Report on the workshop

Technical solutions for biosecurity in synthetic biology

held on April 03rd, 2008 in Munich

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1 Introduction

Synthetic biology is a rapidly evolving field, which is based on the exciting proposition of applying our deepening understanding of biological system to technical problems, creating de novo solutions synthetically. Such solutions can be new features of existing living systems - mostly bacteria - but also the construction of entirely new organisms, such as the Mycoplasma bacterium based on a minimal genome created by Craig Venter.[1]

At the basis of synthetic biology is the ability to create components of biological systems artificially and in a highly efficient way. This ability has widely expanded in the past ten years, leading to a vivid industry of service providers who manufacture custom proteins, DNA fragments and genes on demand. In particular, the field of gene synthesis has seen a dramatic development from a small niche technology to a widely applied standard technique. Today synthetic genes can be produced on demand, with virtually no limits in quantity and length.

In parallel, an exhaustive infrastructure has been created for the collection, storage and processing of genetic information. In combination with software for the design of new genes, these resources can be used to rapidly assemble complex genetic networks.

These developments in the field of synthetic biology open up exciting new opportunities for research and industry. The power of the resulting biotechnological applications has vastly increased in the past decade. Concerns have been raised that this power may be abused.

In its most recent unclassified report on the future global landscape, the U.S. National Intelligence Council predicted that a major terrorist attack employing biological agents will likely occur by 2020. Leading committees and reports on both sides of the Atlantic have uniformly recommended the implementation of technical measures to screen synthetic biology projects for biosecurity risks, including the Fink Report[2] and the report of the Royal Academy/Wellcome Trust[3]. The EU research project synbiosafe[4] described in a recent report on the biosecurity awareness in Europe ongoing public efforts and their reception by the synthetic biology community[5].

In the light of these concerns, the Industry Association Synthetic Biology (IASB) has organized a workshop on technical solutions for improved biosecurity in synthetic biology. The workshop took place on April 3rd, 2008 in Munich. The workshop was attended by leading companies from both sides of the Atlantic as well as representatives of the International Consortium for Polynucleotide Synthesis trade association. Collectively, the group provided a broad, representative cross-section of gene synthesis companies worldwide.

This is a report of the workshop and the resulting action items and agreements. The intention of the workshop was to promote the development and implementation of technical measures that address current and future biosecurity threats.

The workshop was borne out of our responsibility for the scientific field to which we provide services and products. It is our conviction that the still relatively moderate size of the field of synthetic biology opens up a unique opportunity to develop and implement reasonable, cost-effective responses to potential biosecurity threats and build a foundation for future developments.

There is now a clear consensus around several strategies that can and should be part of a unified strategy towards increased biosecurity in synthetic biology. This basic agreement

includes a detailed list of projects in which members agreed to take concrete measures to improve biosecurity over the next year. The resulting Work Packages and other initiatives can be found in Section 12 of this report.

2 Participants

The workshop was organized by the Industry Association Synthetic Biology (IASB), the current members of which are (in alphabetical order) ATG:Biosynthetics GmbH, Biomax Informatics AG, Entelechon GmbH, febit Holding GmbH, MWG Biotech AG, and Sloning Biotechnology GmbH. In addition to representatives from the IASB members, there were a number of distinguished representatives from the academic field, the industry and government-associated organizations present:

First name	Last name	Organization
Tom	Bayer	Eurofins MWG
Alexandra	Beil	Sloning
Hubert	Bernauer	ATG Biosynthetics
Luis	Campos	Drew University
Anthony	Caruso	Febit
Jason	Christopher	Goldman School of Public Policy
Werner	Deininger	Entelechon GmbH
Markus	Fischer	Entelechon GmbH
Sibylle	Gaisser	TESSY
Marcus	Graf	GeneArt
Philipp	Habermeier	Febit
Gudrun	Horn	Sloning
Robert	Jones	Craic Computing LLC
Stephanie	Kretz	Europäische Union
Josef	Maier	IStLS - Information Services to Life Science
Steve	Maurer	Goldman School of Public Policy
Kathryn	Nixdorff	Universität Darmstadt
Catherine	Rhodes	University of Bradford
Maxim	Scheremetjew	Universität Halle-Wittenberg
Heinz	Schwer	Sloning
Peer	Stähler	Febit
Eva	Sterzel	Febit
Karoline	Stürmer	Geneart AG
Terence	Taylor	International Council for the Life Sciences
Damon	Terrill	Integrated DNA Technologies, Inc.
Tobias	Wagner	Eurofins MWG
Anna	Zmorzynska	Universität Hamburg

3 The problem of biosecurity in synthetic biology

The assembly of artificial DNA into functional genes and the production of proteins from such genes is available as a highly standardized commercial service. This international industry currently fills thousands of orders for whole genes and millions of orders for short oligonucleotides each day.

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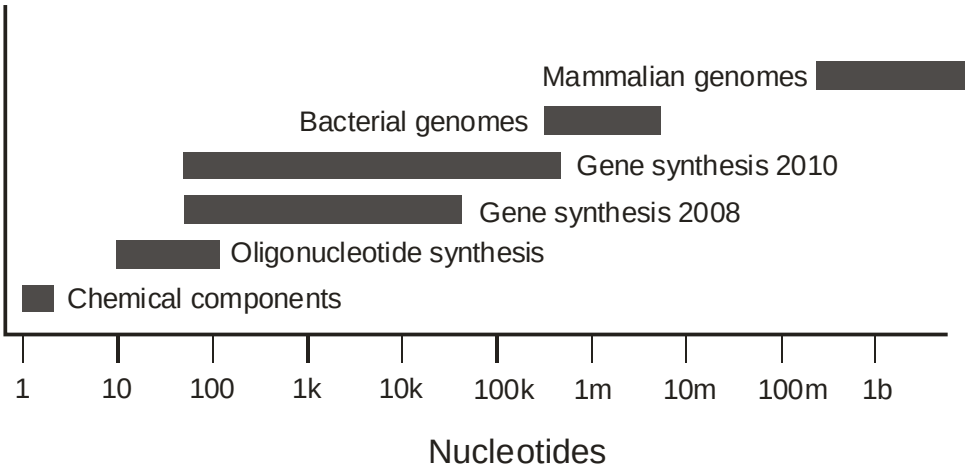

