# Synthetic Biology Challenges and Risks

iGEM european experience

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UMR8030

# Synthetic biology: public opinion challenges and scientific ethics

-Golden rice
-CRISPR in human embryos

# 30 june 2016: 107 Nobel Laureates signed a letter to support GMOs and Golden Rice

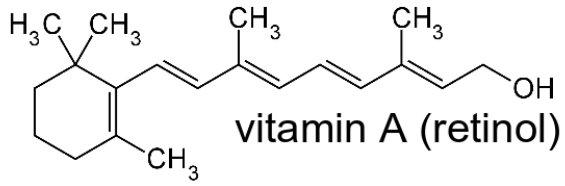
http://supportprecisionagriculture.org/
https://www.washingtonpost.com/news/speaking-of-science/wp/2016/06/29/more-than-100-nobel-laureates-take-on-greenpeace-over-gmo-stance/

Excerpts from the letter:

WE CALL UPON GOVERNMENTS OF THE WORLD to reject Greenpeace's campaign against Golden Rice specifically, and crops and foods improved through biotechnology in general; and to do everything in their power to oppose Greenpeace's actions and accelerate the access of farmers to all the tools of modern biology, especially seeds improved through biotechnology.

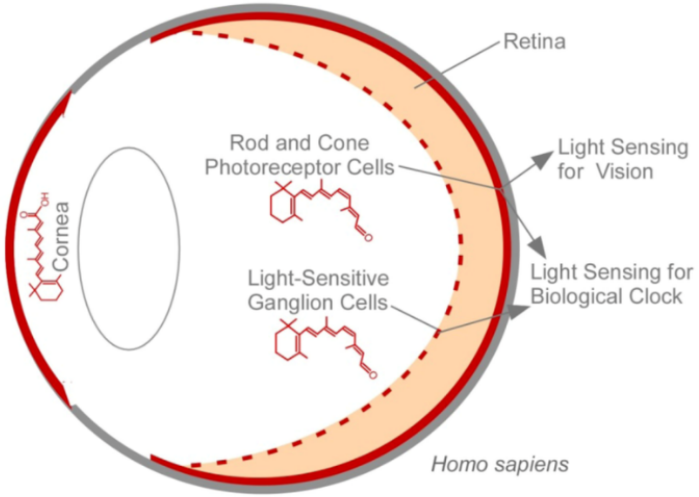
How many poor people in the world must die before we consider this a "**crime against humanity**"?

# Why would we want to produce golden rice?




vitamin A (retinol)

