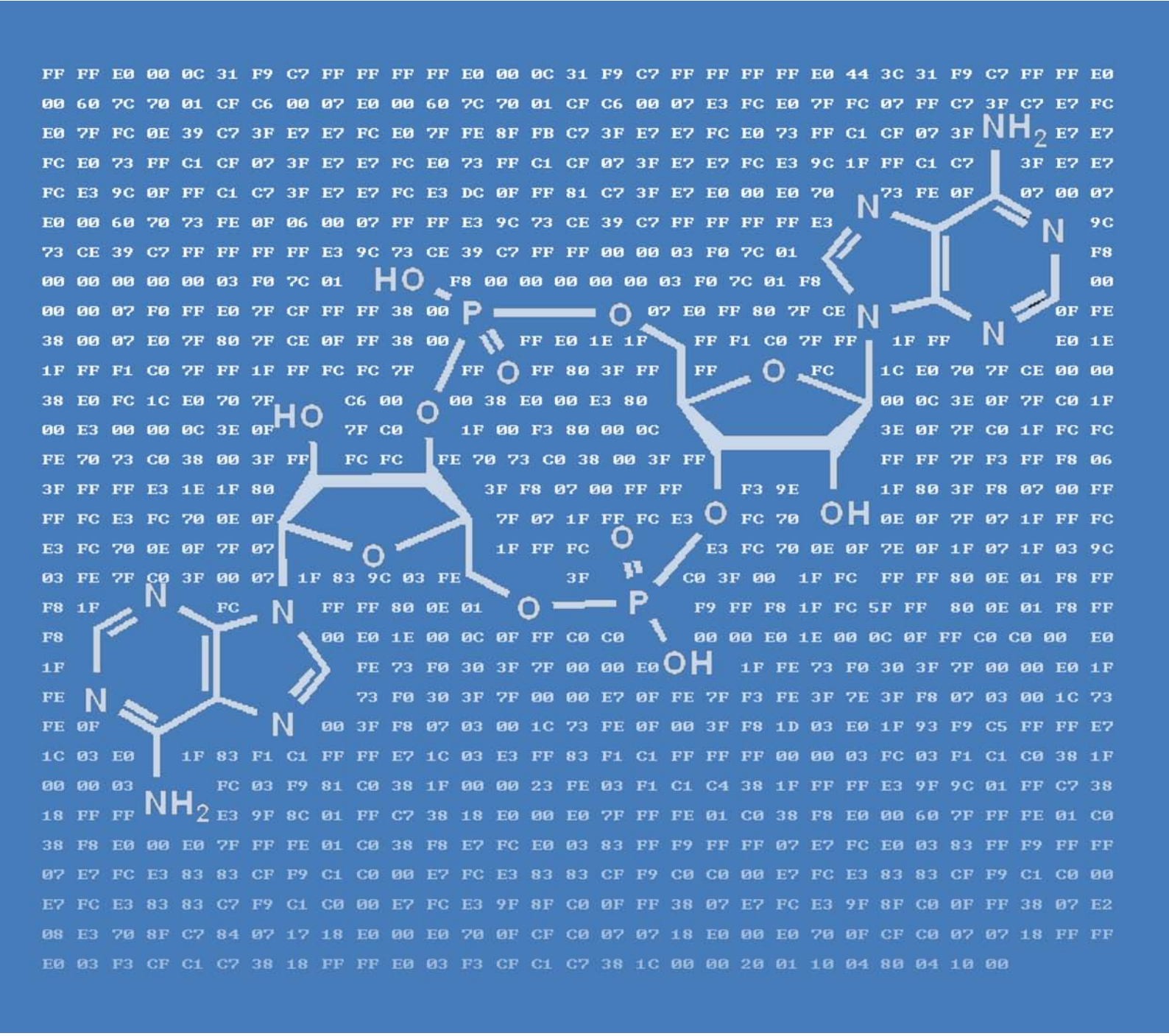
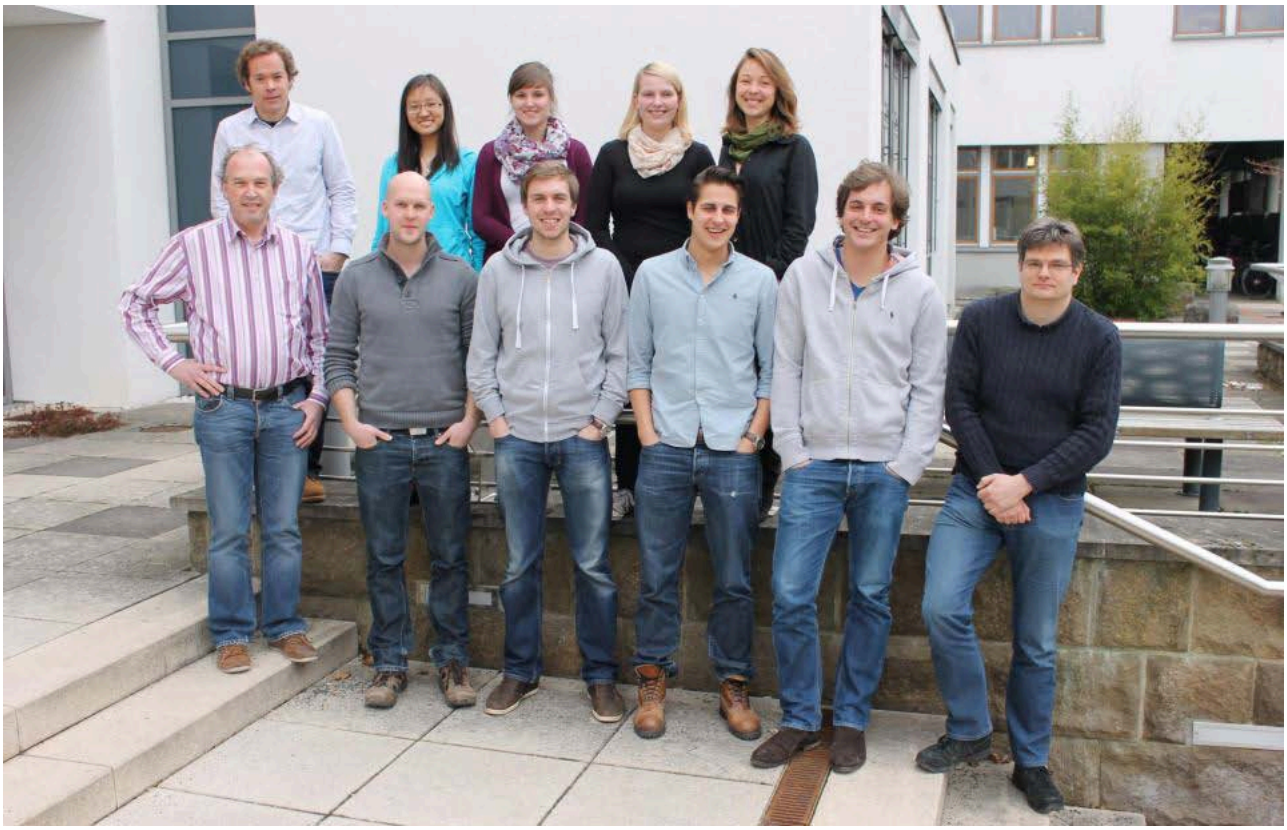