Fig. 1: Scales of naturally occurring nucleic acid species and the capabilities of current and future synthesis processes

In some instances, these orders comprise DNA sequences derived from pathogenic organisms, and in rare cases from pathogens with an extremely high mortality rate, such as the Ebola virus.

So far, none of these orders has been identified as malevolent. Instead, they relate to work on new vaccines, on basic research to better understand mechanisms of pathogenicity or to clinical research and drug discovery.

Nevertheless, it is important for gene synthesis companies to detect such orders for genes of concern effectively and efficiently. To achieve this, almost all gene synthesis houses apply a screening process in which incoming orders are homology matched against a database. However, reports persist that a few gene synthesis companies have yet to embrace this practice and this loophole needs to be closed.[6]

The reference database usually consists of all known genomic sequences of a list of pathogenic agents - oftentimes this list is either the list of select agents or the Australia group list or a combination of both. Unfortunately, this leads to a high number of false positive hits, since for instance harmless housekeeping genes of a pathogenic organism are highly homologous to those of a non-pathogenic relative. Fixing this problem could potentially make screening more affordable for gene synthesis companies that currently do

not screen and make it feasible for companies that produce shorter, oligonucleotide-length sequences to develop their own screening programs.

In addition, it is unclear how a gene synthesis house is supposed to respond to a true positive hit. It is difficult to distinguish between legitimate and malicious use cases, especially since the interaction between customers and suppliers can be very limited.

4 Bioterrorism

Terrorism is a serious threat to the security of the world. The vulnerability of societies to terrorist attacks results in part from the great technological achievements of the past decades resulting in proliferation of chemical, biological, and nuclear weapons of mass destruction, but is also a consequence of highly efficient transportation systems, cheap travel and advanced interconnectedness of people around the world. A brief report by the U.S. Central Intelligence Agency on a workshop on new biosecurity threats states the inherent risk in new biotechnology developments:

> *According to experts, the biotechnology underlying the development of advanced biological agents is likely to advance very rapidly, causing a diverse and elusive threat spectrum. The resulting diversity of new BW agents could enable such a broad range of attack scenarios that it would be virtually impossible to anticipate and defend against, they say. As a result, there could be a considerable lag time in developing effective biodefense measures.*[7]

This report reviews and recommends consensus measures that can materially reduce the risk of a terrorist attack with biological weapons addressing human and agricultural health systems.

The most frequently cited lists of biological agents with potential to pose a severe threat to public health and safety are published by the US Federal Register, FDA, NIAID, CDC, by the American and European Societies for Microbiology and by the WHO. Bio-terrorism agents are listed under three priority categories: priorities A and B are distinguished on the basis of easiness of dissemination and transmission of a pathogen from person to person, the associated mortality and morbidity rates, social disruption potential, and necessity for enhanced disease surveillance. Category C or third priority contains emerging pathogens that could be engineered for mass dissemination in the future. A comprehensive summary of the different pathogens with some of their most important characteristics is attached at the end of this executive summary. The following bio-terrorism agents are currently considered as major threats: Bacillus anthracis (anthrax), Clostridium botulinum toxin, Yersinia pestis (plague), Francisella tularensis (tularemia), Variola major (smallpox) and viral hemorrhagic fevers (e.g. Ebola, Marburg, Lassa, Junin, Machupo virus, etc.) From a public health perspective, Influenza A H1/N1 (Spanish Flu), Influenza A H5/N1 (avian flu) and SARS-CoV represent an additional category of (naturally-)occurring agents with dangerous outbreak potential.

The power and versatility of modern biotechnological methods is inherently of ‘dual use’, and thus bears a considerable security risk. In the words of Matthew Meselson, a leading molecular biologist, at the Symposium on Biological Weapons and Bioterrorism of the American National Academy of Sciences:

Every major technology (metallurgy, explosives, internal combustion, aviation, electronics, nuclear energy) has been intensively exploited, not only for peaceful purposes but also for hostile ones. Must this also happen with biotechnology, certain to be a dominant technology of the coming century? During the century just begun, as our ability to modify fundamental life processes continues its rapid advance, we will be able not only to devise additional ways to destroy life but ... also ... to manipulate it – including the processes of cognition, development, reproduction, and inheritance. A world in which these capabilities are widely employed for hostile purposes would be a world in which the very nature of conflict has radically changed. Therein could lie unprecedented opportunities for violence, coercion, repression, or subjugation.[8]

5 National regulation

There exist of course a number of national regulations that deal with biosecurity. Most countries, including the USA and EU members, have legislation in place for the limitation of the export of dual use goods. This includes synthetic DNA. In Germany, for instance, the export is controlled by the BAFA[9], and export gene sequences involved in the pathogenicity mechanism of a range of pathogenic organisms requires an export permit. This permit will be granted based on an evaluation of the ordered sequence as well as the ordering party.

