“Genomieditoinnin mahdollisuudet”
Suomalainen Tiedeakatemia
BioCity Turku 18.5.2016 klo 14.15–16.00

# Synteettinen biologia ja genominmuokkaus – bioturvaamisnäkökohtia

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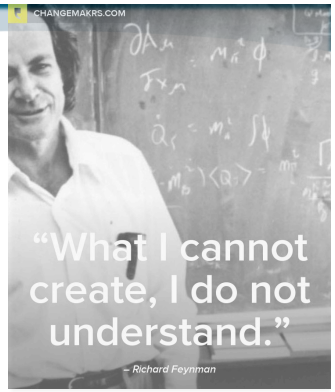
# VÄITE

## Tietyt kehityskulut, jotka liittyvät synteettiseen biologiaan ja genomin-muokkaukseen, nostavat bioturvaamisriskejä.



# MITÄ SYN-TEETTINEN BIOLOGIA ON?

- Synteettinen biologia on nopeasti kehittyvä, useita tieteenaloja yhdistävä tutkimusala, jossa sovelletaan insinööritieteellistä suunnittelu- ja rakennustapaa biologiaan.




Kuvan lähdetieto:
https://www.pinterest.com/pin/229402793251454111/
(12.3.2016)

- Biotekniikan neuvottelukunnan (BTNK) *Synteettinen biologia* -julkaisussa annetaan seuraava määritelmä: ”uusi biologian osa-alue, jossa suunnittelun, mallinnuksen ja rakentamisen avulla valmistetaan biologisia osia, mekanismeja ja molekulaarisia järjestelmiä, joilla on uusia ominaisuuksia” (Ritala ym. 2013, 4).
- Tiedekomiteoiden raportti Euroopan komissiolle (2014) yksilöi 35 määritelmää.



- **J. Craig Venter Institute**
  - ”We report the **design, synthesis, and assembly of the** 1.08–mega–base pair ***Mycoplasma mycoides JCVI-syn1.0*** **genome**”

➔**Seurauksena laaja mediahuomio+komitea-työt&raportit.**

**Gibson et al. (2010)**
***Science* 329(2nd July): 52–56**

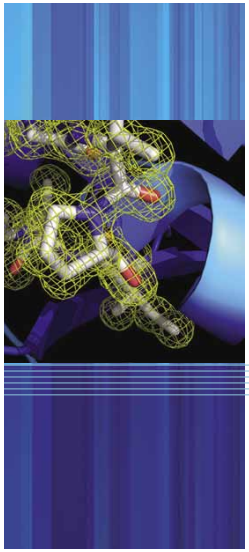
### Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including “watermark” sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

In 1977, Sanger and colleagues determined the complete genetic sequence of phage φX174 (*1*), the first DNA genome to be completely sequenced. Eighteen years later, in 1995, our team was able to read the first complete genetic sequence of a self-replicating bacterium, *Haemophilus influenzae* (*2*). Reading the genetic sequence of a wide range of species has increased exponentially from these early studies. The ability to rapidly digitize genomic information has increased by more than eight orders of mag-

We developed a strategy for assembling viral-sized pieces to produce large DNA molecules that enabled us to assemble a synthetic *M. genitalium* genome in four stages from chemically synthe-sized DNA cassettes averaging about 6 kb in size. This was accomplished through a combination of in vitro enzymatic methods and in vivo recombi-nation in *Saccharomyces cerevisiae*. The whole synthetic genome [582,970 base pairs (bp)] was stably grown as a yeast centromeric plasmid (YCp) (*7*).



FinSynBio

SYNTEETTINEN BIOLOGIA
(FINSYNBIO) 2013–2017

SUOMEN AKATEMIAN TUTKIMUSOHJELMA

LÄHDE:
http://www.aka.fi/Tiedostot/Synteettinen%20biologia/Finsynbio_tutkimusohjelma1_2013_6s_suomiWEB.pdf



# MITÄ GENOMIN-MUOKKAUS ON?

- **Genominmuokkauksella viitataan menetelmiin, joiden avulla perimää voidaan muuttaa tarkoin määrätyistä kohdista.**
- **Tällaisia ovat esimerkiksi CRISPR-Cas9 (engl. *clustered regularly interspaced short palindromic repeats*), ODM (*oligonucleotide directed mutagenesis*), TALEN (*transcription activator-like effector nucleases*) ja ZFN (*zinc finger nucleases*).**