## vitamin A is required for vision




Zhong et al, 2012 *Nutrients* 4(12), 2069-2096

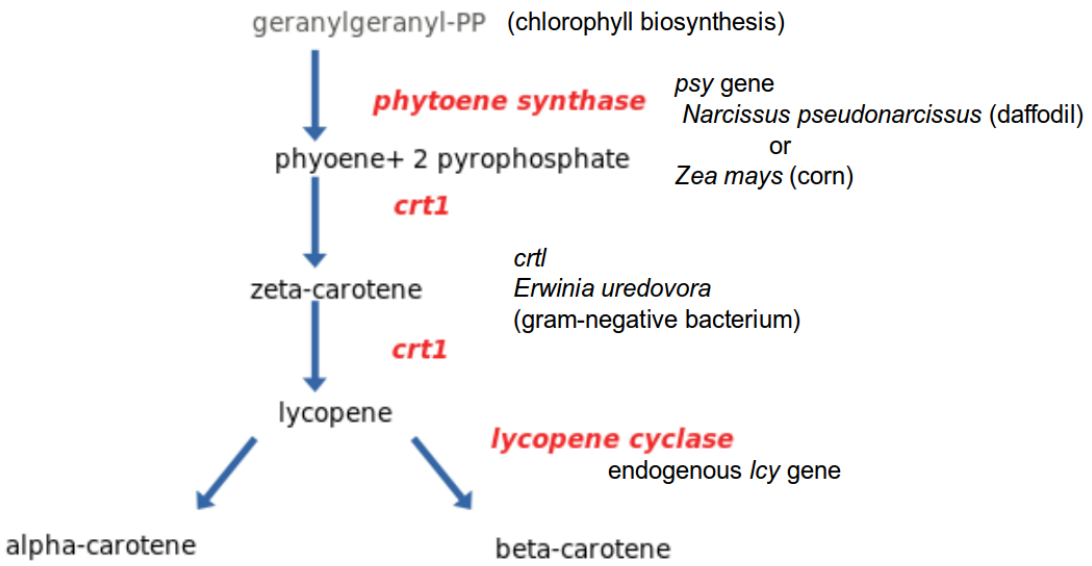
## vitamin A deficiency

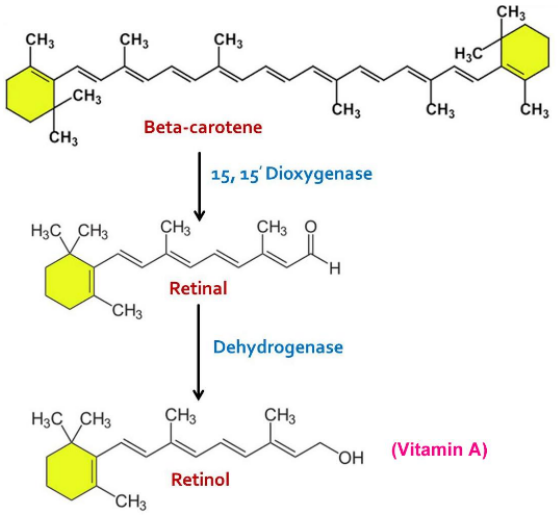
-The leading cause of preventable childhood blindness (UNICEF)

-500,000 cases of childhood blindness annually (National Institutes of Health)

-670,000 childhood deaths annually (*Lancet* 371 9608: 243–60)

# How was golden rice developed?

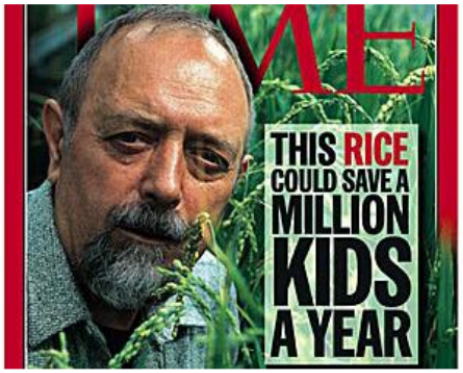



http://genomics.unl.edu/RBC_EDU/car.html

endosperm-specific promoter: *psy-crtI*
add plastid-targeting sequence to *lcy* gene

# Golden Rice is an effective source of vitamin A




http://www.allowgoldenricenow.org

1 cup per day of Golden Rice could supply 50% of required vitamin A (American Society of Nutrition)

Golden Rice is effectively converted into vitamin A in humans
Tang 2009 *Am J Clin Nutr* 89 (6): 1776–83.

Golden Rice B-carotene is as effective as b-carotene in oil at providing vitamin A to children

# Why is golden rice controversial?

(DOI: 10.1126/science.320.5875.468)

## Greenpeace calls Golden Rice "genetic pollution"

-**Golden Rice is corporate plot** to win over a skeptical public and open the door to other GM crops.
-Golden rice does not attack the underlying problem of poverty.

-"There's a real chance here for governments and the philanthropic community to support these endeavours by **investing in climate-resilient ecological agriculture** and empowering farmers to access a balanced and nutritious diet, rather than pouring money down the drain for GE 'Golden' rice"