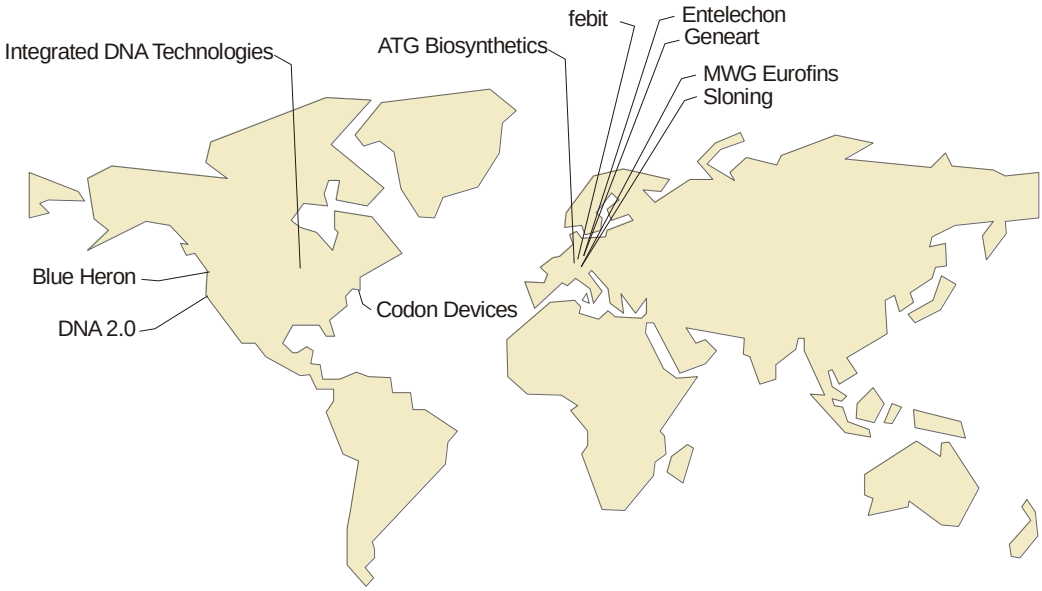

Fig. 2: Place of residence of the IASB and ICPS members who offer gene synthesis services

The largest part of inquiries for risk-associated gene sequences never surfaces in the public or at the authorities. There are two reasons for this: First, domestic inquiries[10] and orders are never submitted to authorities. Secondly, since the bureaucratic steps necessary for export permits are very complicated, customers and suppliers oftentimes discard an order rather

than going through the paperwork for a permit. Therefore, the relatively strong export regulations lead to less, not more control of the executive on security-related activities in the market.

Interestingly enough, legislation for domestic orders is much more relaxed – both in the USA and the EU. Such legislation is focused much more on biosafety than biosecurity and takes the view that the shipping of material that is in itself harmless, i.e. does not pose an immediate biosafety hazard, needs not be restricted nor registered.

Damon Terrill (of IDT) evaluated the significant inter-agency effort now underway in the U.S. to develop the first generation of federal regulations aimed at reducing the perceived security risks of synthetic biology. The regulatory principles developed in the U.S. will affect directly companies and researchers active there, but may also indirectly influence European and other approaches. Mr. Terrill pointed out that there exists a formal mechanism for the interaction between the USA and the EU for regulation, which may form the basis of an emerging regulatory consensus on biosecurity in synthetic biology.

He brought to attention the NSABB (National Science Advisory Board on Biosecurity) report[11] on the question of synthetic biology. This report suggested harmonized guidelines for the application of the select agent rule, in particular with regard to isolated DNA sequences as opposed to whole pathogenic organisms. In addition, the report suggested that US government agencies should implement a requirement for industry suppliers to screen both their customer contacts and incoming gene sequences. In the long run, the current Select Agent Rule may be replaced by a tiered system of checks and screens that accounts for the modular nature of virulence factors and risk-associated genetic elements.

Mr. Terrill pointed out that the current system lacks both in conceptual clarity and in implementation details. There is currently no permanent reporting mechanism in place which could be used by industry suppliers to efficiently report suspicious orders. Member companies of the International Consortium for Polynucleotide Synthesis (ICPS) are currently working with government agencies – in particular the FBI – to implement a suitable reporting system as well as adequate response strategies to positive hits.

A Policy Coordinating Committee (PCC) led by staff in the Homeland Security Council has been established in the US that will address the problem of biosecurity in synthetic biology. The PCC has issued eight taskings, of which two are currently being pursued actively: the creation of harmonized guidelines to the Select Agent Rule[12]; and the engagement of stakeholders, i.e. industry producers and consumers. The latter has been realized in the form of the industry / FBI collaboration, in particular. It is clear that US activities in the field of biosecurity are much more prevalent than those from the EU. Therefore, the IASB will seek the dialog with EU representatives to address this deficit in EU policy making.

Robert Jones noted that there is strong interest from the US executive branch in promoting screening practices and creating technical solutions for this purposes. However, there is currently not yet any funding available from the US government. Therefore, it is important that industry proceed with developing technical solutions immediately rather than waiting for government support to materialize.

6 Biosafety considerations

Sibylle Gaisser raised the issue of balancing biosecurity and biosafety. So far very few concerns have been raised that are specific to synthetic biology. Most biosafety

considerations for general molecular biology and genetic engineering apply to synthetic biology as well, and perhaps to a larger extent due to the efficiency and scalability of the field. However, it is conceivable that new biosafety risks emerge from synthetic biology in relatively short time. IASB wants to anticipate and address these issues, and it was agreed that a similar event to this workshop will be organized. As shown in an analysis[13] recently carried out on behalf of the BBSRC in the UK two of the five areas of concern in Synthetic Biology[14] are directly linked to biosafety issues. These are

- uncontrolled environmental release
- creating artificial life

A number of important lessons can be learnt from the history of recombinant DNA, including that it is essential for the scientific community to play a leadership role in addressing risks and ethical issues, introducing pre-emptive policy initiatives, applying tight regulation in the beginning which can be relaxed over the time and stimulating open public debate. The need for a timely clarification of ethical issues and the development of a coordinated regulation for biorisks, biosafety and biosecurity was shown in the Roadmap for Synthetic Biology in Europe which was developed in the European TESSY project[15] under the coordination of the Fraunhofer Institute for Systems and Innovation Research, Karlsruhe, Germany. IASB wants to anticipate and address these issues, and the IASB aims to organize a workshop specifically on the topic of biosafety.