# MITEN SYNTEETTINEN BIOLOGIA JA GENOMINMUOK-KAUS LIITTYVÄT TOISIINSA?

- **Ne ovat osittain päällekkäisiä siinä mielessä, että tietyissä synteettisen biologian tutkimushaaroissa käytetään genominmuokkausmenetelmiä.**
- **Tiedekomiteoiden raportissa Euroopan komissiolle (2015a) synteettisen biologian sisällä erotetaan seuraavat tutkimushaarat:**
  - **(1) geneettisten osien kirjastot ja metodit**
  - **(2) minimaaliset solut ja isäntäsolualustat**
  - **(3) proto- ja keinotekoiset solut**
  - **(4) ksenobiologia**
  - **(5) DNA-synteesi ja genominmuokkaus**
  - **(6) DIY(tee-se-itse)-biologia eli biohakkerointi**



# BIOTURVAAMINEN

- **Bioturvaaminen (engl. *biosecurity*) ja bioturvallisuus (*biosafety*).**
- **Bioturvaamisella tarkoitetaan periaatteita, käytänteitä ja yksittäisiä toimenpiteitä, joiden avulla pyritään estämään tutkimustiedon ja teknologioiden mahdollista tietoista väärinkäyttöä esimerkiksi bioterrorismitarkoituksessa.**

**Cressey (2007)**
***Nature* 448(16th Aug.): 732–733.**

**KAKSIKÄYTTÖTUTKIMUS (DUAL-USE RESEARCH)**
**”work that could be of use to terrorists as well as to legitimate researchers**
**(…)**
**The prospect of a deliberate release of dangerous biological material is of increasing concern**
**(…)**
**The more security you have, the more impaired the research gets”.**

Responding to an anthrax scare in Liverpool, UK, in 2001.

## Not so secure after all

In the movie *28 Days Later* a deadly virus escapes from a British research lab and wreaks havoc across the country. That was fiction, but concerns about lab safety are not.

It is now nearly certain that the foot-and-mouth virus discovered on 3 August in cattle near Guildford, UK, originated at the nearby animal-research facility in Pirbright. The incident seems to have been due to an accidental leak of the virus from either the government-run Institute for Animal Health (IAH) or commercial vaccine manufacturer Merial Animal Health, which share the Pirbright facility. Merial said last week that it "has complete confidence in its safety and environmental protection". The IAH also says it does not know of any security breaches and is cooperating with the inspectors.

This latest incident highlights the problems that can occur with the security of so-called 'dual-use' research — work that could be of use to terrorists as well as to legitimate researchers (see 'Laboratory lapses').

Investigations into the foot-and-mouth outbreak are ongoing, but engineering or personnel failure must have been to blame if the virus escaped from a secure lab, in the opinion of Keith Plumb, a bioprocess engineer at the Institution of Chemical Engineers in London. It could have emerged only through the ventilation system, in waste, or on people, he says. Waste should be sterilized before disposal in the sewers, either by steam or chemicals. Damage to filters in the negative-pressure air system, for example, could have given the virus a possible exit route, says Plumb.

Lab workers are fully covered by a gown, with only their eyes exposed, and must enter the lab via air-locks. After leaving the lab and removing the gown, researchers must shower to get rid of any contamination that might have occurred. Not taking enough time to shower is another possible exit route for the virus, Plumb says.

"These kinds of breaches happen frequently in labs," he says, although usually with no serious consequences.

The 2001 anthrax attacks in the United States were a rude awakening for biosecurity, and

### Laboratory lapses

● **1998** Imperial College London fined for failure to follow health-and-safety rules in a study that created a chimaera of hepatitis C and dengue-fever viruses.

● **2001** Anthrax spores sent maliciously through the post in the United States. A laboratory source for the bacterium was suspected.

● **2003** Thomas Butler of Texas Tech University charged with infringing regulations on the handling of the plague bacterium, including bringing samples into the United States from Tanzania on a plane without declaring them to customs.

● **2003/2004** SARS cases due to laboratory accidents in China, Taiwan and Singapore.

● **2007** Texas A&M University work on 'select agents' shut down after failure to report a 2006 incident in which members of staff had been infected with *Brucella* and *Coxiella*. D.C.







