Genomic solutions to sustainable development

Week 9 — Synthetic biology

15 April 2025

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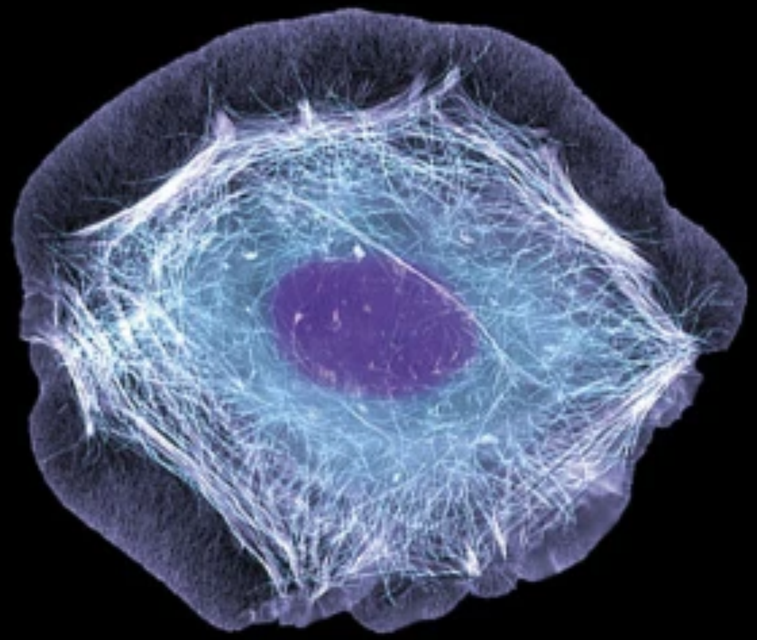
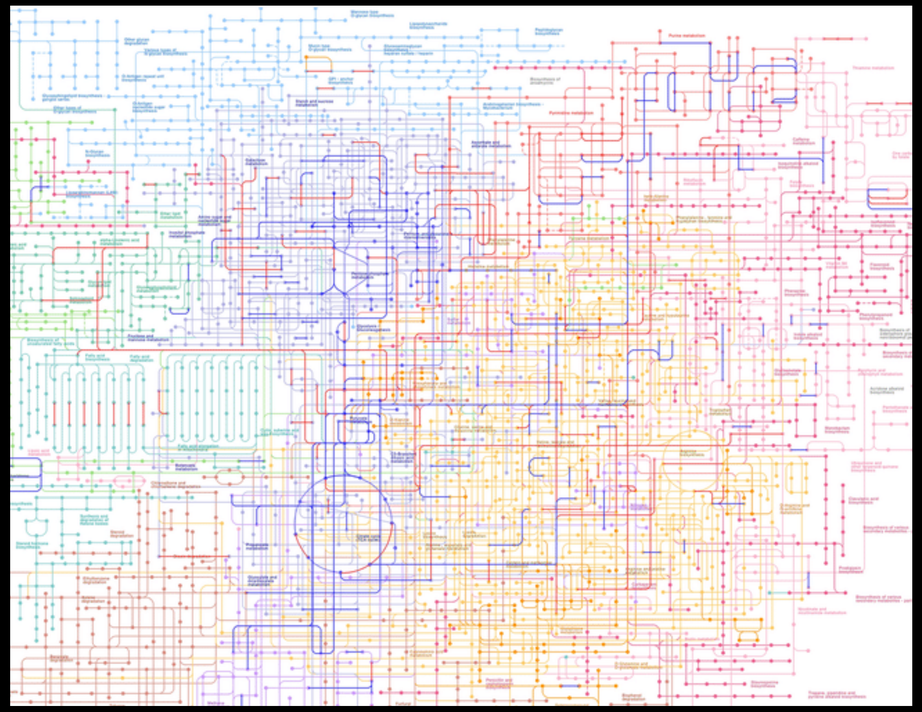
BIO-312 Genomic solutions to sustainable development

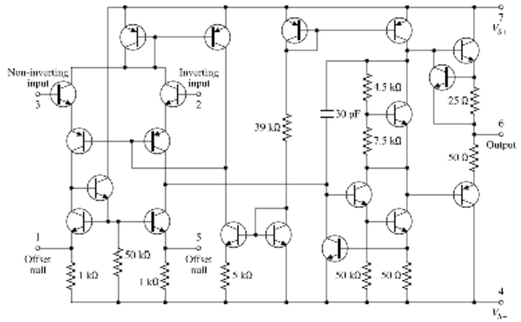


BIO-312 Genomic solutions to sustainable development

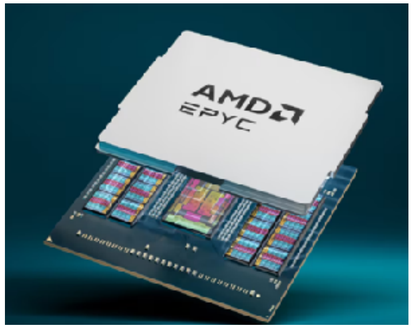


Physical components
(eg, resistor)

