	5 th June, 2009	La Sw
48	Stanislas Noria network ongoing, based in Paris and Hong Kong (www.normalesup.org/~adanchin/causeries/causeries_en.html)	IP (A. Danchin)			
49	Some members of IP are registering at Twitter	IP (A. Danchin)	http://twitter.com/snoria		
50	www.synthbio.org/mailman/listinfo/jeudi	IP (A. Danchin)	Creation of the list "jeudi" for discussions span science, epistemology and ethics		
51	Paper: Journal of Microbes and Infection (4:12)	IP (A. Danchin)	Paper on the model of the cell as a computer (published in Chinese)	2009	Chi
52		UNIL	Course in SB for second year's Biology Bachelor students within the frame of a class on "Biologie et société". The course consisted of a literature search, study of TARPOL activities and oral presentation for all students in the class		



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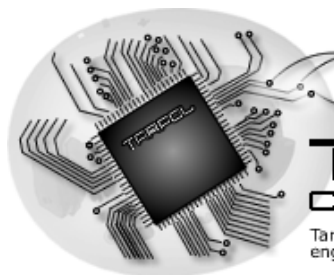
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#	Type of activities ⁴	Main leader	Title	Date	
53		UNIL	Class on SB in its public experimental laboratory called "Eprouvette". The class is aimed at the level of 4 th to 5 th grade High School and consists of an introductory and explanatory lecture plus a transformation of E. coli with a synthetic gene construct allowing the cells to produce banana flavor. Course tutor is Mrs. Sara Tochetti, under auspices of the Eprouvette and with advices from the UNIL-PI in the TARPOL consortium		
54	Meeting of the Swiss Society for Microbiology	UNIL	Plenary meeting on SB organized by UNIL. Plenary speaker was Antoine Danchin	4-5 June 2009	La Fra
55	Presentation. Keynote lecture, European Conference on Synthetic Biology	UNIVE (Bedau)	Beyond BioBricks: synthesizing synergistic biochemical systems from the bottom-up	19 March – 3 April 2009	Sa Gu
56	Conference: Opportunities and Challenges in the Emerging, Field of synthetic biology. Organized by the USA National Academy of Science and the UK Royal Society	UNIVE	Invited participation	9-10 July 2009	Wa
57	Preparation and publication of MA Bedau, Emily C Parke, Uwe Tangent and Brigitte Hantsche-Tangen. Social and ethical checkpoints for bottom-up synthetic biology, or protocells. Systems and Synthetic biology, Springer, forthcoming	UNIVE		2010	
58	Organizer of the Annual Conference of the Hungarian Biochemical Society (http://prof-congress.hu/2008/biokemia/)	BRC-HAS	Talk on minimal genomes by Balint Csorgo	31 August – 3 September 2008	Sze Hu
59	Conference on Environmental Ethics (www.tothjanosszte.extra.hu/konferencia/pdf/program_web.pdf)	BRC-HAS	Talk on Synthetic Biology (Gyorgy Posfai, invited speaker)	25-26 September	Un Sze Sze Hu



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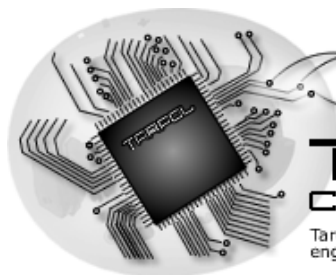
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#	Type of activities ⁴	Main leader	Title	Date	
60	Conference on the Minisymposium of the Hungarian Chemotherapeutic Society	BRC-HAS	Talk on progress in synthetic biology (Gyorgy Posfai, invited speaker)	30 September	Sze Hos Buc Hur
61	Workshop on Synthetic Biology	BRC-HAS	Talk on minimal genomes (Gyorgy Posfai, invited speaker)	27 February 2009	DF Lec Ber Ge
62	Second European Conference on Synthetic Biology	BRC-HAS	Talk and poster on reduced genomes (Tomas Feher et al)	29 March – 3 April 2009	Sar Gu
63	Book Chapter by Tomas Fehér in Microbial Systems Biology – New Directions and New Opportunities (in press, expected publication in second half of 2009) Editor: Nicholas Bergman, Ph. D. Georgia Tech Research Institute. Atlanta, USA. Publisher: John Wiley and Sons, Oxford, UK	BRC-HAS	Systematic Reduction of Microbial Genomes. Abstract: The intent of this chapter is to give a brief overview on the theoretical considerations used to define minimal gene sets as well as to introduce the toolbox available for practical genome reduction projects. Specific works carried out in genome minimization will also be discussed, along with their merits, limitations and future promises.		