7 Current state of screening

Workshop participants uniformly agreed that synthetic biology's principal risk for the immediate future was that terrorists could use artificial DNA to recreate naturally occuring pathogens like smallpox or 1918 influenza. This implies that better screening – including both extending existing methods to the handful of gene synthesis companies that do not use them and the development of more powerful, second generation technologies – are a powerful lever for managing this risk.

7.1 Sequence screening

Virtually all gene synthesis providers in Europe and the USA have a screening system and policy in place. **Robert Jones** described the current implementation of screening procedures: The usual setup consists of a BLAST homology search of DNA or protein sequences of incoming orders / inquiries against a subset database of Genbank. The subset may vary between companies, but generally consists of all available genetic information for selected pathogenic organisms (such as all organisms on the Australia Group common control lists[16] or the Select Agent list[17]).

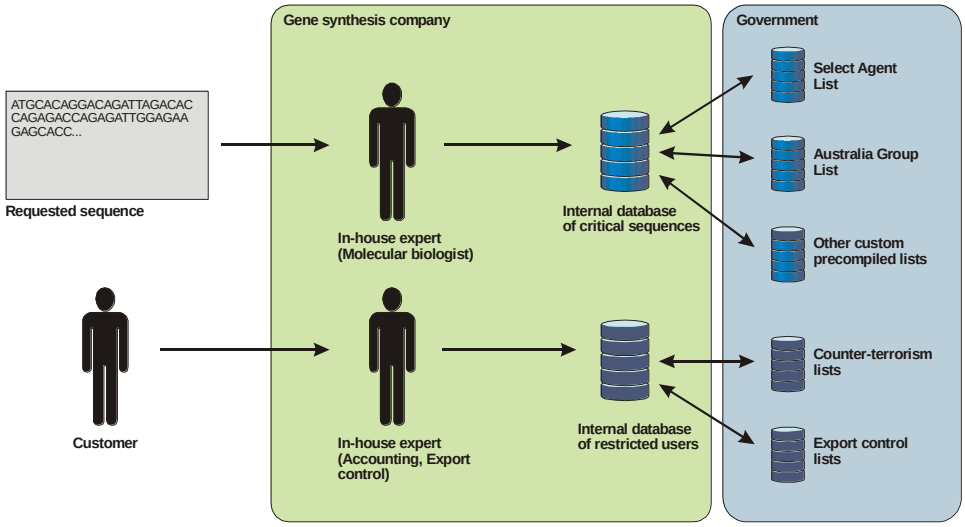

Fig. 3: Schematic of the screening processes implemented at gene synthesis companies. Both biological sequences and customers are screened. The screening is done on the basis of databases that include at least in part government information, such as lists of pathogenic organisms or counter-terrorism person lists. The interaction between companies and governments is very poorly standardized, though, and the details of the implementation as well as the exact contents of the databases vary between companies.

Many companies have created their own implementation of screening software. There are two applications of more general distribution, which are used by a number of companies: pathoGENEDetective[18] by Entelechon and Blackwatch[19] by CRAIC Computing. pathoGENEDetective will be discontinued in favor of Blackwatch, and Entelechon will contribute to the development of future versions of Blackwatch.

CRAIC has also agreed to make the current, first-generation version of Blackwatch available as open source to any gene synthesis or oligonucleotide synthesis company that wants it. This will simultaneously make it easier for those few companies that do not currently screen to adopt first generation technologies and lay the groundwork for an open source community of users to maintain and improve the current code base.

Blackwatch and similar implementations perform a BLAST search against such a database and report a hit if a similarity above a certain threshold is found. Unfortunately this procedures leads to a high number of false positive hits due to the high number of conserved genes between pathogenic and non-pathogenic organisms. These include harmless housekeeping genes, but also genes that are already in Nature being used dually, in a harmless, non-pathogenic context and as part of a pathogenicity mechanism.

It is noteworthy that false positive hits are not recorded in any central place, and often not even within a company. This means that there is little accumulation of knowledge within companies, and even less so between companies. The costs of screening are currently moderate but increasing. Companies can go some distance toward counter balancing this trend by sharing the costs of screening open source-style sharing of best practice methods and, especially, literature searches and other research conducted to investigate false

positive hits as they arise. **Steve Maurer** pointed out that encouraging screening employees to share virulence factors would benefit all companies and that the underlying economic logic is more or less identical to that which drives corporate involvement in more traditional open source collaborations like LINUX and APACHE.

True positive hits pose a problem as there are many conceivable legitimate uses for such sequences, including basic research and vaccine development.

Dr. Jones discussed the problem of false negatives as well. There is a number of ways in which a sequence may be modified in order to avoid matches in screening databases, such as the replacement of synonymous codons or the alteration of the amino acid sequence. In addition, an obvious strategy to evade detection would be to split an order into several smaller fragments that can easily be assembled afterwards. Therefore, an effective screening should be able to detect the smallest possible sequence fragments without an unduly high number of false positive hits.

In order to achieve that, a number of steps are necessary. One is to limit the screening database to those sequences that actually bear a biosecurity risk. This requires a curated database that is peer reviewed and accepted by the community and authorities as being authoritative.