Electronic circuits



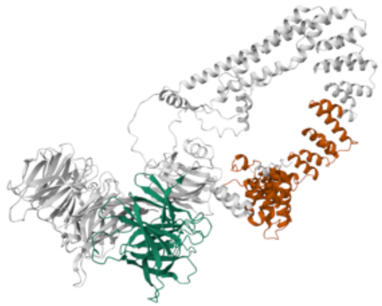
Integrated modules
(eg, RAM, CPUs)



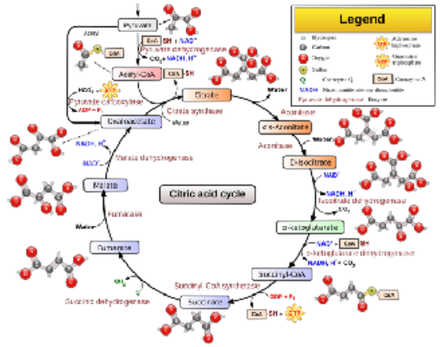
Drone
(individual robot)

Drone swarm/network

Biomolecules
(gene, protein, metabolite)

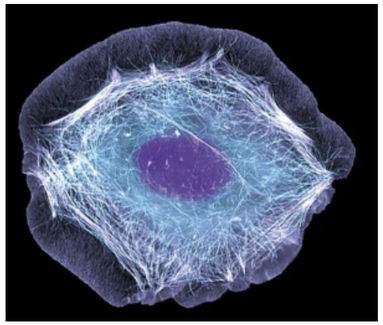


Genetic circuits



Organelle

Cell



Multicellular organism

BIO-312 Genomic solutions to sustainable development

Synthetic biology

- **Definition:** to apply engineering principles to design, construct, and redesign of biological systems for a specific purpose

- **Core concepts**:
  - *Design and construction:* breaking down complex biological systems into parts and assembling them back together
  - *Engineering principles:* abstraction, standardisation, quantification, simulation
  - *Rational design*: predict and optimize biological system behavior
  - *Genetic engineering:* design-driven DNA synthesis, DNA assembly, synthetic genomes

Synthetic biology circuits

- Sensing
- Processing
- Actuation

Biological sensor
(eg, metabolite)

Regulatory circuits
(eg, processing/integration of signals)

Controlled cell behavior
(eg, GFP expression)

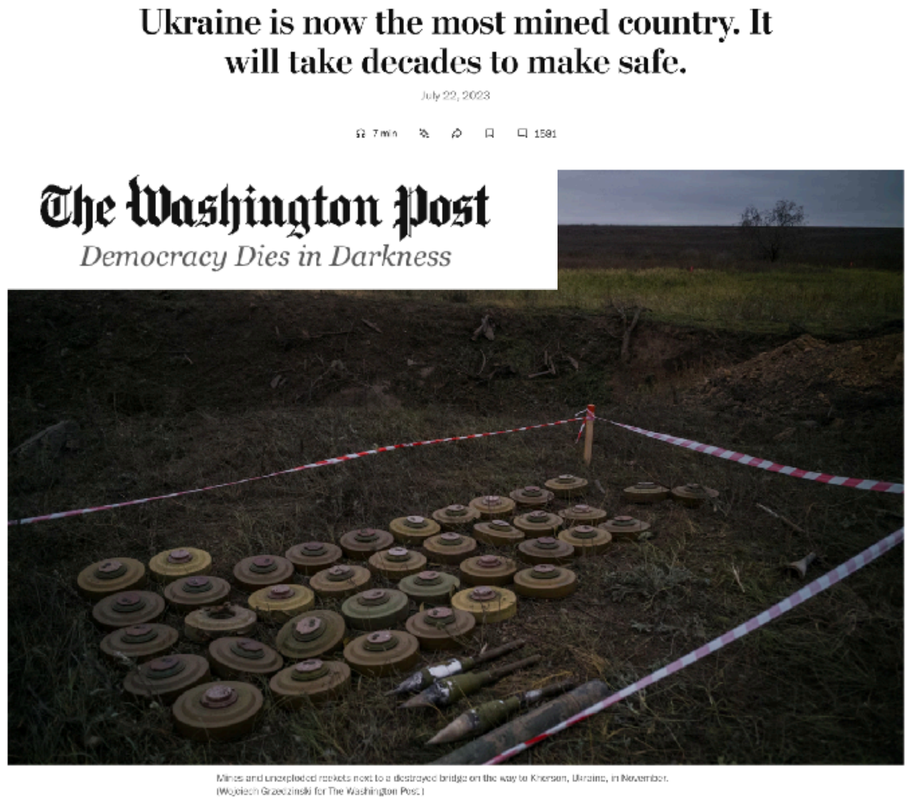
Land mines

- Millions of hectares in 70 countries are contaminated with land mines and 60 million people live on a daily basis with the risk of landmines

- Land mines cause significant land degradation due to (1) restricted access, (2) loss of biodiversity, (3) micro-relief disruption, (4) altered chemical composition, (5) loss of productivity

Land mines in Ukraine and it’s impact on global food production

- Ukraine is one of the world’s top agricultural producers (wheat, corn, sunflower oil)
- About 174,000 km2 (=25 million football fields) of it’s land are contaminated with land mines
- About one third of arable area is currently affected by land mines



Remote detection of buried landmines using bacterial sensors

US005972638A

United States Patent [19]

Burlage et al.