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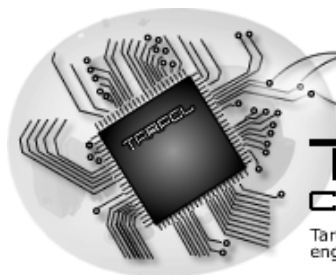
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#	Type of activities ⁴	Main leader	Title	Date	
64	Oral presentation at the 7 th Panhellenic Scientific Chemical Engineering Conference,	IMPERIAL (Koutinas M, Lam M-C, Kiparissides A., Silva- Rocha R, Godinho M, de Lorenzo V, Pistikopoulos EN, Martins dos Santos VAP, Mantalaris A,)	Model driven decisions for the understanding and optimisation of microorganisms genetic circuits: an application in biodegradation of aromatic compounds.	3-5 June 2009	Uni Pat Gre
65	Poster presentation at the 21 st Meeting of the European Society for Animal Cell Technology "Cellular solutions for Clinical Challenges", ESACT	IMPERIAL (Kiparissides A, Pistikopoulos EN, Mantalaris A)	Towards energy-based dynamic optimisation of monoclonal antibody-producing GS-NS0 cultures.	2009	Du



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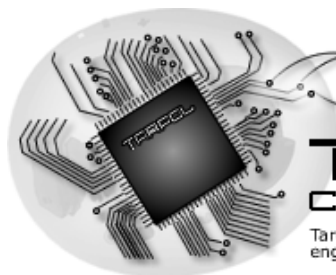
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#	Type of activities ⁴	Main leader	Title	Date	
66	Oral presentation at the Synthetic Biology 4.0, Hong Kong University of Science & Technology	IMPERIAL (Lam M-C, Koutinas M, Kiparissides A., Silva- Rocha R, Godinho M, de Lorenzo V, Martins dos Santos VAP, Pistikopoulos EN, Mantalaris A)	Formal design tools for synthetic biology – engineering the building blocks of genetic circuit in <i>Pseudomonas putida</i> .	10-12 October 2008	Cle Bay Hor
67	Poster presentation at the Synthetic Biology 4.0, Hong Kong University of Science & Technology	IMPERIAL (Lam M-C, Koutinas M, Kiparissides A., Godinho M, de Lorenzo V, Martins dos Santos VAP, Pistikopoulos EN, Mantalaris A)	Towards a model of the biodegradation network in <i>Pseudomonas putida</i> : in silico study of functional units responsible for the degradation of aromatics.	10-12 October 2008	Cle Bay Hor



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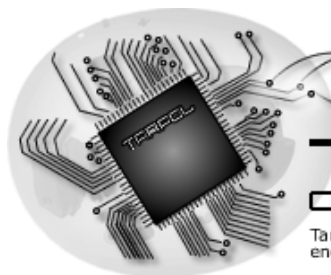
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#	Type of activities ⁴	Main leader	Title	Date	
68	Publication in Microbiology of Hydrocarbons, Oils, Lipids [ed. K.N. Timmis], Springer-Verlag, Berlin	HZI (Puchalka J, Lam CMC, Martins dos Santos VAP)	Genome-Scale Constraint-Based Models to Navigate the Microbial Landscape		
69	Publication in Societal Aspects in Synthetic Biology. Springer-Academic-Press	HZI (Lam CMK, Godinho M, Martins dos Santos VAP)	An Introduction to Synthetic Biology		
70	Conference. Participation in the Synthetic Biology 4.0 Conference. One session organized by Vitor Martins dos Santos	HZI (Carolyn Lam and Vitor Martins dos Santos)		10-12 October 2008	Hor
71	Participation in EMERGENCE workshop synthetic Biology: strategic discussion at DFG/ACA TECH/ Leopoldina	HZI (Vitor Martins dos Santos)		26-28 February 2009	Leo Ber Ge
72	Participation in the ESF meeting ESCBII: design, programming and optimization of biological systems	HZI (Audrey Leprince)		29 March – 3 April 2009	Sar Gu
73	Participation in the Spring School on Systems Biology.	HZI (Jacek Puchalka and Sandra Placzek)		6-8 April 2009	See Ge
74	Participation in the ISMB/ECCB and biopathways meetings (organized through EMERGENCE)	HZI (Vitor Martins dos Santos)		27 June – 2 July 2009	Sto (Sw



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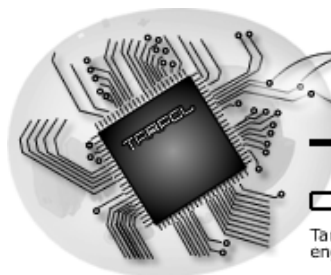
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75	Participation in the symposium Opportunities and challenges in the emerging field of synthetic biology	HZI (Vitor Martins dos Santos)		09-10 July 2009	Wa
76	Communication in conference derived from this project	UPVLC	Cyanobacterial metabolic modeling directed to hydrogen production		
77	Communication in conference derived from this project	UPVLC	Promoter calibrator: one possible application for a biological comparator		
78	Communication in conference derived from this project	UPVLC	Analysis of the capabilities of an autotrophic chassis oriented to synthetic biology applications		
79	Communication in conference derived from this project	UPVLC	Yeast cultures with UCP-1 uncoupling activity as a heating device		
80	Communication in conference derived from this project	UPVLC	Construction and analysis of a genome scale metabolic model for the cyanobacteria <i>Synechocystis</i> sp. PCC6803		
81	Organization of a National symposium in synthetic biology in UPVLC titled "III Jornadas Internacionales de Biología Sintética"	UPVLC			
82	Article in popular Spanish press	UPVLC y UVEG	UV y UPV retan a los mejores universitarios del mundo		
83	Article in popular Spanish press	UPVLC	En busca de energía más barata y limpia		
84	Article in popular Spanish press	UPVLC	Valencianos en el Olimpo de la Ciencia		
85	Article in popular Spanish press	UPVLC	Alumnos de la UPV y la UV consiguen medalla de oro en biología sintética		
86	Article in popular Spanish press	UPVLC	Biohackers: reventar y reinventar la biología desde los grupos		