# KEHITYSKULUT

## (I) Tarvittavan tietotaidon leviäminen

- **Tekniikoiden yhä laajempi käyttö tutkijoiden ja tuotekehittäjien keskuudessa**
- **Yliopistot, ammattikorkeakoulut, lukiot**
- **iGEM-kilpailut (*International Genetically Engineered Machine Foundation*)**
- **DIY-biologialiikkeen kasvu ja siihen liittyvät yhteisölaboratoriot ja -tilat (engl. *community labs and hackerspaces*)**



# KEHITYSKULUT (jatkuu)

## (II) Tekniikoiden, tarvikkeiden ja biologisten osien saatavuuden parantuminen

- **DNA-sekvenssoinnin ja -synteesin hinnan nopea lasku**
- **Genominmuokkaustekniikat (tarkkuus ja helppokäyttöisyys)**
- **DNA-synteesipalveluja tarjoavat yhtiöt**
- **Tutkimusjulkaisut ja geneettisten osien kirjastot (esim. *the Registry of Standard Biological Parts*)**
- **Ohjeet kotilaboratorioiden perustamiseen saatavissa internetistä, samoin tarvikkeiden myynti internetissä sekä lisäksi kekseliäät ratkaisut kalliiden laboratorio-instrumenttien korvaamiseen (engl. *workarounds*)**

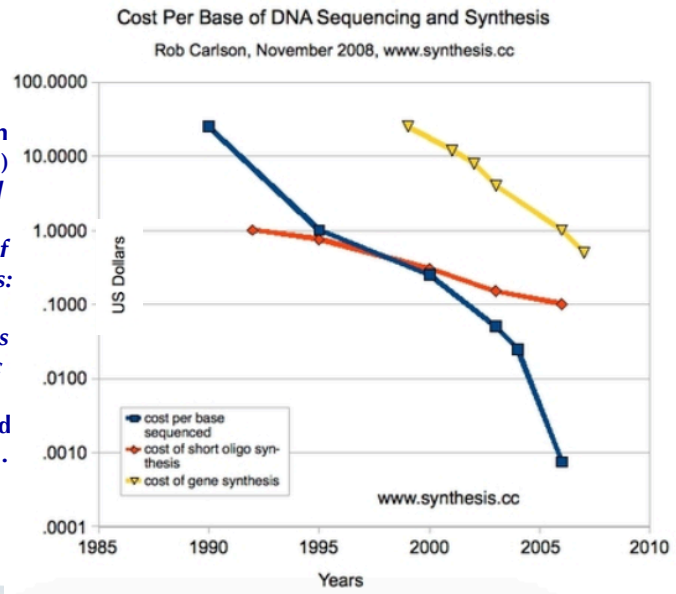


# KEHITYSKULUT (jatkuu)

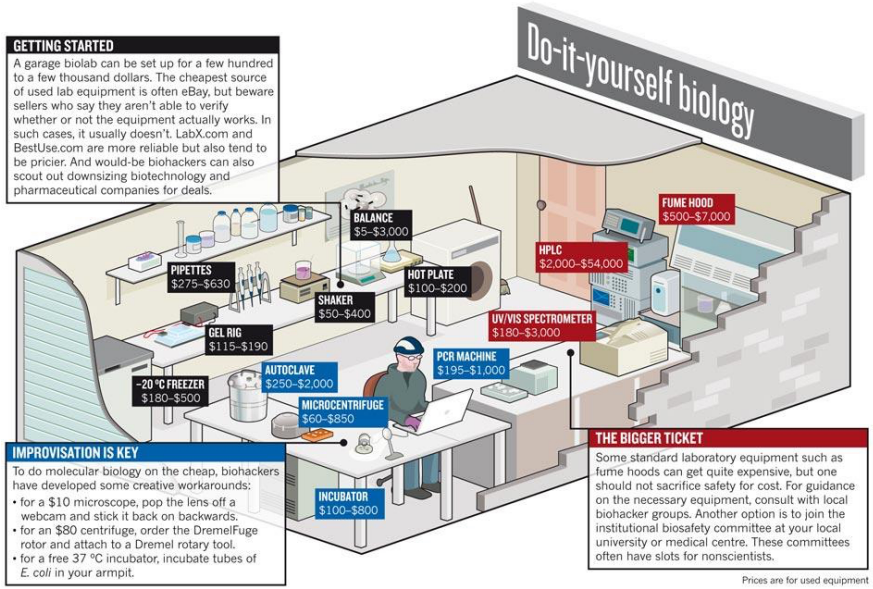
## (III) ”Uudet” mahdollisuudet

- **Jo hävinneiden patogeenien uudelleenrakentaminen (esim. espanjantauti)**
- **Uudenlaiset patogeenit tai (synteettiset) organismit, jotka tuottavat toksiineja**
- **Korkeampi virulenssi**
- **Mahdollinen resistanssi tunnetuille lääkkeille**

Extracted from Oye KA (2012) *Proactive and Adaptive Governance of Emerging Risks: The Case of DNA Synthesis and Synthetic Biology*. A paper prepared for the IRGC).