7.2 Customer screening

In addition to sequence screening, most gene synthesis companies perform a background screen of new customers. Damon Terrill noted that there are several government-maintained lists for the screening of individuals and corporate customers. There exists advanced and affordable standard software for the screening of customers against these lists, for example the Bridger Insight suite.

Customer screening is not well standardized, and government regulations are vague about the requirement to screen customers (except in cases of export of dual-use goods). Moreover, watch lists differ widely between nations and watch list screening may interfere with national privacy laws.

Therefore, this problem would strongly benefit from a closer transnational collaboration of policy makers and from a more standardized regulation.

7.3 Genes versus Oligonucleotides

There is a vast difference in the screening of short and long DNA fragments. Long DNA fragments are pieces of at least 200 nt length, which usually represent one or more functional genes and are delivered as double-stranded, cloned DNA ready for protein expression. Short DNA fragments are so-called oligonucleotides that are single stranded DNA molecules of about 15-70 nt length. Oligonucleotides are usually too short to represent a complete functional gene, but can be used for a large number of purposes, including the retrieval, amplification and modification of existing DNA from natural sources or the assembly of complete genes. An additional rich source of oligonucleotides lacking any attention, regulation or oversight is represented by synthetic DNA microarrays.

Screening of oligonucleotides for pathogenicity is not performed at all at the moment. The reason is that the number of orders is two magnitudes larger than for genes, and due to the short length of oligonucleotides it is very difficult to eliminate false positives (or reach a high enough sensitivity to detect true positives). Nevertheless, a set of well designed

oligonucleotides can easily be assembled into a full-length functional gene by a moderately trained molecular biologist.

Robert Jones suggested a mechanism to address this problem: By determining the homology of each part of a pathogen genome with the genomes of closely related organisms, it is possible to identify genomic sequence fragments that are diagnostic for the pathogen. These diagnostic parts can then be used to identify matching sequences unambiguously, without producing false positive hits. It is planned to incorporate this strategy into the next version of BlackWatch.

7.4 Response to hits

One important gap in current screening strategies is the question of what to do when a positive hit comes up. There are rules under national biosafety laws and under export regulation laws, with different impact on the question of biosecurity.

In particular, only export regulations address forensic scenarios, whereas national laws for domestic processes are not concerned with questions of malevolent use. Therefore, there is a significant number of unregulated cases and loopholes.

In these cases – which might nevertheless be relevant in terms of biosecurity and/or biosafety – there is not currently a clear line of action to take, nor is there a competent point of contact in the executive branch of the EU administration (the situation is similar in the US, but may be changing there due to the activities of the aforementioned PCC).

U.S.-based companies are collaborating with the FBI in a pilot project to establish points of contact and standard procedures for responses to positive hits. As of now, no such initiative exists in the EU or in any EU member state, nor – according to the authors' knowledge – in other countries of the world.

8 Trust and customer confidence

All industry representatives agreed that customer confidence is of the utmost importance for the industry. Since virtually all industrial orders require strict confidentiality and involve trade secrets of the ordering party (in the form of proprietary DNA and protein sequences), it must be ensured that no customer data will be shared with a third party.

All biosecurity measures discussed must maintain this very high level of confidentiality. Virtually all industrial orders require strict confidentiality and involve trade secrets of the ordering party (in the form of proprietary DNA and protein sequences) and any feasible biosecurity initiative must respect this. That said, limited disclosures in which data is presented in ways that conceal customer identities, mask individual orders, and/or occur after a commercially reasonable delay can often accommodate these legitimate needs and may be acceptable to customers if they are (correctly) seen as a necessary step towards improved biosecurity.

9 Current developments

There is a number of efforts for improved biosecurity going on in the synthetic biology community and industry.

9.1 Web-based portal for experiments of concern

Stephen Maurer from the Goldman School of Public Policy and **Jason Christopher** presented current efforts under their supervision for increased biosecurity in synthetic biology. These include the development of a web-accessible advice portal for “experiments of concern” in the life sciences. This portal will address the problem that new experiments are planned on a daily basis that build upon emerging powerful tools such as high throughput gene synthesis and that potentially bear security and safety risks. Such risks may not be obvious to the researcher who plans an experiment, and may require analysis by a panel of peers in order to fully appreciate the level of risk they bear.

Workshop participants agreed that the Portal could potentially benefit industry by giving companies a new source of biosecurity advice, as well as providing a place to send customers whose orders raised security issues. Mr. Maurer pointed out that there were several informal and inexpensive ways that industry could help support the Portal. These potentially include (a) inserting web links into IASB, ICPS, and member company web sites, (b) suggesting that customers consult the Portal when appropriate, and (c) nominating industry members and contacts willing to serve peer experts for the Portal.

The portal will be used by academic and industrial researchers and hosted by the University of California at Berkeley. Experiments will be reviewed by peer experts recruited from the field of the particular experiment that is under review. Readers interested in evaluating beta site versions of Portal software are encouraged to contact jchristo@berkeley.edu. A full-scale version of the Portal will go on-line on January 2, 2009.

The database project for experiments of concern was discussed with regard to its implications for synthetic biology. It was acknowledged that the submission of descriptions of experiments may interfere with the confidentiality of customer data. Jason Christopher explained that the portal does not aim to be exhaustive in covering all planned experiments world-wide, but would be a service to concerned scientists and a reference for current trends in the life sciences which might open up new security and safety risks. Therefore, the system would be based on an opt-in mechanism, where interested customers could share their non-confidential data on a voluntary basis, in order to increase the safety and security of their experiments.

