Challenges for biosafety in the rapid changing field of biotechnology

Gijsbert van Willigen, PhD
Coordinator CBRN Safety & Security
LEIDEN UNIVERSITY MEDICAL HOSPITAL
Trends in biotechnology

• Next generation sequencing
• Gene editing (Crispr/Cas)
• Personalized medicine
• Gene therapy
• GMO-insects
• Gene silencing
• 3D bio printing
• Synthetic biology
Trends in biotechnology

- Next generation sequencing
- Gene editing (Crispr/Cas)
- Personalized medicine
- Gene therapy
- GMO-insects
- Gene silencing
- 3D bio printing
- Synthetic biology
Gene editing: Crispr/Cas

Crispr/Cas9 double-strand DNA break

NHEJ
Gene disruption by small insertions or deletions

HDR
Gene correction or insertion by assisted recombination
Gene editing: Crispr/Cas

• Advantages:
  • Simple to use
  • Highly specific
  • Highly efficient
  • Flexible

• Disadvantages:
  • Off target events
  • Mosaicism
Crispr/Cas: number of publications

![Graph showing the number of Crispr/Cas publications from 2002 to 2015. The number of publications increases significantly from 2013 onwards.]

- 2002: 1
- 2003: 2
- 2004: 0
- 2005: 5
- 2006: 5
- 2007: 10
- 2008: 22
- 2009: 32
- 2010: 47
- 2011: 75
- 2012: 123
- 2013: 246
- 2014: 520
- 2015: 820

7th Biosafety & Biosecurity symposium, Milan, Italy

March 23, 2017
Crispr/Cas: Applications

The future of CRISPR-Cas9-mediated genome engineering

- Human gene therapy
- Screens for drug target ID
- Agriculture: crops, animals
- Ecological vector control: mosquito sterilization, etc.
- Synthetic biology; pathway engineering
- Viral gene disruption; pathogen gene disruption
- Programmable RNA targeting
Crispr/Cas: Applications

Human gene

Embryo editing gets green light
UK decision sets precedent for research on editing genomes of human embryos.

CRISPR gene editing tested in a person
Trial could spark biomedical duel between China and US.

Gene editing could improve the ability of immune cells (spherical) to attack cancer.

Viral gene disruption; pathogen gene disruption

THE LANDSCAPE FOR HUMAN GENOME EDITING
A view of international regulations suggests where in the world a CRISPR baby could be born.
Crispr/Cas: Safety challenges

- Off target events
  - GuideRNA binds to a site homologous to the target site

- Gene drives:
  - Genetic changes are distributed rapidly and irreversibly within a population
  - Prerequisites:
    - Crispr/Cas and the guide RNA are located on one DNA-vector that integrates in the genome
    - Sexual reproduction
  - Risk: A gene drive cannot be repaired
Crispr/Cas: Safety challenges

Normal inheritance

Altered gene  Wild type

Male  Female

Altered gene without gene drive: One copy inherited from one parent.
50 percent chance of passing it on.

Altered gene does not spread

Gene drive inheritance

Gene drive  Wild type

Cut  Repair

Altered gene as gene drive: One copy converts gene inherited from other parent.
More than 50 percent chance of passing it on.

Altered gene is almost always inherited
Crispr/Cas: Ethical issues

- Accepted applications:
  - Repairing genetic defects:
    - Cure genetic diseases in germ line cells
    - Correction of recessive genetic disorders

- Controversial application:
  - Improving mankind:
    - Protection against cancer
    - Enhance properties of human (length, IQ, build-up of muscles)
    - Equip humans with new features (new sensors, improving genes)
Crispr/Cas: Biosecurity issues

- Available, easy to use and cheap technique
- Can be used by anyone: DIY bio, neighborhood labs, biohacking
- Weaponizing micro-organisms
  - Not recognizable for diagnostic labs
- Weaponizing gene drives
  - Creating killer mosquitos using gene drives
- Gain of function research using Crispr/Cas
Crispr/Cas: Is the resulting organism a GMO?

- The application of Crispr/Cas is considered a GMO technique
- The resulting gene-edited organism does not contain the Crispr/Cas machinery any more
- Hard to detect whether the organism is made using gene editing
- How can we make sure these products are labelled properly?
Crispr/Cas: Is the resulting organism a GMO?

- The application of Crispr/Cas is considered a GMO technique.
- The resulting gene-edited organism does not contain the Crispr/Cas machinery any more.
- Hard to detect whether the organism is made using gene editing.
- How can we make sure these products are labelled properly?