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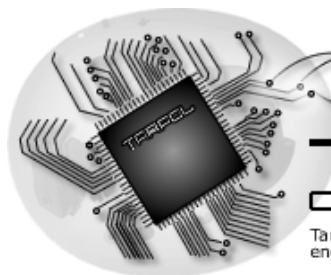
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#	Type of activities ⁴	Main leader	Title	Date	
87	Article in popular Spanish press	UPVLC	Estudiantes y valencianos trabajan en la creación de una pantalla de televisión a partir de células		
88	Article in popular Spanish press	UPVLC	Una pantalla de televisión hecha de células		
89	Conference at "Public Science conference, Festivale della Scienza)	IDC (Markus Schmidt)	Societal aspects of synthetic biology	November 2008	Ital
90	Conference. Hearing to the European Group on Ethics in Science and New Technologies (EGE) during their XXXIInd meeting on February 17, 2009 in Brussels on "Ethics of synthetic biology	IDC (Markus Schmidt)	Safety, security and ethical issues in synthetic biology.	February 2009	
91	Conference. Public lecture and debate	IDC (Markus Schmidt)	Sinful science and synthetic biology	February 2009	The Mu Lor
82	Conference – European conference on Synthetic Biology (ECSB) II: Design, programming and optimization of biological systems.	IDC (Markus Schmidt)	Societal aspects of Synthetic Biology (tutorial)	April 2009	
83	Special Issue of Biological Theory	CNRS-IHPST			
84	Hosting of the EMERGENCE website (Coordination project FP6, NEST program).Regularly update of the contents with the TARPOL website	ETH-Zurich			
85	Emergence and TARPOL were introduced at the Emergence stakeholder meeting at Synthetic Biology 4.0 in Hong Kong	ETH-Zurich			Hon
86	Publication in Sensory Mechanisms in Bacteria (Ed. S. Spiro and R. Dixon). Horixzon Scientific Press. Norwich	CSIC (de Lorenzo, V; Silva-Rocha, R; Carbajosa, G; Galvão, TC and Cases, I	Sensing xenobiotic compounds: lessons from bacteria that face pollutants in the environment	2009	



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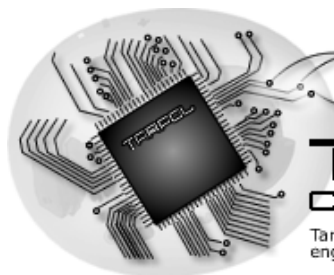
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87	Publication in Bacterial Chromatin (Ed. Charles Dorman Springer-Verlag Berlin Heidelberg (Chapter 16)	CSIC (Muñoz, A; Valls, M and de Lorenzo, V)	Extreme DNA bending: molecular basis of the regulatory breadth of IHF	2009	
88	Publication in Handbook of Hydrocarbon and Lipid Microbiology (Ed. K.N. Timmis) Springer-Verlag Berlin Heidelberg, vol. 2 Microbiology of utilization of hydrocarbons, oils and lipids (Chapter 96)	CSIC (de Lorenzo, V; Fraile, S; Jimenez, JI)	Emerging Systems and Synthetic biology Approaches to Hydrocarbon Biotechnology	2009	
89	Publication in Handbook of Hydrocarbon and Lipid Microbiology (Ed. KN Timmis) Springer-Verlag Berlin vol. 3. Consequences of microbial interactions with hydrocarbons, oils and lipids (Chapter 19)	CSIC (de las Heras, A and de Lorenzo, V)	Genetic constructs: molecular tools for the assembly of environmental bacterial biosensors		
90	Publication in Handbook of Hydrocarbon and Lipid Microbiology (Ed. KN Timmis) Springer-Verlag Berlin vol. 3. Consequences of microbial interactions with hydrocarbons, oils and lipids (Chapter 1)	CSIC (de Lorenzo, V)	Exploiting Microbial diversity: the challenges and the means.		
90	Publication in Handbook of Hydrocarbon and Lipid Microbiology (Ed. KN Timmis) Springer-Verlag Berlin vol 4 Experimental Protocols and appendices (Chapter 96)	CSIC (Carreño, CA and de Lorenzo, V)	Genetic traps for surveying new catalysts in (meta)genomic DNA.		
91	European Science and Society Summer School (E4S): Deconstructing and Reconstructing life: from classification to design	UVEG (Andrés Moya)	Synthetic biology: some philosophical considerations	25-30 August 2008	Hei Ge
92	Seminars on Frontiers in Genomics. Licenciatura en Ciencias de Estudios Genómicos	UVEG (Andrés Moya)	Genomic analysis of bacterial adaptation to intracellular symbiosis	5 September 2008	UN
93	Participation/talk at the SB Summer School in Basel organized by ETH-Zurich	BCR-HAS		24-27 September 2010	ETI (Sw)