The beast and its Achilles heel: A novel target to fight multi-resistant pathogenic bacteria

iGEM

GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

2013



The iGEM team 2013

An international group of ambitious and enthusiastic master students of the master programs "Microbiology and Biochemistry", "Developmental, Neural, and Behavioral Biology" and "Molecular Biology" of the Georg-August-Universität Göttingen, has teamed up once again to participate in the iGEM competition 2013.

Our team is supported by Prof. Dr. Stülke, Dr. Fabian Commichau (both Department of General Microbiology) and Prof. Dr. Neumann (Department of Synthetic Biology). They act as advisors for our project and support us in planning and organizing.

The achievements of our iGEM team 2012 impressed us and attracted our attention to the iGEM competition. The possibility to do our own research project from planning and organizing up to designing of experiments, convinced us to continue Participating in 2013.

This year's iGEM preparation started with several brainstorming sessions to discuss ideas, followed by meetings with the professors and members of the previous team. We discussed challenges that appeared last year and got a lot of helpful advice. Because of the great support preliminary, uncertainties were quickly resolved and we are well prepared for the work to come. The first steps are made but there is much more to do.

Due to the successful cooperation with your company during the iGEM competition 2012, we would like to kindly ask you for your continued help. We noticed the enthusiasm of the KWS representatives for the iGEM competition at the meeting in December 2012 and hope to attract your attention for our new research project. Since we are convinced that your continued attendance would be a great support for our team, it would be a real pleasure for us to have the KWS logo on top of all our creations.