[11] **Patent Number: 5,972,638**

[45] **Date of Patent: *Oct. 26, 1999**

[54] **METHOD FOR DETECTION OF BURIED EXPLOSIVES USING A BIOSENSOR**

[75] Inventors: **Robert S. Burlage**, Knoxville; **David R. Patek**, Loudon; **Kirk R. Everman**, Knoxville, all of Tenn.

[73] Assignee: **Lockheed Martin Energy Research Corp.**, Tenn.

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: **08/792,251**

[22] Filed: **Jan. 31, 1997**

[51] **Int. Cl.**[6] **C12N 1/21**; C12Q 1/02; C12Q 1/24

[52] **U.S. Cl.** **435/29**; 434/4; 434/252.3; 434/252.31; 434/252.33

[58] **Field of Search** 435/4, 252.3, 252.31, 435/252.33, 254.11, 29

[56] **References Cited**

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4,683,195	7/1987	Mullis et al.	435/6
4,683,202	7/1987	Mullis	435/91.2
4,965,188	10/1990	Mullis et al.	435/6
5,491,084	2/1996	Chalfie et al.	435/189

Higson, "Microbial Degradation of Nitroaromatic Compounds," *Adv. App. Microbiol.*, 37:1–19 (1992).

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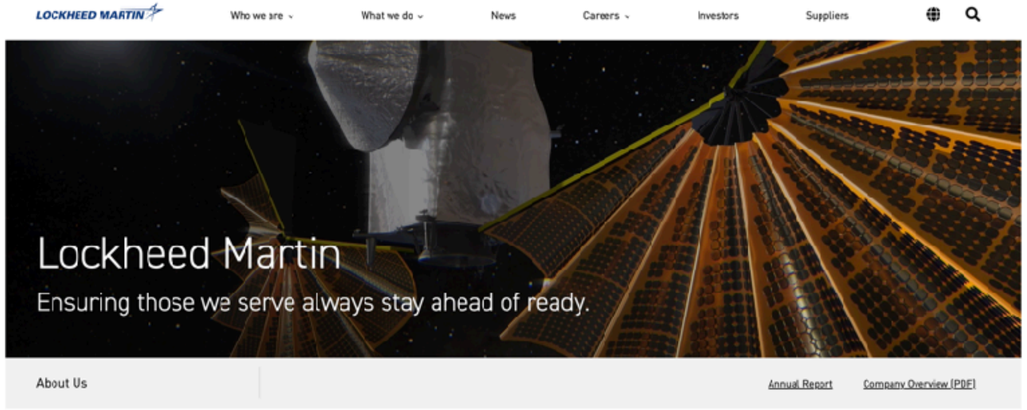
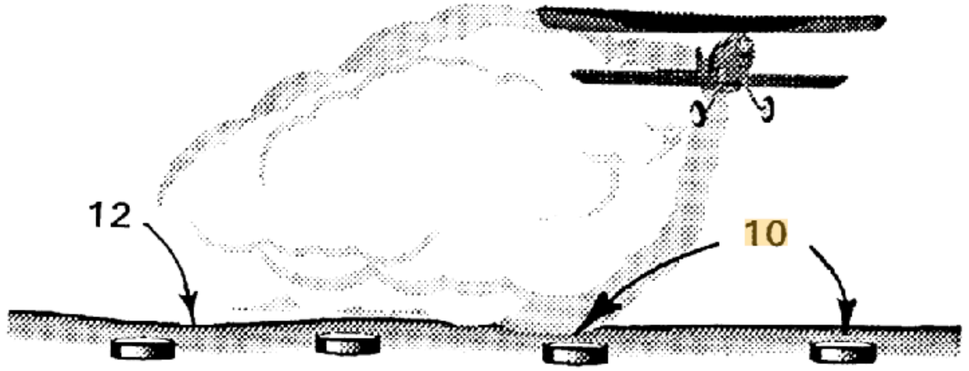
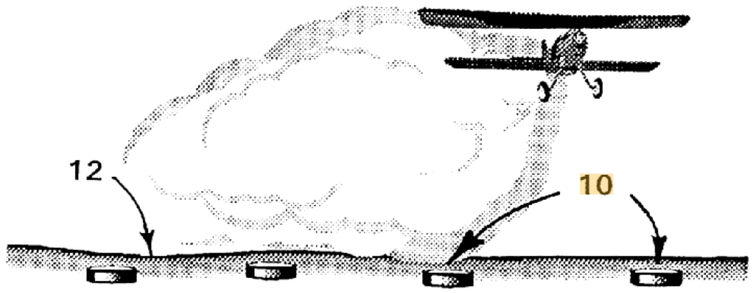


We specialize in defense tech, solving complex challenges, advancing scientific discovery and delivering innovative solutions that help our customers keep people safe.