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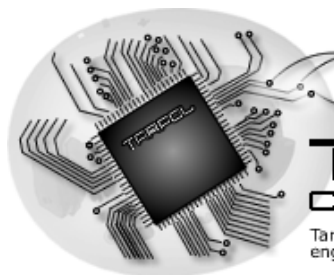
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#	Type of activities ⁴	Main leader	Title	Date	
94	Conference organisation and talk	BCR-HAS (G Posfai)	Annual Conference of the Hungarian Biochemical Society	31. Aug.–03. Sept. 2008	BR Sze Hun
95	Conference talk	BCR-HAS (G Posfai)	Conference on Environmental Ethics	25-26. Sept. 2008	Uni Sze Sze Hun
96	Conference talk	BCR-HAS (G Posfai)	Minisymposium of the Hungarian Chemotherapeutic Society	30. Sept. 2008.	Buc Hun
97	Workshop talk	BCR-HAS (G Posfai)	Workshop on Synthetic Biology	27. Febr. 2009.	DF Lec Ber Ger
98	Conference talk	BCR-HAS (T Feher)	Second European Conference on Synthetic Biology	29. March – 03. Apr. 2009.	Sar Gu
99	Workshop talk	BCR-HAS (T Feher)	International Summer School on Advanced Techniques in Bacterial Genome Research	28.09.2009 – 02.10.2009	Uni Bie Ger
100	Conference talk	BCR-HAS (T Feher)	4th European Conference on Prokaryotic Genomics	4-7, 10. 2009.	Gö Ger
101	Workshop talk	BCR-HAS (G Posfai)	Workshop on Streamlined and Synthetic Genomes	16-17, 11. 2009.	Val



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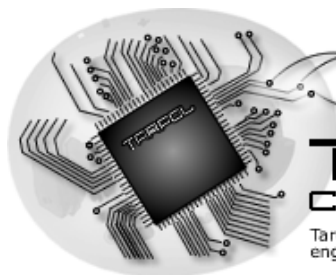
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#	Type of activities ⁴	Main leader	Title	Date	
102	Advisory working group meeting on SB	BCR-HAS (G Posfai)	EASAC (European Academies Science Advisory Council)	09.03.2010.	Fra Ge
103	Conference talk	BCR-HAS (G Posfai)	SynBioNT Symposium	18-22. 03. 2010.	No
104	Conference talk	BCR-HAS (G Posfai)	FEMS, NoE EuroPathoGenomics and ERA-NET PathoGenoMics Conference	22-24. 04. 2010.	Péc
105	Workshop talk	BCR-HAS (G Posfai)	TARPOL SB Summer School	24-27. 09. 2010.	Bas Sw
106	Popular press article	BCR-HAS (T Feher)	Bacteria by design	13-14. 08. 2010.	Me
107	Summer course	BU	TARPOL summer school	19-23 April 2010	Val
108	-Webcast-Conference	IDC	<i>Does your research raise security concerns? Strategies for promoting responsible research in the life sciences</i>	23rd September 2010	Wa DC
109	conference	IDC	<i>United Nations Bioweapons Convention's seminar on synthetic biology. Engineering a safer future</i>	25th August, 2010	Ge Sw
110	workshop	IDC	<i>J. Craig Venter Institute's 3rd Workshop on Synthetic Genomics: Scientists' Understanding of Society's Concerns, Society's Understanding of the Science and Scientists</i>	22-23 July, 2010	Wa DC



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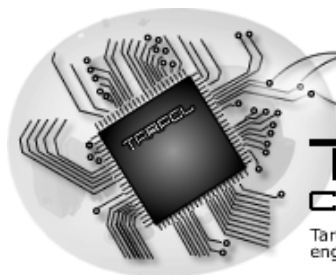
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#	Type of activities ⁴	Main leader	Title	Date	
111	conference	IDC	<i>First Meeting of the US Presidential Commission for the Study of Bioethical Questions.</i>	<i>8-9 July, 2010</i>	<i>Wa DC</i>
112	conference	IDC	<i>TEXTURES - The 6th European Meeting of the Society for Literature, Science and the Arts. Roundtable Biopalimpsest. „Synthetic Biology: (De)constructing eternal dreams</i>	<i>15-19 June, 2010</i>	<i>Rig</i>
113	conference	IDC	<i>National Academy of Technologies of France (NATF) and the French Ministry of Industry Seminar: „Technologies convergentes : enjeux et perspectives“.</i>	<i>8th June 2010.</i>	<i>Pa</i>
114	conference	IDC	<i>TA'10 Die Ethisierung der Technik und ihre Bedeutung für die Technikfolgenabschätzung</i>	<i>31st May - 1st June</i>	<i>Vie</i>
115	conference	IDC	<i>European Commission's DG SANCO Risk Assessment Workshop on Synthetic Biology: From Science to Governance.</i>	<i>18th -19th March 2010.</i>	<i>Br Be</i>
116	workshop	IDC	<i>BBSRC-EPSRC Synthetic Biology and Society Workshop</i>	<i>16-17 March, 2010.</i>	<i>Sw</i>