9.2 Virulence factor information repository - VIREP

We have already noted that it makes good economic sense for companies to establish open-source-style arrangements for preserving and sharing the virulence factor information generated by their respective screening programs. Such a database would enable the step from an organism-centric view of biosecurity to a gene-centric view. It would enable technology providers to screen for risk-associated sequences in a much more efficient way, avoiding a large number of false positive hits. In the near future a board of scientific experts should be built to give further guidance and support further diligence and curation.

Initial steps have already been made to create such a database. The Lawrence Livermore National Laboratories have created a database of virulence factors as part of the BioWatch project which could be used as an initial basis for a new database with a broader scope and the ability for user-provided content. Entelechon has created an initial proof of concept database – which can be used for the refinement of feature lists and specification.

VIREP will serve three purposes: It will be a community resource that company screening programs can turn to when they encounter unfamiliar gene sequences and, more generally, discuss whether those sequences are virulent and should be considered a biosecurity risk. It

will form the underlying data basis for improved, second generation screening technologies. And it will be a valuable resource of categorized sequence data for the development and training of machine learning algorithms which can then be used to annotate and improve virulence factor database – including VIREP itself.

9.3 Biosafety and biosecurity reporting site

The Goldman School of Public Policy is currently in the early stages of constructing a central reporting site where industry members and researchers could pool information about emerging biosafety and biosecurity issues as they emerged. Such a site will facilitate the discussion of experience that could otherwise be overlooked or over sensationalized. It will turn anecdotes into peer-reviewable and structured information.

Steve Maurer and Jason Christopher made it clear that the public and the industry can help to shape the results of the ongoing efforts at the University of Berkeley. Since the described three components – the database of experiments of concern, VIREP, and the biosafety and biosecurity reporting site – are currently still in development, there is a unique opportunity for the intended target users (industry and academia) to contribute and to have an impact on the end result.

It is hoped and anticipated that these projects will translate into community activity not just once they are finished, but already during their development. Workshop participants agreed that this sharing could begin by writing a jointly authored review article in which several leading firms described the history of their respective screening program and what this experience has taught them about the experiments of concern problem in general (see Section 12.3).

Workshop participants agreed that all three components would be useful projects. IASB endorses them and IASB members plan on actively contributing to the design, specification, implementation and usage of the system. At least for an interim time, IASB is willing to contribute to the curation of a virulence factor database.

9.4 Next version of BlackWatch

CRAIC Computing is currently working on the next version of BlackWatch, under the working title Safeguard. Safeguard will include a number of improvements, including the sensitivity analysis of sequence parts for diagnostic specificity and the usage of a curated database of virulence factors.[20]

Another step is the analysis of database sequences to detect diagnostic versus non-diagnostic portions of a gene or genome. When comparing the smallpox viral genome[21] to that of other pox viruses, for instance, it becomes obvious that some sequence parts have a much lower homology to neighboring genomes than others. These parts – without which the genome would not be functional – could be used to efficiently detect attempts at synthesizing smallpox without any false positive hits.

Safeguard will likely incorporate data gathered via VIREP, and will be loosely associated with a biosecurity portal to be created at the Goldman School of Public Policy.

9.5 Reporting infrastructure

As mentioned, certain U.S. companies are already working on the standardization of operating procedures for reporting suspicious DNA synthesis orders. In collaboration with

the FBI, manageable rules and points of contact are established and tested. The two industry representations could work together to generalize these procedures and make them applicable in the various national contexts.

10 Technical approaches

Josef Maier suggested replacing or complementing the current homology-based screening approach with a pattern recognition approach. This would entail the training of a pattern recognition algorithm with selected DNA sequences that are diagnostic for a certain type of virulence factor, for a pathogenicity islands or other sequences or motifs of concern.

Such a method would have the benefit of detecting closely related sequences, even if they were not known at the time of the pattern generation. Therefore, much as a heuristics-based virus scanner in the IT world, this system would be adaptable and could respond to new sequences in a meaningful way.

It was agreed that such an approach could have a strong impact on the quality of screening, but requires a significant amount of software development. Therefore, the implementation was deferred until more funding is available.

Klaus Heumann suggested a knowledge-based approach to the screening problem, using a metric that represents the risk associated with each individual sequence. Such a metric could be derived from parameters collected from various sources, including a knowledge base. The knowledge base would in turn be maintained by experts from the field, including those responsible for biosecurity screening at the gene synthesis houses. It was noted that there could be more than one metric, with different weights or integration methods for the available parameters, so that the system is adaptable to the requirements of individual users.

11 Discussion

In the discussion, it became obvious that the industry does not view biosecurity as a field for competition, but that all industry representatives were very interested in collaboration. Therefore, a certain degree of sharing of information would be relatively easy to implement. It was pointed out that confidentiality of information and data integrity are of the utmost concern of both industry producers and consumers, and there was consensus that all implementations must ensure the confidential and secure handling of sensitive customer data.

It was agreed that the IASB and ICPS should promote best practices and codes of conduct, but that it is outside of their scope to act as standard defining organizations. The implementation of a code of conduct would raise the question of auditing and generally of how compliance with such a code would be ensured. It is clear that such an auditing cannot and should not be organized by the industry associations, but that the formulation of a code of conduct would be an important first step. Ultimately, the definition of standards and the enforcement of compliance with these is a government task.

However, since any government decision in this direction can potentially put a strong burden on customers and suppliers, it is in our best interest to interact with the authorities to promote standards that are efficient and effective.

In the discussion, it was noted that Blackwatch and Safeguard serve an important role in driving specifications and viable pathways for technical solutions. Blackwatch is currently adopted by Blue Heron and Codon Devices as their screening tool of choice. Several IASB

members contemplate the adoption of Blackwatch, and Entelechon has already committed to using Blackwatch in the future.