The time has come again. . .

It is middle of February and the registration for the iGEM competition 2013, one of the most popular international Synthetic Biology competitions for undergraduate students, is approaching. Once again, student groups from all over the world start planning and organizing their individual research projects. The "biobricks" will be sent out and everyone will start with the lab work.

The time will quickly pass until October, when the European jamboree takes place. Each team has the chance to present its project and the best ones will make it to the World Championship in Cambridge, USA. In 2012 about 190 teams participated in the European jamboree and the members of the previous team felt it might become even more this year.

About iGEM

iGEM is an international competition created by the MIT (Massachusetts Institute of Technology) in Cambridge, USA, for undergraduate students of disciplines related to molecular biology. iGEM stands for "International Genetically Engineered Machine competition".

On the one hand, iGEM is aimed at combining aspects of education and social collaboration among undergraduate students; on the other hand, it provides a growing library for standardized and interchangeable parts which can be used in living systems, particularly in model organisms like *Escherichia coli, Bacillus subtilis* or yeast.

Student teams from all over the world will receive a kit of biological parts, also called "biobricks", and work on an individual research project over the summer. Each year the "Registry of Standard Biological Parts" is upgraded with further "biobricks" by the participating iGEM teams. These biobricks will be accessible to the iGEM community in the following years in order to use these parts for novel projects.

The iGEM competition started in 2003 as a course for students of the MIT only. In 2011, already 165 universities from all over the world competed with each other. This year it will be the second participation of the University of Göttingen. The iGEM competition is a unique opportunity for students to gain and improve a multitude of skills that are necessary during a career as a molecular biologist. Those skills include planning and organizing projects, fund raising and team recruitment, co-ordination of lab work, designing experiments and working together as a team. In addition to intensive lab work during the summer, literature search and well-structured documentation of the experiments is crucial.

The lack of novel antibiotics – the problem that we address

The discovery of penicillin by Alexander Fleming in 1928 and the broad application of antibiotics marked a major victory of mankind in the battle against infectious diseases.

However, shortly after the beginning of industrial penicillin production in 1942 some human pathogens had already acquired resistance against penicillin and related antibiotics.
Moreover, the frequent use of antibiotics in the past and the rapid adaptation of bacteria to these compounds had led to the development of many multi-resistant pathogens.

Some of the pathogenic isolates are even resistant against most commercially available antibiotics. Therefore, both the discovery and the development of new antibacterial substances are extremely important to fight these threats of

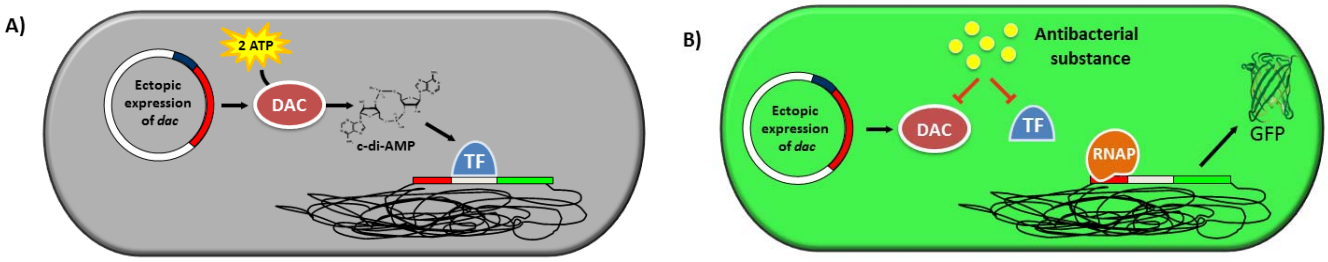


Figure 1: Screening system to identify novel antibacterial compounds that perturb homoeostasis of the essential dinucleotide c-di-AMP in *E. coli*. a) Ectopic production of c-di-AMP by DAC leads to repressor binding and inhibition of reporter gene expression. b) Changes in c-di-AMP homeostasis can be visualized by expression of GFP or *β*–galactosidase.

human health. Unfortunately, the pipeline of novel compounds with antibiotic activity and appropriate pharmaceutical properties is empty. This makes the challenge of finding new compounds even more urgent!