Companies like Calyxt have portrayed gene editing more like moving the cursor in a word processor to a particular location and making a small change to the text.
Technique of sterile insect techniques already used in the 1950’s
- Irradiated male insects

Seen as tool to reduce infectious diseases spread by insects

Mating with sterile males does not result in new insects:
- Insect population decreases

Advantage: reduction in use of insecticides
GMO insects

- Recent developments: use of GMO-insects

- Examples:
  - Insects with a suicide gene
  - Use of Crispr/Cas as gene drive
GMO insects: suicide gene

• Advantage:
  • Have same fitness as normal male insects
  • Easy selection (100% contains suicide gene)
  • GMO insects will die
  • Insect population will not die out completely

• Disadvantage:
  • After some time the population will be back to normal
GMO insects: suicide gene

• Safety and security issues:
  • Effect on the eco system
  • Affects biodiversity

• Ethical issues:
  • Decrease of infectious diseases
  • No freedom of choice for the general public
  • Developing countries will be dependent of Western countries to use the technique
Gene drive:
- Spreads through the population very quickly (non Mendelian transfer of the effect)
- Considered stable in the genome

First example:
- No egg production female mosquito’s
GMO insects: Crispr/Cas – Gene drive

• Advantages:
  • Mutation will spread quickly
  • Mutation will eventually affect the entire population

• Disadvantages:
  • In theory it will eventually extinct an entire population
  • Gene drive affects the fertility and fitness of the insects
Safety and security issues:
- Effect on the ecosystem and affects biodiversity
- What happens when an entire population of an insect will be extinct?
- Is it really safe to release these insects already? Can we assess the risk?

Ethical issues:
- Decrease of infectious diseases
- No freedom of choice for the general public
- Developing countries will be dependent of Western countries to use the technique
Gene silencing

• Plant breeding techniques:
  • Non-GMO:
    • Selective breeding – natural process
    • Cross breeding – natural process
    • Reverse breeding – hybrid cells
    • Infiltration – transfer of genes into a plant
    • Targeted mutagenesis – use of chemical or radiation mutagenesis
  • GMO:
    • Gene silencing - RNAi
    • Genome editing – Crispr/Cas, TALEN, Zinc finger
Gene silencing

• Plant breeding techniques:
  • Non-GMO:
    • Selective breeding – natural process
    • Cross breeding – natural process
    • Reverse breeding – hybrid cells
    • Infiltration – transfer of genes into a plant
    • Targeted mutagenesis – use of chemical or radiation mutagenesis
  • GMO:
    • Gene silencing - RNAi
    • Genome editing – Crispr/Cas, TALEN, Zinc finger
Gene silencing

- Technique of gene silencing:

DNA → RNA → No Protein

Double-stranded RNA is destroyed
Gene silencing

• Types of gene silencing:
  • Silencing a gene in the organism itself
    • GMO
      • Artic apple (Polyphenol oxidase free)
    • Amylose free potato
  • Non GMO
    • Spraying dsRNA
  • Silencing a gene in a different organism
    • Pest control
Gene silencing

- Safety issues:
  - Are the GMO’s safe for food and feed? Impossible to test?
  - Safety issues for persistence of RNAi in the environment

- Are there security issues related to the technique?

- Ethical issues:
  - No freedom of choice for the general public
  - Developing countries will be dependent of Western countries to use the technique
Gene silencing

• Safety issues:
  • Are the GMO’s safe for food and feed? Impossible to test?
  • Persistence of RNAi in the environment
  • Are there security issues related to the technique?

• Ethical issues:
  • No freedom of choice for the general public
  • Developing countries will be dependent on Western countries to use the technique
• What is synthetic biology:
  • Synthetic genomics
    • Synthesing genes and genomes from scratch
  • Minimal genomes
    • Organisms that contain only the most essential genes
  • Synthetic cells
    • Synthetic genomes in synthetic hulls
  • Xenobiology
    • Using synthetic non-natural nucleotides
  • Metabolic pathway engineering
    • Production of specific molecules by genetically altering the organism
Safety and security issues:

- Can we predict the risks of organisms created by synthetic biology using the present risk assessment techniques?

- Can we predict the environmental impact of organisms created by synthetic biology are released

- Can be used by anyone: DIY bio, neighborhood labs etc
Synthetic biology

• Advantages:
  • Cheaper production of raw materials and chemicals
    • Biofuels, food supplements
  • Easier production of medication
  • Production of synthetic vaccines (cheaper and quicker)
General conclusions

• New techniques in biotechnology are considered beneficial

• General questions:
  • Are the techniques safe to use?
  • Do the techniques bring biotechnology into an uncontrolled environment?
  • Are the techniques an issue in biosecurity?
  • Ethical issues of the techniques
General conclusions

• Challenges for biosafety:
  • More research is needed to identify potential risks of the new techniques before placing products on the market
  • More oversight is needed for the use of the new techniques
  • Identify potential biosecurity issues related to the new techniques
Bioterrorism could kill more people than nuclear war, Bill Gates to warn world leaders

By Ben Farmer, DEFENCE CORRESPONDENT, IN MUNICH
18 FEBRUARY 2017 · 9:30AM

Bioterrorists could one day kill hundreds of millions of people in an attack more deadly than nuclear war, Bill Gates will warn world leaders.
General conclusions

Bioterrorism could kill more people than nuclear war, Bill Gates to warn world leaders

Rapid advances in genetic engineering have opened the door for small terrorism groups to tailor and easily turn biological viruses into weapons.

By Ben Farmer, DEFENCE CORRESPONDENT, IN MUNICH
18 FEBRUARY 2017 • 9:30AM

Bioterrorists could one day kill hundreds of millions of people in an attack more deadly than nuclear war, Bill Gates will warn world leaders.
Gijsbert van Willigen, PhD
Leiden University Medical Center
Leiden, the Netherlands

Phone: +31 71 526 4304
E-mail: g.van_willigen@lumc.nl