Therefore, there is a significant user basis for Blackwatch present in the current gene synthesis community, and even companies that do not use Blackwatch acknowledge its important role as a reference implementation of a screening system. IASB intends to promote Blackwatch and participate in the development of Safeguard and associated framework technology.

It was agreed that Blackwatch will aim for a close integration with the virulence factor database proposed and developed by Steve Maurer and Jason Christopher.

Any biosecurity screening infrastructure that is initially developed with or by the industry must eventually be put under the control of an independent, international body. It is the declared goal of the IASB to promote such a transition. In the meantime, it is important to coordinate today's industry-level initiatives across countries. This will make more formal international bodies much easier to negotiate and implement when the time comes. IASB and ICPS will work closely together to this end.

Markus Fischer suggested a time line for the implementation of missing functionality:

- Development of standards and a code of conduct: 3rd quarter of 2008
- Development and implementation of harmonized screening procedures: 1st quarter of 2009
- Basic implementation of a virulence factor database: 1st quarter of 2009
- Acquisition of funding: In 2009
- Transition to a non-profit organization: Beginning in 2010 or 2011

Concerns were raised that the accumulating information in the database for experiments of concern and in VIREP is in itself a security risk due to the centralized form in which it is provided. Therefore, access to these databases must be controlled.

However, at the same time care must be taken to not exclude interested parties from access to the system. A certain level of openness is important in allowing the engagement of academics and industry representatives. These two goals – security and openness – must be balanced carefully. A subscription-based system was proposed where interested parties would be authenticated through personal interaction with the curating organization, but where such authentication is not limited a priori to a particular user group. Instead, interested parties must provide plausible arguments why they want or need access to the system.

It was noted that although there is an added security risk due to the centralized nature of the provided information, at least in the case of VIREP virtually no information will be in the system that isn't readily available from other sources. The situation thus is similar to the acquisition of publications on select agents where there had been a debate for a long time whether access to such publications – for instance in the form of abstracts in PubMed[22] – should be restricted. In the end, the decision was for open access to this information, since any access restrictions would impede benevolent users much more than those with malicious intent. This is in agreement with the Fink Report which clearly advocates open access to information on pathogenicity.[23]

It was clear that at least companies active in the field of synthetic biology as suppliers should have access to the databases, so that they can implement technical solutions to counteract malevolent orders.

The workshop acknowledged the increasing complexity of biosecurity risks emerging from synthetic biology. This includes new and unprecedented experiments, new ways of interfering with human health (such as the synthesis, modification and application of bioregulators[24]), new delivery technologies and vastly increased scales of analytical and synthetic methods. Therefore, it is clear that no single technical measure will provide a universal solution to the problem of biosecurity. Instead, it is important to create solid technical foundations upon which to build, and to promote an enabling environment for the detection of and response to malevolent behavior. Furthermore, it would be wrong to defer initiatives simply because they might eventually be revised or supplemented later on. There is no such thing as a perfect, 100% solution and it is pointless to wait for one. Industry can and should mount reasonable initiatives to improve biosecurity in the immediate future.

12 Results

The workshop defined three work packages that can be immediately addressed by the IASB members and/or in a collaboration of the IASB, ICPS member companies, and the academic participants. In addition, the IASB members resolved to advertise their commitment to biosecurity screening. Attendees agreed that it would be a useful first step for both IASB and ICPS to (a) announce on their web sites that all of their members practice responsible screening, and (b) encourage their members to make similar representations on their individual company sites. This simple, inexpensive step would empower consumers and encourage the handful of companies that do not currently screen to reform their practices. It would also mark a useful first step towards more detailed best practice codes that may be developed in the future.

12.1 Work package 1: Harmonized screening strategies

IASB and ICPS member companies will share information about the screening strategies currently implemented at their corporate members. They will create a wiki-style non-public forum to discuss shortcomings and possible improvements and to share technical resources on a non-competing basis. In cooperation with CRAIC Computing, the current Blackwatch software will be set up as a reference implementation for a screening workflow. This reference implementation will be freely available to other organizations who wish to screen gene sequences for biosecurity risks.

12.2 Work package 2: Central virulence factor database

IASB will cooperate with the Goldman School of Public Policy in building the infrastructure for a virulence factor database. This resource, the 'Virulence Factor Information Repository - VIREP' will be a web-based, publicly accessible database containing the annotated genomes of selected viruses, bacteria and possibly eukaryotic pathogens. The database will provide means to annotate genomic information on the level of genes and genetic elements, and will include both virulence factors and other, 'harmless' genes. The purpose of VIREP will be threefold: First, it will provide the data basis for future screening software applications. In contrast to current implementations, such software will not screen against all available genomic information for a set of pathogens, but against selected sequences with an associated biosecurity or biosafety risk. Second, VIREP will provide an open forum for

the discussion and definition of virulence factors and mechanisms of pathogenicity. And third, it will provide reference data for the training and testing of machine learning algorithms for the (semi-)automated identification of virulence factor sequences.

As Steve Maurer pointed out, a centralized virulence factor database or similar resource may also facilitate the sharing of information on false positives between gene synthesis companies. A human screening operator would use VIREP to retrieve information about a homology hit, including what previous researchers have learned about the sequence's pathogenicity. This would reduce the cost of screening significantly.

12.3 Work package 3: Publication on status quo of synthetic biology

The workshop participants agreed to write an article describing the synthetic biology industry; the history of screening and other biosecurity / biosafety programs within their respective companies; a description of the false positives and other challenges which these programs typically face; and what these programs have taught them about experiments of concern and how to move forward. Representatives of Entelechon, febit, CRAIC have already agreed to participate and the Goldman School's Steve Maurer will serve as lead author. Companies and individuals who would like to join the project should contact smaurer@law.usc.edu.