Design of a genetically engineered bioreporter for detection of land mines

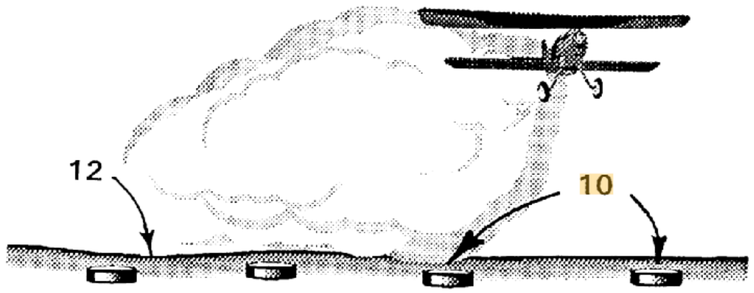
Sensing
Direct of indirect
detection of explosives



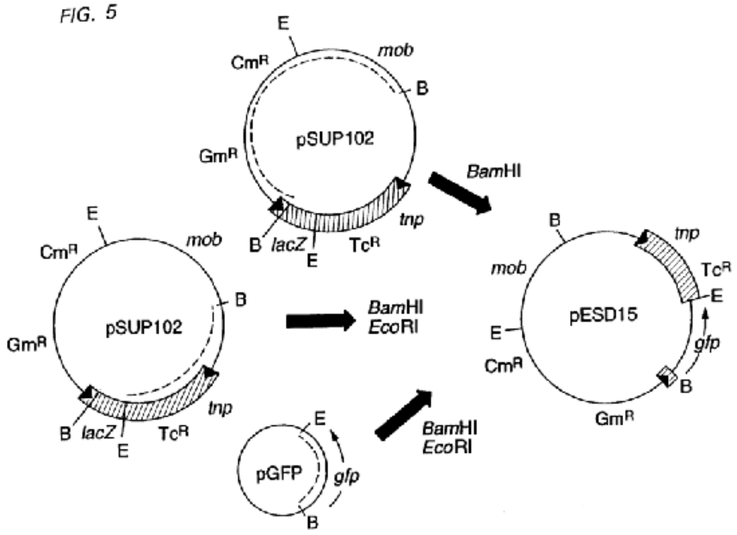
US Patent 5,972,638

Design of a genetically engineered bioreporter for detection of land mines

Sensing
Direct of indirect
detection of explosives



Processing
salt stress gene promoter
upstream of reporter gene



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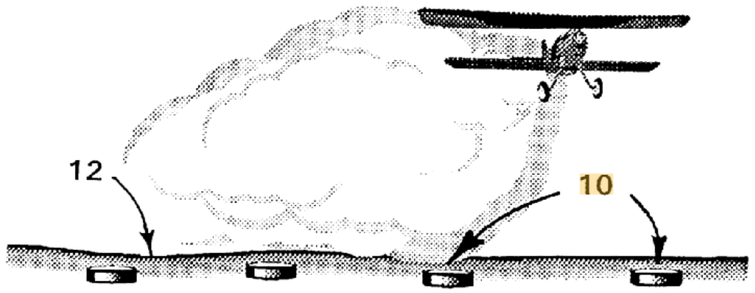

