BIORISK-BIOTERRORISM:
Genetic editing & CRISPR Technology
Is it a national security threat?

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The Transgenic Technology Lab (TTL) is a Research Infrastructure of Hellenic Pasteur Institute founded in 1996 and supported by General Secretariat for Research and Technology as part of the program for improvement of Specialized Services in Universities / TEI and Research Institutions. The main activity of TTL is the generation of genetically modified mice by using cutting-edge transgenic technologies with the aim to establish models for human diseases, as well as to perform preclinical drug evaluations in terms of high quality services or research collaborations to the national and international research community. TTL is the first founded in Greece and the place where the first "Greek" transgenic mice were generated. Since 2012 a new Technique of genetic modification has been established. CRISPR[1] (clustered regularly interspaced short palindromic repeats)/Cas technology has made gene editing cheap and easy. Worldwide Transgenic Core Facilities, like ours at Hellenic Pasteur Institute, uses CRISPR gene editing platform in order to specifically target and modify sequences within complex genomes. Genetically Modified Organisms (GMO's) can now easily be created.

WHAT is CRISPR Technology. CRISPR is an adaptive antiviral defense system of prokaryotes and archaea that scientists translated and used in genome editing, as a tool. CRISPR-Cas immunity is a natural process of bacteria and archaea preventing bacteriophage infection, conjugation and natural transformation by degrading foreign nucleic acids that enter the cell. CRISPR-Cas9 is used as a genome editing tool that is able to induce a double-strand break into DNA at selected sites in the genome of any cell and species. In practice, a guide RNA (gRNA) leads the DNA endonuclease Cas9 to a specific sequence to instruct a cut through the DNA strands. So, this technique allows an endonuclease system to break DNA and through either NHEJ or HEJ a new sequence can be added to the genome, allowing a gene to be introduced or being switched off.

WHY is CRISPR Technology a threat? The possibility about potential misuse and that gene editing technologies may be used for the development of genetic weapons of mass destruction raises concerns.[2,8] Global progress in the field of biotechnology has increased the potential for the development of genetically engineered pathogens that express enhanced or unique virulence properties.[3,4] This is of concern as highly virulent and highly resistant organisms may be constructed for which there may be no known effective treatment for exposed and infected persons or animals.[6,7] Public health (diagnostics, vaccines), Clinical applications (gene therapy, antimicrobials), Agriculture (disease-resistant crops, vector control) and Industry are sectors which have already assimilated this technology. Alteration of a selected DNA sequence in a cell using targeted nucleases isn't new. Engineered meganucleases, zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN)[17] is being used in order to develop these modifications. The revolution that Crispr technology has brought to these type of modifications is that it is as robust and accurate as others but it is way cheaper and faster. Neither sophisticated instruments nor special expertise and infrastructure are needed. The cost of ordering a guide RNA and enzymes required is less than 100€. This means that terrorist or radical groups would not find it difficult to establish a CRISPR infrastructure[5,18]. Also many labs worldwide work on these topics, without any obligated biosafety framework, meaning it also increases risk of accidental release of engineered pathogens. gRNA sequences for CRISPR experiments can be ordered online from many suppliers. Oversight appears to be limited and identification of all possible harmful sequences is unachievable.

TABLE A-1 Summary of the relative maturity of selected synthetic biology concepts, approaches, and tools. NOTE: For each column, darker shading indicates routine use for that community, lighter shading indicates emerging use, and white background indicates little or no use. Adoption flows from left to right in most cases.

	In development	In use by developers of the technology	In use by the synthetic biology community	In use by the molecular biology community	In use by amateur biologists
CRISPR/Cas9					
Genetic logic					
Machine learning					
Multiplexed genome editing (MAGE/CRISPR)					

National Academies of Sciences, Engineering, and Medicine. 2018. **Biodefense in the Age of Synthetic Biology**. Washington, DC: The National Academies Press

Gene drive approaches could be applied for pest control where a CRISPR-Cas9 cassette is able to self-perpetuate, thereby rapidly spreading any genetic information among all individuals of a population(e.g. insects). Such application proposed the delivery into mosquitoes of genes that can block transmission of malaria or dengue fever to combat insect-borne diseases.[11,13] Environment and biodiversity are also clearly among the potentially affected areas.
Tools which are potentially more efficient like Cas12a (Cpf1)[14] or Tild-CRISPR[12] have also been established. Interestingly, it was recently shown that a bacteriophage protein can switch-off the CRISPR/Cas9 activity, which should permit a certain level of control of CRISPR/Cas9-mediated gene editing, although this approach does not revert a modification already initiated[9].

HOW CRISPR Technology can be controlled? Concerning the above it is necessary to establish a control framework[6,7,15] and enactments covering gene editing and somatic genome editing through policy makers regarding Public Health and Environmental concerns. Gene drive policies, biosafety and biosecurity measures and approaches need to be harmonized and integrated to labs through Biosafety committees. Dedicated guidelines published on the required risk assessment and minimal control measures applicable are essential for setting barriers and

contained use of genetically modified organisms.[10] We shouldn't neglect the significance of CRISPR Technique in contributing to our understanding of biological functions and disease mechanisms. CRISPR technology can be proved a beneficial tool to develop treatments for human diseases. The new genome editing tools are expected to empower innovation in all sectors of employment. As with other tools, there may also be potential for misuse, either inadvertently and associated with biosafety concerns or deliberately and associated with biosecurity concerns. International dialog is essential for resolving contentious points and evaluating the implications for ensuring responsible research and innovation.[16]

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