Ledford H (2010) Life Hackers. *Nature* 467(Oct. 7th): 650–652.




Science

Randerson (2006) *The Guardian* (14th June).

## Revealed: the lax laws that could allow assembly of deadly virus DNA

Urgent calls for regulation after Guardian buys part of smallpox genome through mail order




Tumpey et al. (2005) *Science* 310 (7 Oct.): 77–80.

## Characterization of the Reconstructed 1918 Spanish Influenza Pandemic Virus

Terrence M. Tumpey,[1]* Christopher F. Basler,[2] Patricia V. Aguilar,[2] Hui Zeng,[1] Alicia Solórzano,[2] David E. Swayne,[4] Nancy J. Cox,[1] Jacqueline M. Katz,[1] Jeffery K. Taubenberger,[3] Peter Palese,[2] Adolfo García-Sastre[2]

The pandemic influenza virus of 1918–1919 killed an estimated 20 to 50 million people worldwide. With the recent availability of the complete 1918 influenza virus coding sequence, we used reverse genetics to generate an influenza virus bearing all eight gene segments of the pandemic virus to study the properties associated with its extraordinary virulence. In stark contrast to contemporary human influenza H1N1 viruses, the 1918 pandemic virus had the ability to replicate in the absence of trypsin, caused death in mice and embryonated chicken eggs, and displayed a high-growth phenotype in human bronchial epithelial cells. Moreover, the coordinated expression of the 1918 virus genes most certainly confers the unique high-virulence phenotype observed with this pandemic virus.

# Jackson et al. (2001) *J Virol 75(3):* 1205–1210.

JOURNAL OF VIROLOGY, Feb. 2001, p. 1205–1210 Vol. 75, No. 3
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.3.1205–1210.2001


## Expression of Mouse Interleukin-4 by a Recombinant Ectromelia Virus Suppresses Cytolytic Lymphocyte Responses and Overcomes Genetic Resistance to Mousepox

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*Pest Animal Control Cooperative Research Centre, CSIRO Sustainable Ecosystems,[1] and Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University,[2] Canberra, Australia*

Received 25 July 2000/Accepted 13 November 2000

**Genetic resistance to clinical mousepox (ectromelia virus) varies among inbred laboratory mice and is characterized by an effective natural killer (NK) response and the early onset of a strong $CD8^+$ cytotoxic T-lymphocyte (CTL) response in resistant mice. We have investigated the influence of virus-expressed mouse interleukin-4 (IL-4) on the cell-mediated response during infection. It was observed that expression of IL-4 by a thymidine kinase-positive ectromelia virus suppressed cytolytic responses of NK and CTL and the expression of gamma interferon by the latter. Genetically resistant mice infected with the IL-4-expressing virus developed symptoms of acute mousepox accompanied by high mortality, similar to the disease seen when genetically sensitive mice are infected with the virulent Moscow strain. Strikingly, infection of recently immunized genetically resistant mice with the virus expressing IL-4 also resulted in significant mortality due to fulminant mousepox. These data therefore suggest that virus-encoded IL-4 not only suppresses primary antiviral cell-mediated immune responses but also can inhibit the expression of immune memory responses.**



# Imai M et al. (2012) *Nature* 486 (21 June): 420–430.

doi:10.1038/nature10831

## Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

Masaki Imai[1], Tokiko Watanabe[1,2], Masato Hatta[1], Subash C. Das[1], Makoto Ozawa[1,3], Kyoko Shinya[4], Gongxun Zhong[1], Anthony Hanson[1], Hiroaki Katsura[5], Shinji Watanabe[1,2], Chengjun Li[1], Eiryo Kawakami[2], Shinya Yamada[2], Maki Kiso[2], Yasuo Suzuki[6], Eileen A. Maher[1], Gabriele Neumann[1] & Yoshihiro Kawaoka[1,2,3,5]