## Francesco Branca (WHO)

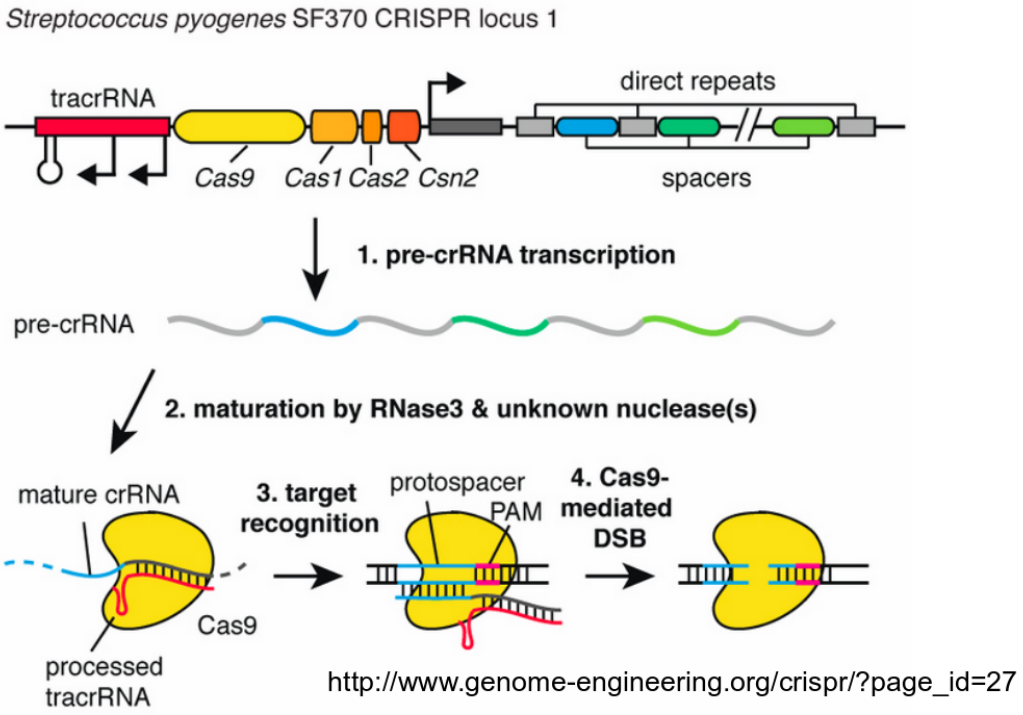
-**Teaching people to grow carrots or certain leafy vegetables** is a more promising way to fight the problem.

Do you think Golden Rice is a good idea?

If so, what would be required to convince the public?

# CRISPR: programmable RNA-guided DNA nuclease

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats




http://www.genome-engineering.org/crispr/?page_id=27

tracrRNA binds repeats
spacer directs Cas9 nuclease

immune system: bacteria 'remember' viral sequences and cut them.

**tracrRNA**: hybridizes spacer RNA to form complex

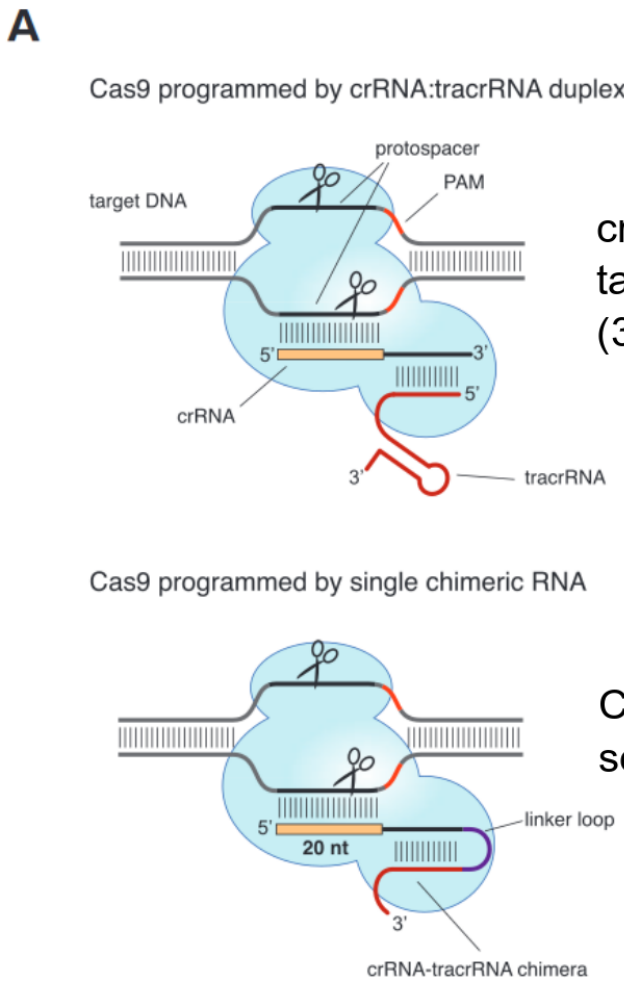
**CAS9**: DNA nucleases guided by crRNA:tracrRNA complex