US Patent 5,972,638

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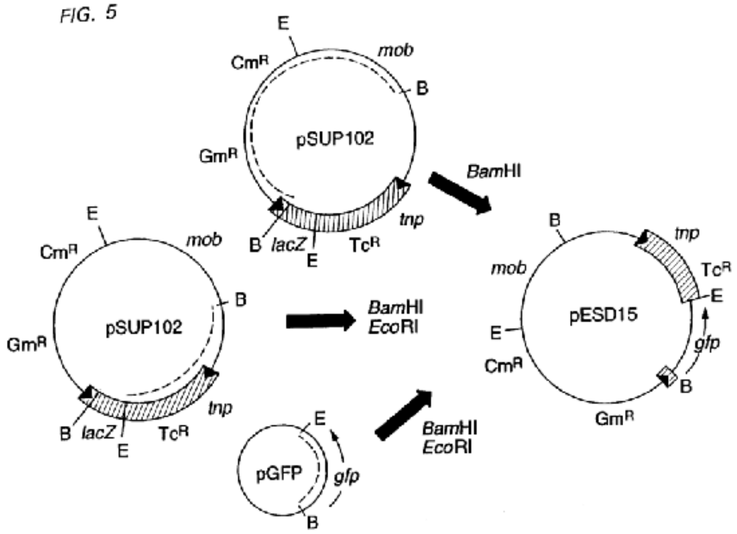
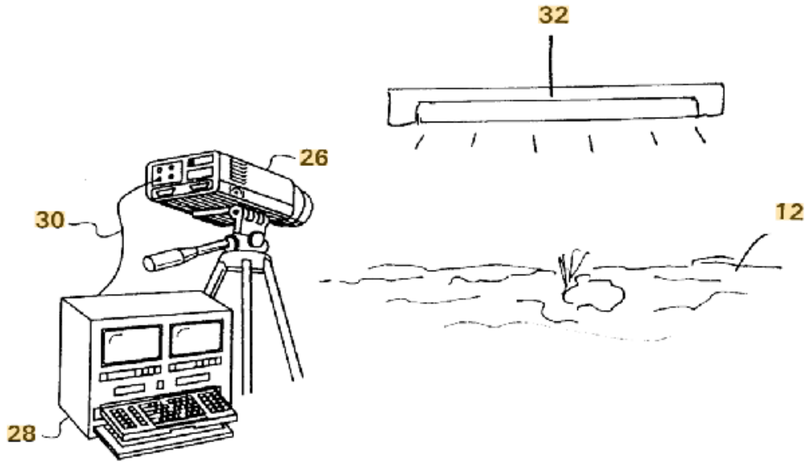
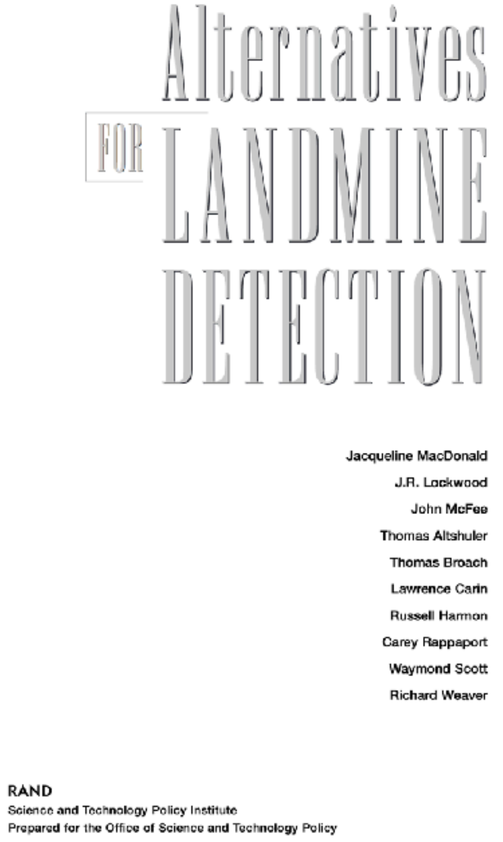


Actuation
glowing bacterial colony under
UV light



First field tests reported by the US Army in 1999

USING FLUORESCENCE IMAGERY AND MICROBES FOR ORDNANCE AND MINE DETECTION



Alternatives FOR LANDMINE DETECTION

Jacqueline MacDonald
J.R. Lockwood
John McFee
Thomas Altshuler
Thomas Broach
Lawrence Carin
Russell Harmon
Carey Rappaport
Waymond Scott
Richard Weaver

RAND
Science and Technology Policy Institute
Prepared for the Office of Science and Technology Policy

4 out of 5 TNT sites were detected within a radius of 2m

Patent hindered commercial development for 20 years

United States Patent [19]

Burlage et al.

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Bruns–Nagel et al., "Microbial Transformation of 2,4,6–trinitrotoluene in Aerobic Soil Columns," *Appl. Environ.*

Nonpayment of patent fees released the approach after 10 years

Legal Events

Date	Code	Title	Description
1997-06-23	AS	Assignment	**Owner name**: LOCKHEED MARTIN ENERGY RESEARCH CORP., TENNESSEE **Free format text**: ASSIGNMENT OF ASSIGNORS INTEREST;ASSIGNORS:BURLAGE, ROBERT S.;PATCK, DAVID R.;EVERMAN, KIRK R.;REEL/FRAME:008600/0567;SIGNING DATES FROM 19970617 TO 19970619
2002-12-05	FEPP	Fee payment procedure	**Free format text**: PAYER NUMBER DE-ASSIGNED (ORIGINAL EVENT CODE: RMPN); ENTITY STATUS OF PATENT OWNER: LARGE ENTITY **Free format text**: PAYOR NUMBER ASSIGNED (ORIGINAL EVENT CODE: ASPN); ENTITY STATUS OF PATENT OWNER: LARGE ENTITY
2003-03-26	FPAY	Fee payment	**Year of fee payment**: 4
2007-05-16	REMI	Maintenance fee reminder mailed	
2007-10-26	LAPS	Lapse for failure to pay maintenance fees	
2007-11-26	STCH	Information on status: patent discontinuation	**Free format text**: PATENT EXPIRED DUE TO NONPAYMENT OF MAINTENANCE FEES UNDER 37 CFR 1.362
2007-12-18	FP	Lapsed due to failure to pay maintenance fee	**Effective date**: 20071026

https://patents.google.com/patent/US5972638A/

First scientific demonstration for remote detection of land mines with bacterial sensors

with a novel mode of action is approved for marketing?

We submit that three decades of soliciting public engagement has not improved the public acceptance cited by advocates as a justification for the efforts, and that subordinating evidence-based policy making to emotional or political calculations has neither increased public acceptance nor encouraged innovation. We would prefer to heed the caveat of Barbara Keating-Edh, who testified before the National Biotechnology Policy Board in 1991, representing the consumer group Consumer Alert:

"For obvious reasons, the consumer views the technologies that are *most* regulated to be the *least* safe ones. Heavy involvement by government, no matter how well intended, inevitably sends the wrong signals. Rather than ensuring confidence, it raises suspicion and doubt" [emphasis in original][11].