**Highly pathogenic avian H5N1 influenza A viruses occasionally infect humans, but currently do not transmit efficiently among humans. The viral haemagglutinin (HA) protein is a known host-range determinant as it mediates virus binding to host-specific cellular receptors[1–3]. Here we assess the molecular changes in HA that would allow a virus possessing subtype H5 HA to be transmissible among mammals. We identified a reassortant H5 HA/H1N1 virus—comprising H5 HA (from an H5N1 virus) with four mutations and the remaining seven gene segments from a 2009 pandemic H1N1 virus—that was capable of droplet transmission in a ferret model. The transmissible H5 reassortant virus preferentially recognized human-type receptors, replicated efficiently in ferrets, caused lung lesions and weight loss, but was not highly pathogenic and did not cause mortality. These results indicate that H5 HA can convert to an HA that supports efficient viral transmission in mammals; however, we do not know whether the four mutations in the H5 HA identified here would render a wholly avian H5N1 virus transmissible. The genetic origin of the remaining seven viral gene segments may also critically contribute to transmissibility in mammals. Nevertheless, as H5N1 viruses continue to evolve and infect humans, receptor-binding variants of H5N1 viruses with pandemic potential, including avian–human reassortant viruses as tested here, may emerge. Our findings emphasize the need to prepare for potential pandemics caused by influenza viruses possessing H5 HA, and will help individuals conducting surveillance in regions with circulating H5N1 viruses to recognize key residues that predict the pandemic potential of isolates, which will inform the development, production and distribution of effective countermeasures.**

before a pandemic. Therefore, we studied the molecular features that would render H5-HA-possessing viruses transmissible in mammals.

Previous studies suggested that HA has a major role in host-range restriction of influenza A viruses[1–3]. The HA of human isolates preferentially recognizes sialic acid linked to galactose by α2,6-linkages (Siaα2,6Gal), whereas the HA of avian isolates preferentially recognizes sialic acid linked to galactose by α2,3-linkages (Siaα2,3Gal)[3]. A small number of avian H5N1 viruses isolated from humans show limited binding to human-type receptors, a property conferred by several amino acid changes in HA[4–9]. None of the H5N1 viruses tested transmitted efficiently in a ferret model[10–13], although, while our paper was under review, one study[14] reported that a virus with a mutant H5 HA and a neuraminidase (NA) of a human virus in the H5N1 virus background caused respiratory droplet transmission in one of two contact ferrets.

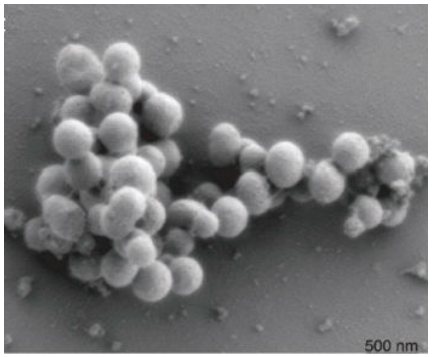
To identify novel mutations in avian H5 HAs that confer human-type receptor-binding preference, we introduced random mutations into the globular head (amino acids 120–259 (H3 numbering), which includes the receptor-binding pocket) of A/Vietnam/1203/2004 (H5N1; VN1203) HA (Supplementary Fig. 1). Although this virus was isolated from a human, its HA retains avian-type receptor-binding properties[6,15]. We also replaced the multibasic HA cleavage sequence with a non-virulent-type cleavage sequence, allowing us to perform studies in biosafety level 2 containment (http://www.who.int/csr/resources/publications/influenza/influenzaRMD2003_5.pdf). The mutated polymerase chain reaction (PCR) products were cloned into RNA polymerase I plasmids[16] containing the VN1203 HA complementary DNA, which resulted in *Escherichia coli* libraries representing the randomly generated HA variants. Sequence analysis of 48 randomly selected clones indicated an average of 1.0 amino acid