The project will create a secure website hosted at Berkeley for the collection of data. This effort will continue after the publication of the article, to create a record of the current state of synthetic biology.

12.4 Technical biosecurity group

The members of IASB and ICPS will each nominate one technical expert for the assembly of a 'Technical Biosecurity Group'. This group will regularly hold virtual meetings to discuss improvements and next steps for biosecurity measures. In particular, this group will work with CRAIC Computing to define requirements and specifications for the second generation of screening software and future versions of BlackWatch. These specifications will be made available to anyone who is interested in creating similar screening software, and will thus serve as an open resource to facilitate the development of such software.

The technical biosecurity group will support efforts to create international policies, as recommended by the Fink report:

> *We recommend that the international policymaking and scientific communities create an International Forum on Biosecurity to develop and promote harmonized national, regional, and international measures that will provide a counterpart to the system we recommend for the United States.*[25]

12.5 Commitment to biosecurity screening

The IASB members are committed to biosecurity screening. Each member already screens incoming gene orders for potential biosafety and biosecurity risks, and each member has implemented mechanisms to establish and authenticate the identity of customers.

In order to promote screening, the IASB members will describe and advertise their screening efforts. This will include public resources on the IASB website[26] and an 'IASB Biosecurity Seal' on their individual websites.

12.6 Next steps

Apart from the work packages, the next steps will be the approach of government representatives in Europe and the networking of IASB efforts in biosecurity with other organizations in the USA and Asia. It was agreed that the IASB should reach out to the international community and especially the local Asian market players at the Synthetic Biology 4.0 conference in Hong Kong.[27] In addition, IASB plans to hold a similar follow-up workshop in 2009. The exact place and time is yet to be determined and will be announced on the IASB website.

Terence Taylor argued that the process of developing standards should be organized as openly as possible. Kathryn Nixdorff argued for a close collaboration with the Biological Weapons Convention (BWC). In 2008, the BWC is putting a focus on awareness raising, which would be an ideal opportunity to initiate a dialog on biosecurity in the commercial sector.

13 About the Industry Association Synthetic Biology

The Industry Association of Synthetic Biology (IASB) is a consortium of leading companies in synthetic biology. Founding members include ATG:Biosynthetics GmbH, Biomax Informatics AG, Entelechon GmbH, febit synbio GmbH, MWG Biotech AG and Sloning BioTechnology GmbH. The main focus of the association is the advancement and future development of synthetic biology. For more information on the IASB please visit the IASB website at www.ia-sb.eu.

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2 National Research Council of the National Academies, 'Biotechnology Research in an Age of Terrorism', 2004, National Academies Press, Washington DC

3 The Royal Society and Wellcome Trust, 07 October 2004, "Do no harm: reducing the potential for the misuse of life science research", royalsociety.org/displaypagedoc.asp?id=13647

4 www.synbiosafe.eu

5 www.synbiosafe.eu/uploads/pdf/Synbiosafe-Biosecurity_awareness_in_Europe_Kelle.pdf

6 Aldhouse P, New Scientist, November 2004, 'The Bioweapon is in the Post.'

7 Federation of American Scientists, Central Intelligence Agency, Unclassified Report, 'The darker bioweapons future', 3 November 2003, www.fas.org/irp/cia/product/bw1103.pdf

8 Meselson, M. 2000. 'The Problem of Biological Weapons,' remarks at the Symposium on Biological Weapons and Bioterrorism, National Academy of Sciences, May 2.

9 BAFA- Bundesamt für Ausfuhrkontrolle

10 It is noteworthy that orders from one EU country into another one are considered domestic, i.e. shipping of goods within EU borders is not considered an export activity

[11] National Science Advisory Board for Biosecurity, 'Addressing Biosecurity Concerns Related to the Synthesis of Select Agents', December 2006. www.biosecurityboard.gov/pdf/Final%20NSABB%20Report%20on%20Synthetic%20Genomics.pdf

[12] www.cdc.gov/od/sap/final_rule.htm

[13] Palmer A, Martin P, Institute for Science and Society, University of Nottingham, May 2008, 'Synthetic Biology – Social and Ethical Challenges'

[14] The other three areas are biological weapons, IP, generation of monopolies, and trade and global justice.

[15] www.tessy-europe.eu

[16] www.australiagroup.net/en/biological_agents.html

[17] www.cdc.gov/od/sap/docs/salist.pdf

[18] www.p-detective.org

[19] biotech.craic.com/blackwatch/

[20] cf. Robert Jones, 'Assessment Criteria for Select Agent Sequences', CRAIC Computing Tech Report 2008-01, www.craic.com

[21] The smallpox virus genome consists of 197 genes and 186 kbp. It is fully sequenced and publicly available from Genbank under the accession number X69198.

[22] www.ncbi.nlm.nih.gov/pubmed/

[23] National Research Council of the National Academies, 'Biotechnology Research in an Age of Terrorism', 2004, National Academies Press, Washington DC

[24] Kagan E., Clin Lab Med. 2001 Sep;21(3):607-18, 'Bioregulators as instruments of terror'

[25] National Research Council of the National Academies, 'Biotechnology Research in an Age of Terrorism', 2004, National Academies Press, Washington DC

[26] www.ia-sb.eu

[27] The Synthetic Biology 4.0 conference is organized by the BioBricks Foundation in partnership with the Hong Kong University of Science and Technology (HKUST), the University of Hong Kong (HKU), and the Chinese University of Hong Kong (CUHK). It takes place in Hong Kong on October 10-12, 2008.