The first step in designing efficient novel antibacterial compounds is the identification of a suitable target. Very recently, a potentially interesting target has been identified. The emerging signaling molecule cyclic dinucleotide c-di-AMP is essential in the Gram-positive model organism *B. subtilis* and in closely related pathogenic bacteria such as *Listeria monocytogenes, Streptococcus pneumoniae, and Staphylococcus aureus*.

These bacteria cause serious diseases and are often multi-resistant. c-di-AMP is needed for the control vital cellular processes in these pathogens because both lack and accumulation of c-di-AMP strongly inhibits growth of the bacteria.

Therefore, any substance that disturbs the homeostasis of the important signaling molecule c-di-AMP and the interaction with its target is of substantial interest to fight human pathogens. The next important step in identifying efficient antibacterial compounds, which either inhibit c-di-AMP biosynthesis or interfere with the essential function of c-di-AMP in the cell, is the development of a powerful screening system.

Our project

Our project is aimed at the development of a simple screening system, which allows the rapid identification and characterization of substances that disturb c-di-AMP homeostasis in pathogenic bacteria.

The principle of how such a screening system could look like is illustrated in Figure 1. The screening system will be established in the non-pathogenic bacterium *E. coli*.

First, we want to construct a promoter-reporter gene fusion which allows us to monitor the activity of a transcription factor that only binds to a specific DNA sequence (operator) in the presence of c-di-AMP. The operator sequence will be placed between a constitutively active promoter and a reporter gene, such as *lacZ* and *gfp* encoding the *β*-galactosidase and the green-fluorescent protein (GFP), respectively.

The activity of either of the two proteins is very easy to detect. Then, we will evaluate whether binding of the transcription factor and thus inhibition of the promoter-reporter gene fusion can be controlled by exogenous c-di-AMP.

Finally, we want to express a diadenylate cyclase from *B. subtilis* to inhibit the promoter reporter gene fusion by endogenously synthesized c-di-AMP. There are two big advantages of using *E. coli* as a host for the development of a screening system to identify antibacterial compounds that interfere with c-di-AMP homoeostasis in Gram-positive pathogenic bacteria.

First, c-di-AMP is not synthesized in *E. coli*. Thus, compounds that inhibit c-di-AMP synthesis will specifically inhibit growth of Gram-positive bacteria. Second, the use of a nonpathogenic *E. coli* strain, which is easy to cultivate will keep the costs very low.

We are confident that our screening system will facilitate the identification of novel antibacterial substances because any change in the activity of the c-di-AMP-dependent promoter-reporter gene fusion, either by inhibition of c-di-AMP synthesis or by activation of DNA-binding activity of the transcription factor will indicate perturbation of c-di-AMP homeostasis.

Human practice – another dimension of iGEM

Beside the challenge to tackle a scientific problem there are more than 20 other categories in the iGEM competition that will be addressed and may deliver additional points for the final scoring of our team. One of these tasks is termed "human practice".

The goal of human practice is not only to explain our scientific problem and the solution we found, but also the field of synthetic biology in a demonstrative and understandable way to a public audience. There is no instruction how this has to be implemented, but creativity is important!

We want to present our project by attending at a contest, which is called "Science Slam". The challenge of "Science Slam" is to explain a scientific problem in an understandable and entertaining way. The contest will give us also the opportunity to acknowledge the sponsors and the supporters of our project.