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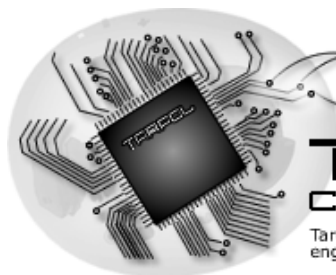
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#	Type of activities ⁴	Main leader	Title	Date	
117	workshop	IDC	<i>J. Craig Venter Institute's 2nd Workshop on "Synthetic Genomics: Scientists' Understanding of Society's Concerns, Society's Understanding of the Science and Scientists"</i>	<i>23-24 February, 2010.</i>	<i>Sa US</i>
118	workshop	IDC	<i>Biosafety and Synthetic Biology Workshop</i>	<i>15 January, 2010</i>	<i>Be</i>
119	conference	IDC	<i>Synthetic biology: new (and old) issues in biosafety and biosecurity. Synthetic Bio(techno)logy.</i>	<i>. 9-10. November, 2009.</i>	<i>Fra Ge</i>
120	conference	IDC	<i>Herausforderungen der Synthetischen Biologie: Biosafety und Biosecurity. Internationales Symposium: Ethik in der Synthetischen Biologie</i>	<i>1-2 October, 2009.</i>	<i>Fra Ge</i>
121	conference	IDC	<i>Societal ramifications of synthetic biology. EuroBIO</i>	<i>September 25, 2009</i>	<i>Lill</i>
122	<i>Conference</i>		<i>European Conference on Nanotechnologies</i>	<i>26 February 2010</i>	
123	Conference	IMPERIAL (Lam Ming-Chi)	<i>Synthetic Biology 4.0</i>	<i>10-12 October 2008</i>	<i>Cle Bay Hor</i>
124	Conference	IMPERIAL (Lam Ming-Chi)	<i>Synthetic Biology 4.0</i>	<i>10-12 October 2008</i>	<i>Cle Bay Hor</i>



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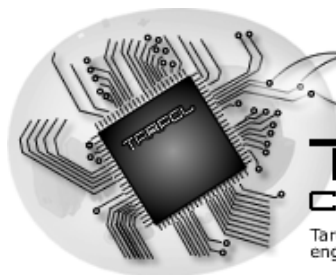
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#	Type of activities ⁴	Main leader	Title	Date	
125	Conference	IMPERIAL (Kiparissides Alexandros)	21 st Meeting of the European Society for Animal Cell Technology "Cellular Solutions for Clinical Challenges", <i>ESACT 2009</i>	7-10 June 2009	Du
126	Conference	IMPERIAL (Koutinas Michalis)	7 th Panhellenic Scientific Chemical Engineering Conference	3-5 June 2009	Pat
127	Conference	IMPERIAL (Kiparissides Alexandros)	20 th European Symposium on Computer Aided Process Engineering – ESCAPE-20	6-9 June 2010	Iscl
128	Conference	IMPERIAL (Koutinas Michalis)	20 th European Symposium on Computer Aided Process Engineering – ESCAPE-20	6-9 June 2010	Iscl
129	Conference	IMPERIAL (Koutinas Michalis)	Metabolic Engineering VIII: Metabolic Engineering for Green Growth	13-17 June 2010	Jej Sou
130	Conference	IMPERIAL (Kiparissides Alexandros)	Metabolic Engineering VIII: Metabolic Engineering for Green Growth	13-17 June 2010	Jej Sou



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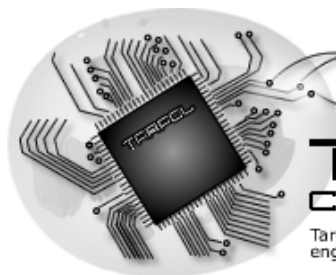
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#	Type of activities ⁴	Main leader	Title	Date	
131	Workshop	UMIL	<i>Pseudomonas in the test tube and in the environment</i>	28-29 January 2010	Mil
132	Web	UMIL	Description of TARPOL within the host department web page	May 2009	Mil
133	Web	UMIL	Insertion of TARPOL string in an institutional web page listing and describing the FP7 projects in which UMIL is involved.	May 2009	Mil