**Direct repeats**: cleavage sites between spacers

**Spacers**: processed into crRNA (~30 bp) to program Cas9 specificity

# Target CRISPR to cut at desired genomic sequence

Cas9 can be targeted to cut any genome sequence using a single guide RNA (<100 bp)



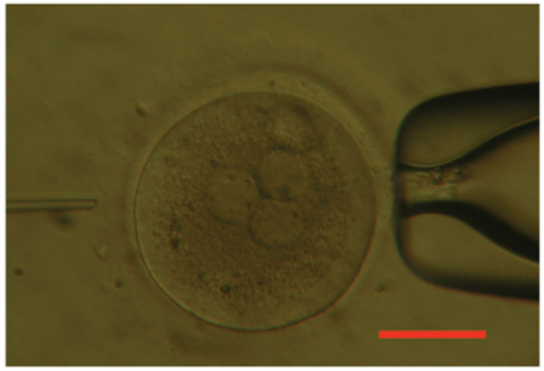

crRNA recognizes and binds homologous target (protospacer) + PAM sequence (3' Protospacer Adjacent Motif NGG)

Cas9 cuts genome at protospacer sequence

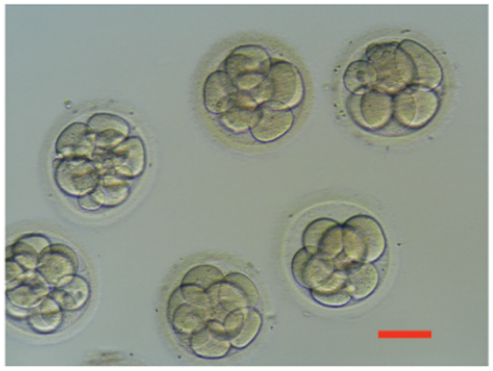
Jinek et al, 2012 (PMID 22745249)

# Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing

Kang et al, 2016 *J. Assisted Reproduction and Genetics*
May 2016, Volume 33, Issue 5, pp 581-588



3PN Zygote Injection



8-16 Cell Stage

development of edited embryos

CRISPR-mediated introduction of CCR5Δ32 allele (HIV resistance) into non-viable human embryos.

# Introduction of CCR5Δ32 into human 3PN embryos

| Groups[a] | Injected 3PN zygotes | Development (%) | | Genetic modification | |
|---|---|---|---|---|---|
| | | Cleavage | 8–16 cell | NHEJ mutations (percent) | Δ32 allele (%) |
| Control water | 18 | 15 (83) | 13 (72) | 0 | 0 |
| Cas9 + gRNA1 | 11 | 9 (82) | 7 (64) | 4 (57) | 0 |
| Cas9 + gRNA2 | 13 | 10 (77) | 8 (62) | 5 (63) | 0 |
| Cas9 + gRNA1 + ssODN1 (PN injection) | 23 | 14 (61) | 11 (48) | 4 (36) | 0 |
| Cas9 + gRNA1 + ssODN1 | 32 | 28 (88) | 20 (63) | 10 (50) | 1 (5) |
| Cas9 + gRNA2 + ssODN2 | 46 | 39 (85) | 27 (59) | 13 (48) | 0 |
| Cas9 + gRNA1 + 1 kb dsDonor | 25 | 21 (84) | 15 (60) | 7 (47) | 1 (7) |
| Cas9 + gRNA1 + gRNA2 | 45 | 37 (82) | 26 (58) | 13 (50) | 4 (15) |

[a] CRISPR/Cas system was delivered to 3PN zygotes by cytoplasmic injection in all groups, except the group labeled with PN (pronuclear) injection

# Author's conclusions

Improved methods needed to define the modification efficiency and to select the embryos containing the desired allele.

Genetic mosaicism needs to be resolved.

Improved methods are needed to eliminate off-target mutations.

The genome-wide effects of changing even a single allele need to be fully understood.

Many scientists called for a moratorium on clinical applications of germline gene editing (Baltimore et al, *Science*. 2015;348(6230):36–8)

Many iGEM teams have developed tools to edit human cells (CRISPR, TALENs, Zinc finger nucleases)

What needs to be done to ensure that these editing tools are used ethically?