We believe that the current excessive and unscientific regulation at the US Environmental Protection Agency (EPA), USDA and FDA (and other agencies) erodes both public understanding and trust, and exacerbates skepticism about the safety of genetically engineering products. The NAS could have helped repair this policy miscarriage. It did not.

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11. *Biotechnol. Law Rep.* **12**, 127–182 (10.1089/blr.1993.12.127 (2009).

Remote detection of buried landmines using a bacterial sensor

To the Editor:

Finding where landmines and improvised explosive devices are buried remains risky. Currently, landmines are detected by personnel in the field using methods that, in principle, have remained largely unaltered for the past 75 years. The obvious risks to life that this poses, the large proportion of false-positive identifications, as well as an extremely limited ability to detect non-metallic landmines, means that a need exists for alternative methods for detecting landmines from a safe standoff distance. Here we report a small-scale field trial that uses a bacterial biosensor for the remote detection of anti-personnel mines.

The use of genetically engineered bacteria for landmine detection was first proposed by Burlage *et al.* in a patent dated 1959 (ref. 1) based on the observation that the residues from explosives can accumulate in the soil surrounding a buried explosive device[2]. We hypothesized that a few hours after spraying the bacterial sensor strain on the soil, the location of buried landmines would be pinpointed by localized areas of fluorescence generated by the response of genetically engineered bioreporters to the explosives' vapors. This concept was field-tested in 1998 (refs. 3,4) with mixed results: four out of five sub-surface targets containing up to 4.5 kg of 2,4,6-trinitrotoluene (TNT) were discovered within 2 m of the target, along with two false positives, using a fluorescent *Pseudomonas putida*-based sensor strain and a laser imaging system. To the best of our knowledge, the results of this test have never been published in the scientific literature, nor has any follow-up been reported.

Most landmines contain TNT, either by itself or in combination with other explosives[5]. Previously, we have engineered an *Escherichia coli* reporter strain capable of micromolar-level detection of both vapor-phase TNT and its main impurity and degradation product, 2,4-dinitrotoluene (DNT)[6,7]. The latter compound is more volatile than TNT and is considered to be an excellent signature chemical for TNT-based explosive devices[5]. Our bacterial TNT sensor strain harbors a plasmid-borne reporter in which the DNT/TNT-inducible *yqjF* gene promoter[6] is fused to the green fluorescent protein gene *gfpmut2*. Activation of *yqjF*, by an as-yet unidentified DNT degradation product, is controlled by the transcriptional regulator *YhaJ*[8]. We also developed a laser based optoelectronic system for the remote detection and quantification of bacterial fluorescence[9,10].

We proceeded to combine our biosensor and remote detection technologies in a small-scale field trial. Our experimental site, 3.8 m × 1.1 m in size, comprised 12 buried plastic boxes, each of which was 40 cm × 70 cm × 30 cm in size, and in which the sample was covered either with natural sand (Mediterranean coastal sand), lab-