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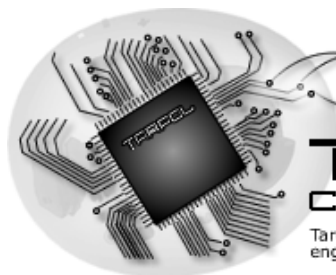
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#	Type of activities ⁴	Main leader	Title	Date	
134	Presentation	UMIL	The dissemination of the concept(s) of SB and its results within 1° and 2° levels degree courses of biotechnological relevance at University of Milan	November-December 2009	Mil
135	Summer course	UVEG	Summer course on synthetic biology, lecture on "Basic molecular biology: the transmission of information in biology"	April 2010	Val
136	Conference	UPVLC (A.Montagud)	Sins, ethics and biology, a comprehensive approach	2009	
137	Conference	UPVLC (J.Urchueguía)	Engineering biological systems: approaching genetics through mathematics	2006	Val
138	Conference	UPVLC (E.Navarro)	Cyanobacterial metabolic modelling directed to hydrogen production	2007	Gin
139	Conference	GENEART	Promoter calibrator: one possible application for a biological comparator	2007, 2008, 2008	Gin Hor
140	Seminar	GENEART	Synthetische Biologie in Deutschland	31.07.2008	He Ge



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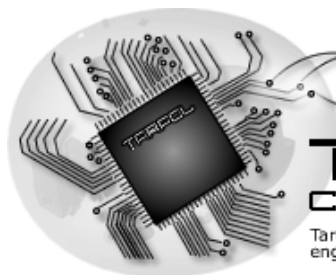
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#	Type of activities ⁴	Main leader	Title	Date	
141	Conference	GENEART	SB4.0	08.-13.10.2008	Ho Ch
142	Workshop	GENEART	The future of synthetic biology	14.11.2008	Lon
143	Symposium	GENEART	DFG Symposium Synthetische Biologie	27.02.2009	Be Ge
144	Symposium	GENEART	Forum Life Science	19.03.2009	Mu Ge
145	Lecture	GENEART	EPFL	23.03.2009	La Sw
146	Conference	GENEART	ESF Synthetic biology	02.04.2009	Sp



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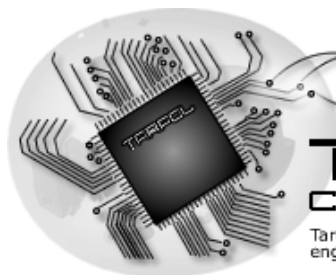
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#	Type of activities ⁴	Main leader	Title	Date	
147	Conference	GENEART	Biofine	16.04.2009	Fre Ge
148	Symposium	GENEART	OECD Symposium	10. 07.2009	Wa US
149	Lecture	GENEART	Impact of high throughput gene synthesis on synthetic biology	28.07.2009	Zu Sw
150	Workshop	GENEART	Biomolecular Systems Conference Mallorca	18.10. 2009	Ma
151	Conference	GENEART	Synthetic Biotechnology	10.11. 2009	Fra Ge
152	Conference	GENEART	35th FEBS Congress	27.06.2010	Go Sw



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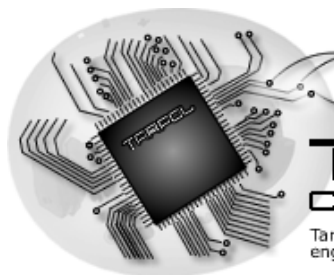
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#	Type of activities ⁴	Main leader	Title	Date	
153	Symposium	GENEART	BMBF Symposium Synthetische Biologie	08.07.2010	Be Ge
154	Conference	UVEG (Rosario Gil)	Towards the synthesis of minimal (living!) cells.	November 11, 2010	8th the Ne Ge Pro Val
155	Enciclopedia entry	UVEG (Rosario Gil)	Minimal cell. In <i>Encyclopedia of Astrobiology</i> (Gargaud, M. et al, eds). Springer	May 2, 2011 (accepted)	
156	Enciclopedia entry	UVEG (Rosario Gil)	Minimal genome. In <i>Encyclopedia of Astrobiology</i> (Gargaud, M. et al, eds). Springer	May 2, 2011 (accepted)	
157	Symposium – First European Meeting on Life and Cognition	UVEG (Juli Peretó)	Top-down strategies for the synthesis of minimal (living!)	22-26 June 2010	Sar Spa