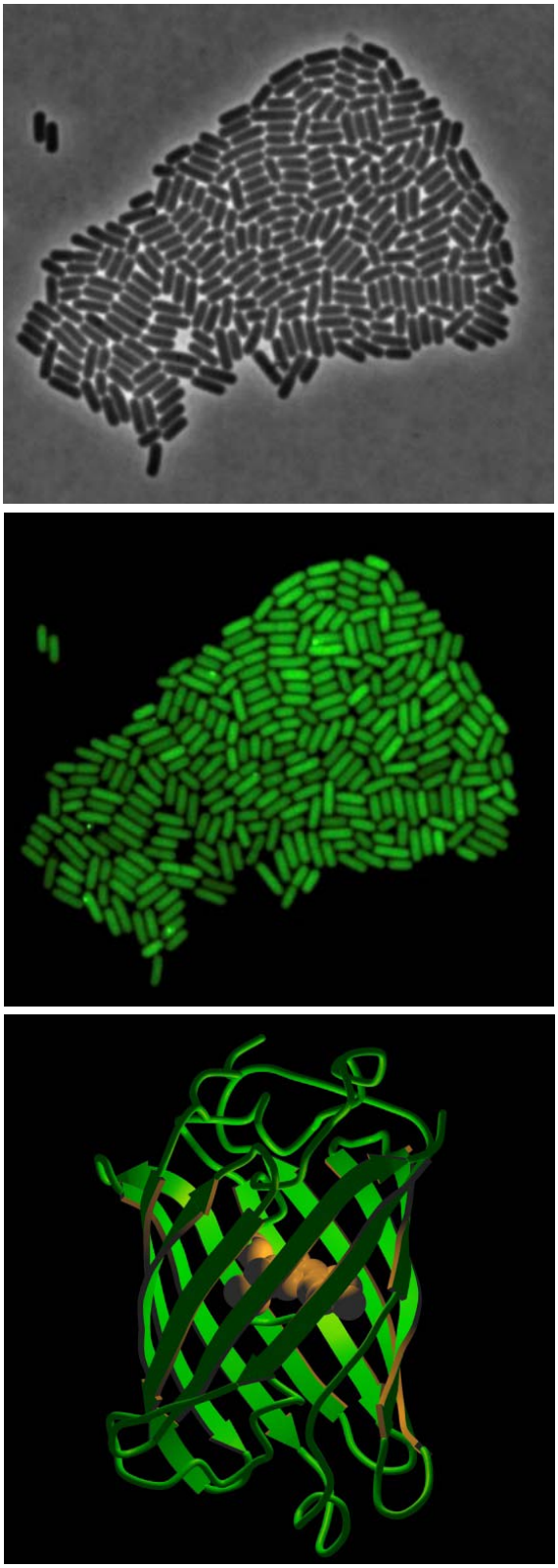


Figure 2: Image of a microcolony of *B. subtilis* expressing the fluorescence marker gene GFP. The same colony is shown using a) bright field microscopy image and b) fluorescence microscopy image. c) Molecular structure of GFP.

Supporters, Sponsors and Sympathizers

KWS Saat AG, Einbeck

KWS, founded 1856 near Magdeburg, Germany (Klein Wanzlebener Saatzucht), is a word wide acting company focused on plant breeding and biotechnology. The product range includes variaties of sugar beet, corn, rapeseed, and cereals. In 2011/12 KWS employed 3.850 people and was active in around 70 countries.

The Göttingen Center for Molecular Biosciences (GZMB)

GZMB is a joint initiative of more than 30 research groups, affiliated with the Faculty of Biology and Psychology, the Medical School (University Medicine), Faculty of Chemistry, the Faculty of Agricultural Sciences, and the Faculty of Forest Sciences at the Georg August University. All of these research groups work in the field of molecular biology. The GZMB is represented by **Prof. Dr. Ivo Feußner**.

Georg-August-Universität Göttingen, Faculty of Biology

The faculty of biology represented by **Prof. Dr. Martin Göpfert** (Dean of faculty) is supporting our iGEM team by paying the registration fee and accrediting the project. Moreover, the Master program "Microbiology and Biochemistry" (speaker: **Prof. Dr. Volker Lipka**) supports our project.

Universitätsmedizin Göttingen(UMG)

The Faculty of Medicine and the University Hospital Göttingen together operate under the umbrella "University Medical Center Göttingen". This structure (integration model) provides the organizational basis for the integration of healthcare, teaching, and research in Göttingen.

The University Medical Center Göttingen has been managed by a three-member Management Board since 01 May 1999, consisting of the divisions Research and Teaching, Healthcare, and Management and Administration.

The University Medical Center Göttingen and the Georg August University Göttingen together form a Foundation under Public Law since 01 January 2003.

Göttingen Genomics Laboratory(G2L)

Microbial genome analysis provides a new research paradigm in the fields of microbiology, molecular genetics, biotechnology and infectious disease biology. Göttingen Genomics Laboratory (G2L) at the Institute of Microbiology and Genetics, University of Göttingen, is a major centre of activities in these areas within Germany. G2L was founded in 1997 by Hans-Joachim Fritz and Gerhard Gottschalk with financial support from the Ministry of Science and Culture of Lower Saxony. Chairman of the laboratory is Rolf Daniel, vice-chairmen are Gerhard Braus and Burkhard Morgenstern. Gerhard Gottschalk is an emeritus professor at the laboratory.