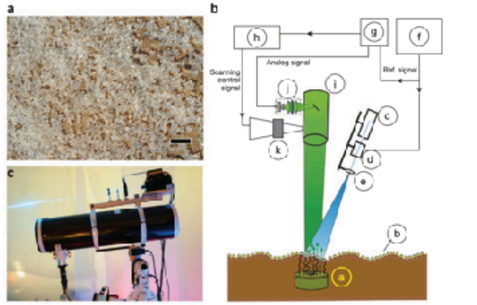
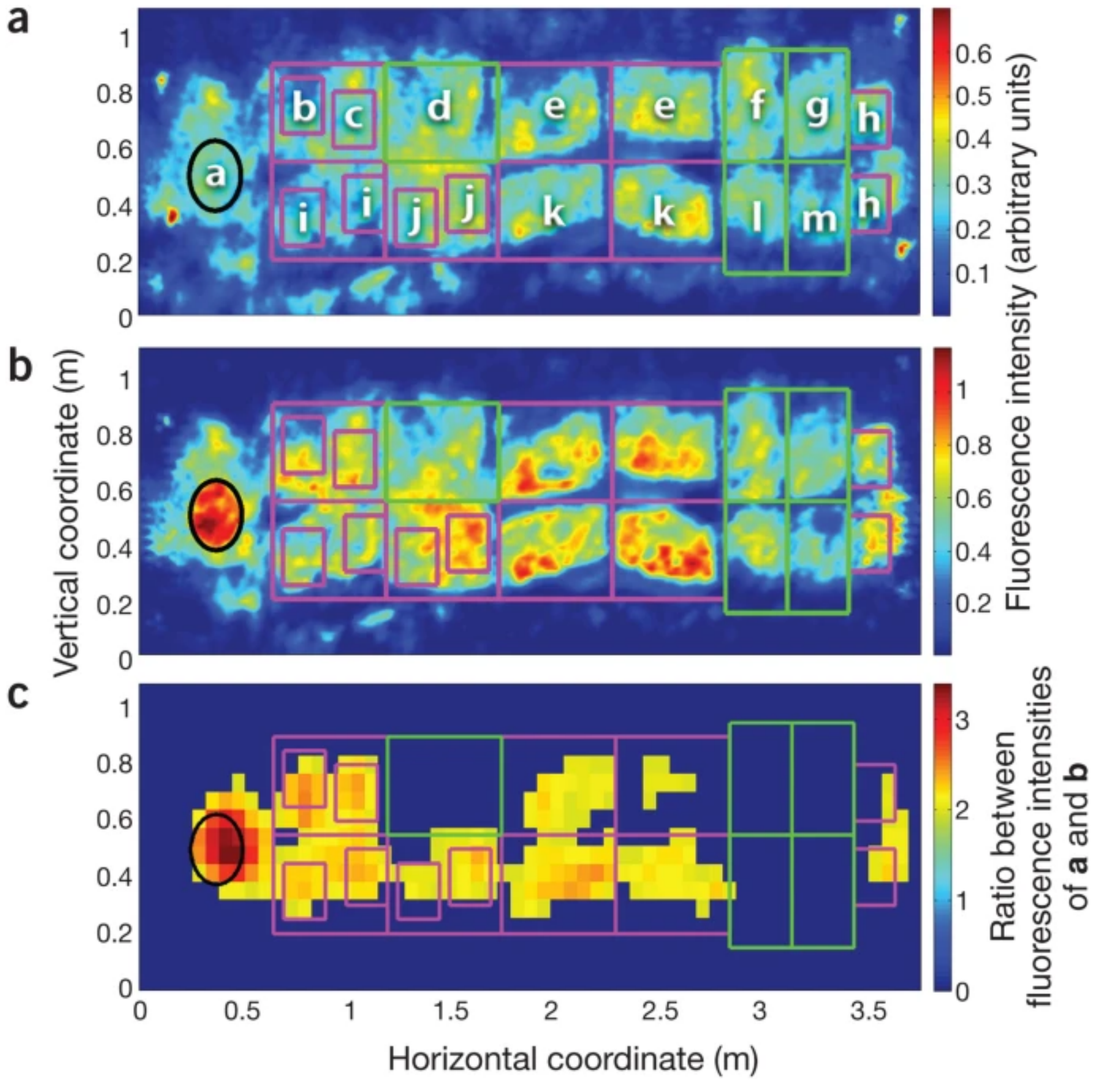


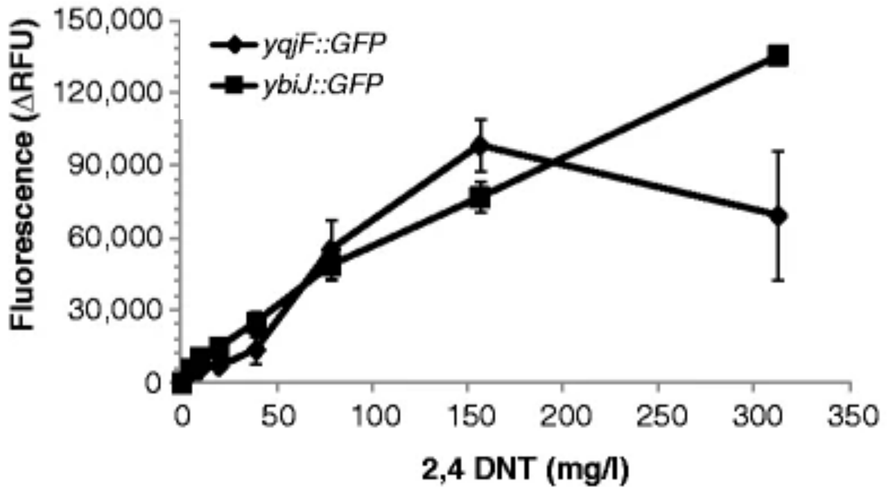
Figure 1 Optical scanning system and alginate-encapsulated bacterial sensors. (**a**) Biosensor beads spread over target areas. Scale bar, 2 cm. (**b**) Schematic of the optical scanning system. Key: (a) Buried landmine; (b) beads containing encapsulated bacteria; (c) laser system producing a Gaussian beam with 0.5 W at 473 nm; (d) laser modulator; (e) optical aiming system; (f) oscillator; (g) digital data acquisition card; (h) computer; (i) collecting telescope; (j) detection module; (k) scanning apparatus. (**c**) Photograph of the optical scanning system.