# Pennisi E (2012) *Science* (13th March).

## 111 Organizations Call for Synthetic Biology Moratorium

By Elizabeth Pennisi | Mar. 13, 2012 , 2:57 PM

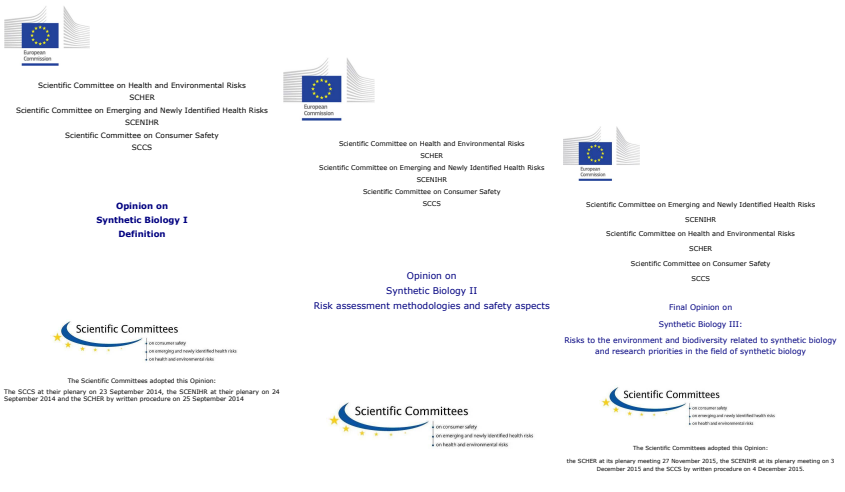



Synthetic cells D. Gibson et al., Science/AAAS

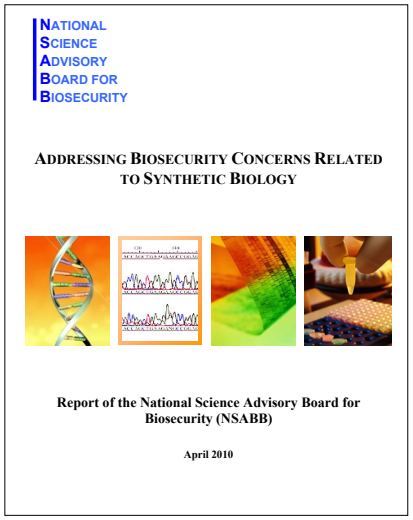


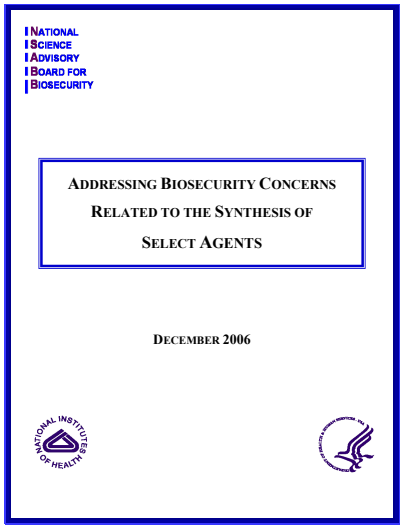
# LOPUKSI

- **VÄITE: Tietyt kehityskulut, jotka liittyvät synteettiseen biologiaan ja genominmuokkaukseen, nostavat bioturvaamisriskejä.**
  - **HUOM! Tästä EI SEURAA, että nämä kehityskulut itsessään olisivat (eettisesti tai muuten) ongelmallisia tai että niitä pitäisi rajoittaa.**
  - **Uudenlaiset uhat ja aiempaa korkeampi riskitaso voivat kuitenkin toimia perusteena tarkistaa lainsäädäntöä sekä hallinnollisia ja viranomaisvalvonnan menettelytapoja.**
  - **Tämä taas EI TARKOITA sitä, että sääntelyä pitäisi kiristää, vaan vain, että näyttäisi olevan hyviä perusteita arvioida, saavutetaanko valittu hyväksyttävän riskin ja suojelun taso nykyisillä bioturvaamisriskienhallinnan toimenpiteillä.**
    - **Viime kädessä moraalispoliittinen valinta.**
  - **Tiedekomiteoiden kolme raporttia Euroopan komissiolle**








# LUKEMISTO

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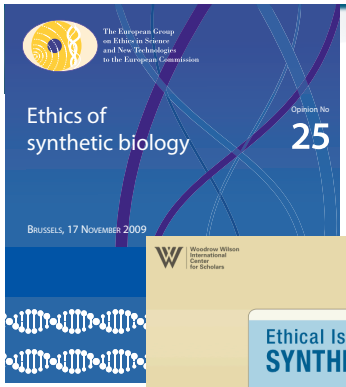


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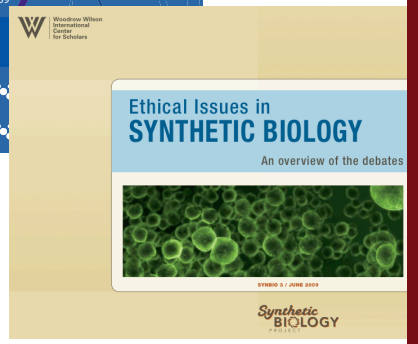


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**Presidential Commission for the Study of Bioethical Issues (2010) Washington DC.**