G2L carries out genome analysis projects on the basis of long-term research interests established at the Institute of Microbiology and Genetics; these include the biochemistry and molecular biology of microorganisms, extrachromosomal genetic elements, evolution and biodiversity. In addition, G2L plays a pivotal role in various sequencing projects of BMBF funded research projects within the research initiatives GenoMik-Transfer and Medizinische Infektionsgenomik, and it is a centre of cooperations with external research groups.

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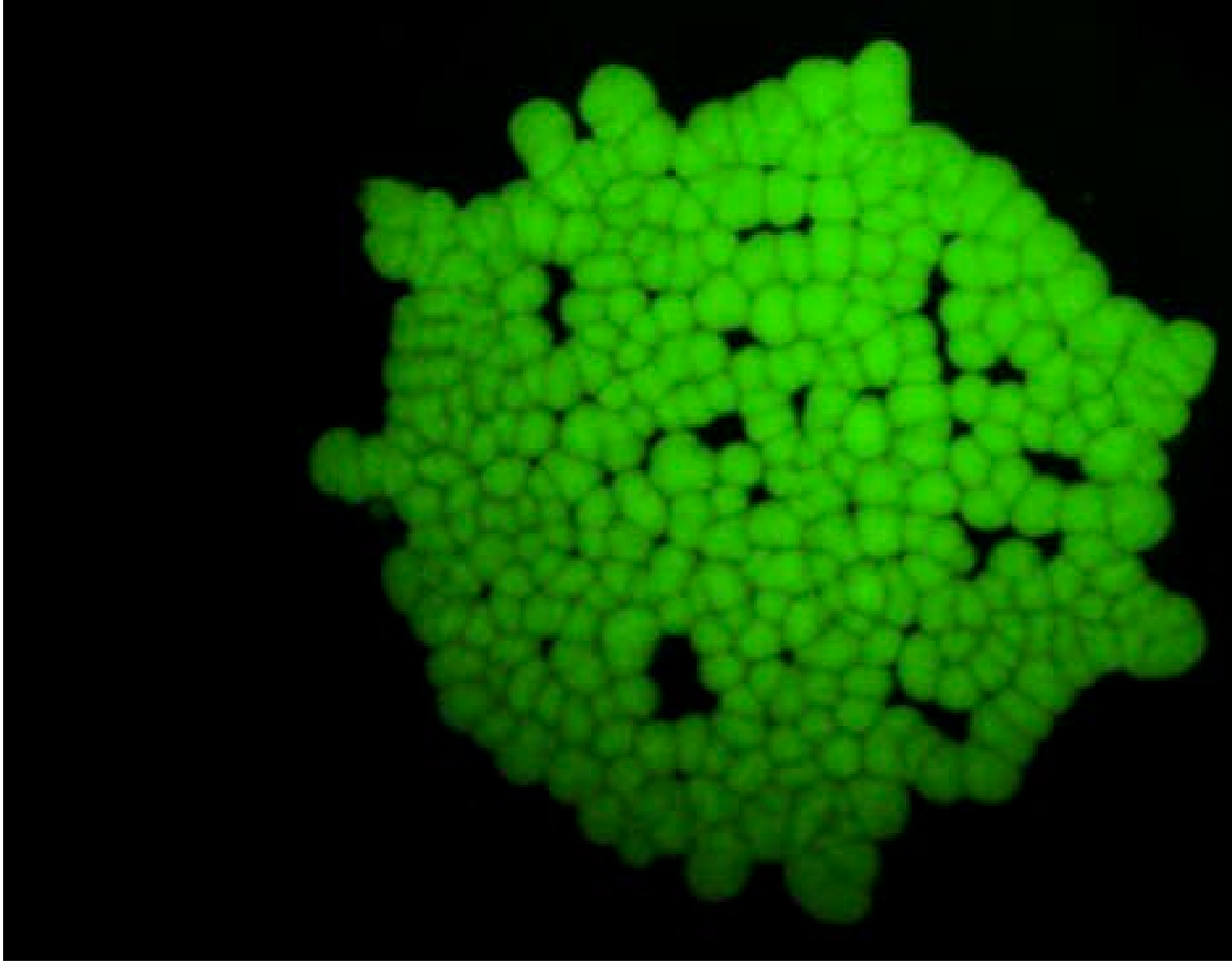

c/o iGEM 2013

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