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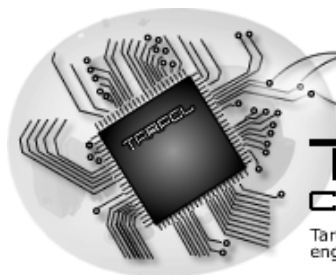
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#	Type of activities ⁴	Main leader	Title	Date	
158	Symposium – NIS Colloquium: First Chemical Steps Towards the Origin of Life	UVEG (Juli Peretó)	Origin of life: an overview of the possible scenarios	16-17 September 2010	Tor
159	Symposium – Evolución de la observación del cambio a la formalización de los mecanismos	UVEG (Juli Peretó)	Simbiosis y bricolaje de redes metabólicas	6-7 October 2010	Cer Ast Ma
160	Book.- 91 pp. Obrapropia editorial	Palanca, C., Vilanova, C., Hueso, A., Porcar, M, et al.,	Sins, ethics and Biology: a comprehensive approach	2009	Spa
161	Book chapter - Editado por J.L. Rubio en XVII Foro Universitario Juan Luis Vives. Ayuntamiento de València.	UVEG (L. Delaye, M. Porcar y A. Moya)	La biología sintética frente al reto de la energía		Spa
162	Course		Advance Lecture Course on: "Quantitative Bioscience"	10-17 September 2010	Spe Gre
163	WORKSHOP	V. de Lorenzo	5 ^o Meeting of the Spanish Network of Systems Biology (REBS) <i>Fostering Systems and Synthetic Biology in Southern Europe</i>	December 13-15, 2009	Ma



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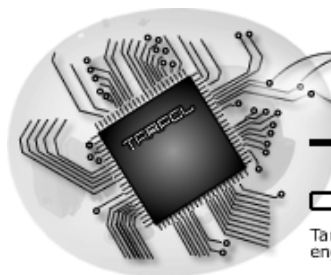
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#	Type of activities ⁴	Main leader	Title	Date	
164	SANDPIT	V. de Lorenzo	Transcription standars: settings criteria for measuring and exploiting promoter activity in engineering prokraryotic systems Illetes (Mallorca, Spain) 21 - 22 October 2009	October, 21-22, 2009	Illet Ma (Sp
165	WORKSHOP	Evolution and Design of Biomolecular Systems		October, 18-20, 2009	Illet Ma (Sp



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2.2 Section B (Confidential⁶ or public: confidential information to be marked clearly)

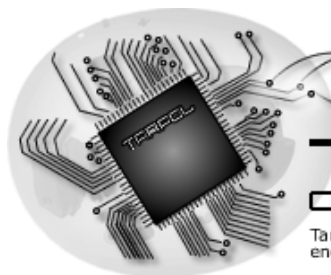
2.2.1 Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights ⁷ :	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)

⁶ Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

⁷ A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.



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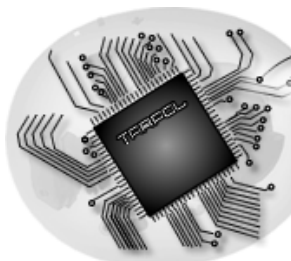
2.2.2 Part B2

Type of Exploitable Foreground ⁸	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application ⁹	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	<i>Ex: New superconductive Nb-Ti alloy</i>			<i>MRI equipment</i>	<i>1. Medical 2. Industrial inspection</i>	<i>2008 2010</i>	<i>A materials patent is planned for 2006</i>	<i>Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC</i>

N.A.

¹⁹ A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

⁹ A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html



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3 Report on societal implications

A General Information (completed automatically when *Grant Agreement number* is entered).

Grant Agreement Number:

212894FP7-KBBE

Title of Project:

TARPOL – Targeting environmental pollution with engineered

Name and Title of Coordinator:

Dr. Andrés Moya Simarro

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)?

- If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports?

0Yes XNo

Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 'Work Progress and Achievements'

2. Please indicate whether your project involved any of the following issues (tick box) :

NO

RESEARCH ON HUMANS

- Did the project involve children?
- Did the project involve patients?
- Did the project involve persons not able to give consent?
- Did the project involve adult healthy volunteers?
- Did the project involve Human genetic material?
- Did the project involve Human biological samples?
- Did the project involve Human data collection?

RESEARCH ON HUMAN EMBRYO/FOETUS

- Did the project involve Human Embryos?
- Did the project involve Human Foetal Tissue / Cells?
- Did the project involve Human Embryonic Stem Cells (hESCs)?
- Did the project on human Embryonic Stem Cells involve cells in culture?
- Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?

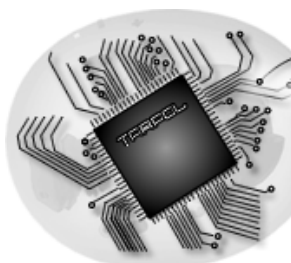
PRIVACY

- Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?
- Did the project involve tracking the location or observation of people?

RESEARCH ON ANIMALS

- Did the project involve research on animals?
- Were those animals transgenic small laboratory animals?
- Were those animals transgenic farm animals?

• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	0 Yes X No
• Research having the potential for terrorist abuse	X No
C Workforce Statistics	
3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).	
Type of Position	Number of Women Number of Men
Scientific Coordinator	1
Work package leaders	6
Experienced researchers (i.e. PhD holders)	5 13
PhD Students	1 6
Other	4 1
4. How many additional researchers (in companies and universities) were recruited specifically for this project?	0
Of which, indicate the number of men:	



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D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project? ☒ Yes ☐ No

6. Which of the following actions did you carry out and how effective were they?

	Not at all effective	Very effective
<input checked="" type="checkbox"/> Design and implement an equal opportunity policy	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input checked="" type="radio"/> <input type="radio"/>	<input type="radio"/>
<input checked="" type="checkbox"/> Set targets to achieve a gender balance in the workforce	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input checked="" type="radio"/> <input type="radio"/>	<input type="radio"/>
<input type="checkbox"/> Organise conferences and workshops on gender	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	<input type="radio"/>
<input checked="" type="checkbox"/> Actions to improve work-life balance	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input checked="" type="radio"/> <input type="radio"/>	<input type="radio"/>
<input type="radio"/> Other: <input type="text"/>		

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

☐ Yes- please specify

☒ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

☒ Yes- please specify

Open Science Day in the Institute. Lectures at Biology School Camp for high school students, Szeged, Hungary 2009 and 2010, TARPOL Summer Schools on Synthetic Biology held at Valencia and Basel; Master students from mSSB, Valencia iGEM team.

☐ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

☒ Yes- please specify UNIL developed a DVD for dissemination of the results corresponding to the WP they worked on

☐ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

☒ Main discipline¹⁰: 2.1; 1.5

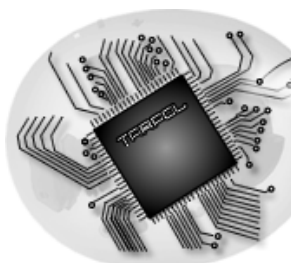
☒ Associated discipline¹⁰: 5.2; 5.4, 1.1

☐ Associated discipline¹⁰:

G Engaging with Civil society and policy makers

¹⁰ Insert number from list below (Frascati Manual).

11a	Did your project engage with societal actors beyond the research community? (if 'No', go to Question 14)	X O	Yes No
11b	If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)? <input type="radio"/> No <input type="radio"/> Yes- in determining what research should be performed <input type="radio"/> Yes - in implementing the research <input checked="" type="radio"/> Yes, in communicating /disseminating / using the results of the project		
11c	In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	O X	Yes No
12.	Did you engage with government / public bodies or policy makers (including international organisations)		
	<input type="radio"/> No <input checked="" type="radio"/> Yes- in framing the research agenda <input type="radio"/> Yes - in implementing the research agenda <input checked="" type="radio"/> Yes, in communicating /disseminating / using the results of the project		
13a	Will the project generate outputs (expertise or scientific advice) which could be used by policy makers? <input checked="" type="radio"/> Yes – as a primary objective (please indicate areas below- multiple answers possible) <input checked="" type="radio"/> Yes – as a secondary objective (please indicate areas below - multiple answer possible) <input type="radio"/> No		
13b	If Yes, in which fields?		
Agriculture X Audiovisual and Media X Budget X Competition X Consumers X Culture Customs Development Economic and Monetary Affairs X Education, Training, Youth X Employment and Social Affairs X	Energy X Enlargement Enterprise X Environment X External Relations X External Trade X Fisheries and Maritime Affairs Food Safety X Foreign and Security Policy Fraud Humanitarian aid	Human rights Information Society Institutional affairs Internal Market X Justice, freedom and security X Public Health X Regional Policy Research and Innovation X Space Taxation Transport X	



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13c If Yes, at which level?

- ☐ Local / regional levels
☒ National level
☒ European level
☒ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?

80

To how many of these is open access¹¹ provided?

22

How many of these are published in open access journals?

16

How many of these are published in open repositories?

6

To how many of these is open access not provided?

58

Please check all applicable reasons for not providing open access:

- ☒ publisher's licensing agreement would not permit publishing in a repository
☐ no suitable repository available
☒ no suitable open access journal available
☒ no funds available to publish in an open access journal
☒ lack of time and resources
☐ lack of information on open access
☒ other¹²: ...no additional funds are available to cover the costs.....

15. How many new patent applications ('priority filings') have been made?

0

("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).

16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).

Trademark

Registered design

Other

17. How many spin-off companies were created / are planned as a direct result of the project?

0

Indicate the approximate number of additional jobs in these companies:

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

- ☒ Increase in employment, or
☐ Safeguard employment, or

- ☒ In small & medium-sized enterprises
☒ In large companies

¹¹ Open Access is defined as free of charge access for anyone via Internet.

¹² For instance: classification for security project.

- ## FINAL REPORT

- 1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)
 - 1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)
2. ENGINEERING AND TECHNOLOGY
- 2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)
 - 2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]
 - 2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)
3. MEDICAL SCIENCES
- 3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)
 - 3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)
 - 3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)
4. AGRICULTURAL SCIENCES
- 4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)
 - 4.2 Veterinary medicine
5. SOCIAL SCIENCES
- 5.1 Psychology
 - 5.2 Economics
 - 5.3 Educational sciences (education and training and other allied subjects)
 - 5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical SIT activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].
6. HUMANITIES
- 6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)
 - 6.2 Languages and literature (ancient and modern)
 - 6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other SIT activities relating to the subjects in this group]