The design

Sensing explosives with *E. coli*

- **Step 1:** GFP assay to monitor promoter activity in *E. coli* after exposure with 2,4-DNT (2,000 promoter library screened)
- **Step 2:** 11 promoters with positive 2,4-DNT hits screened in a concentration series
- **Step 3**: 2 promoters selected with dose-dependent response to 2,4-DNT (*yqiF* and *ybiJ*)
- **Step 4**: Test for specificity with compounds that are structurally relevant or similar
- **Step 5**: Enhance sensitivity based on random promoter mutagenesis (eg, PCR or synthetic)
- **Step 6**: Study mechanism for biosensing (eg, *yqiF/ybiJ* involved in removal, degradation, or neutralization of 2,4-DNT)



Chemical	Max response ratio[a]		EC_{200} (mg/L)[b]	
	ygjF	*ybiJ*	*ygjF*	*ybiJ*
2,4,6-Trinitrotoluene (2,4,6-TNT)	+++	+++	7.84±1.12	8.65±0.05
2,4-Dinitrotoluene (2,4-DNT)	+++	+++	19.4±2.9	27.0±3.8
2,6-Dinitrotoluene (2,6-DNT)	–	–	–	–
1,2-Dinitrobenzene (1,2-DNB)	–	–	–	–
1,3-Dinitrobenzene (1,3-DNB)	+++	+++	6.02±1.56	10.91±4.53
1,4-Dinitrobenzene (1,4-DNB)	+	+	13.43±2.63	19.24±0.1
2-Nitrotoluene (2-NT)	–	–	–	–
3-Nitrotoluene (3-NT)	–	–	–	–
4-Nitrotoluene (4-NT)	–	–	–	–
Benzene	–	–	–	–
Toluene	–	–	–	–
Nitrobenzene	–	–	–	–
Phenol	+/–	+/–	–	–
Aniline	+/–	+/–	–	–

Appl Microbiol Biotechnol (2014) 98:885–895

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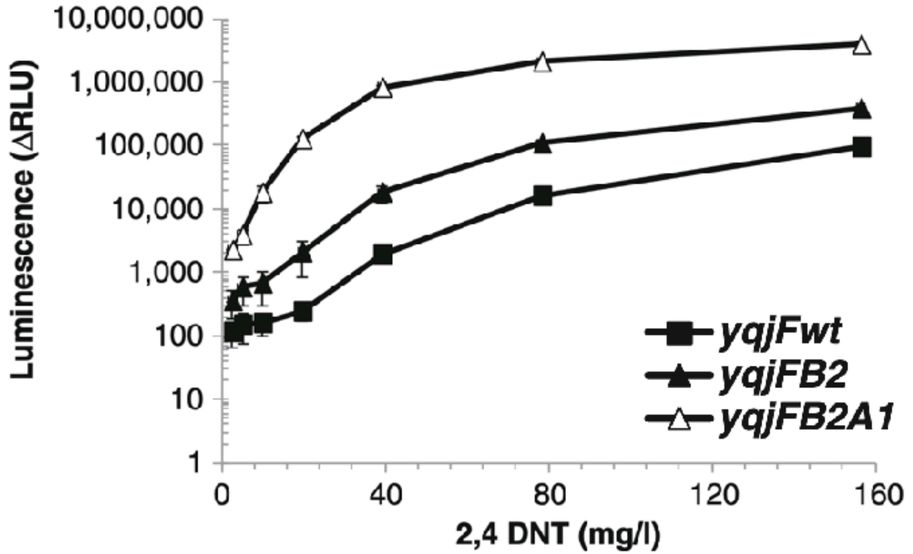
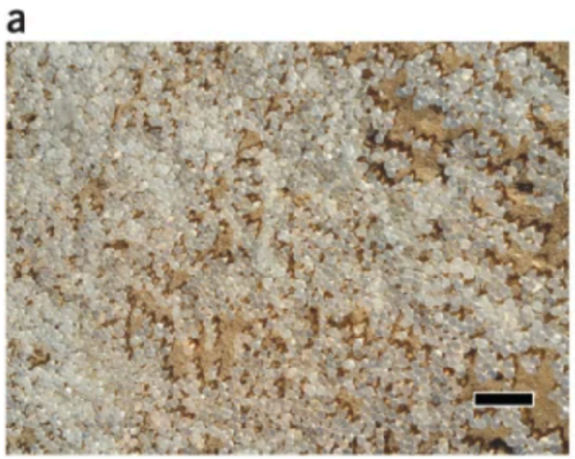
- **Step 3**: 2 promoters selected with dose-dependent response to 2,4-DNT (*yqiF* and *ybiJ*)
- **Step 4**: Test for specificity with compounds that are structurally relevant or similar
- **Step 5**: Enhance sensitivity based on random promoter mutagenesis (eg, PCR or synthetic)
- **Step 6**: Study mechanism for biosensing (eg, *yqiF/ybiJ* involved in removal, degradation, or neutralization of 2,4-DNT)

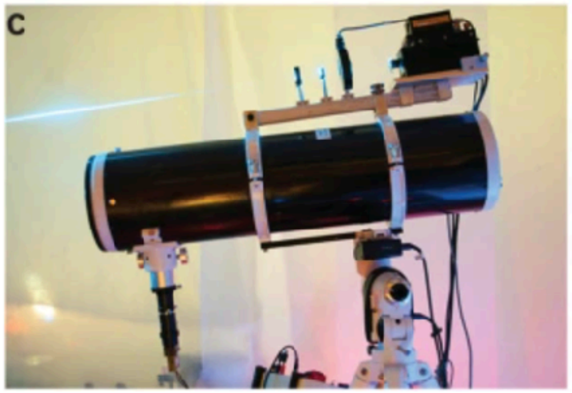


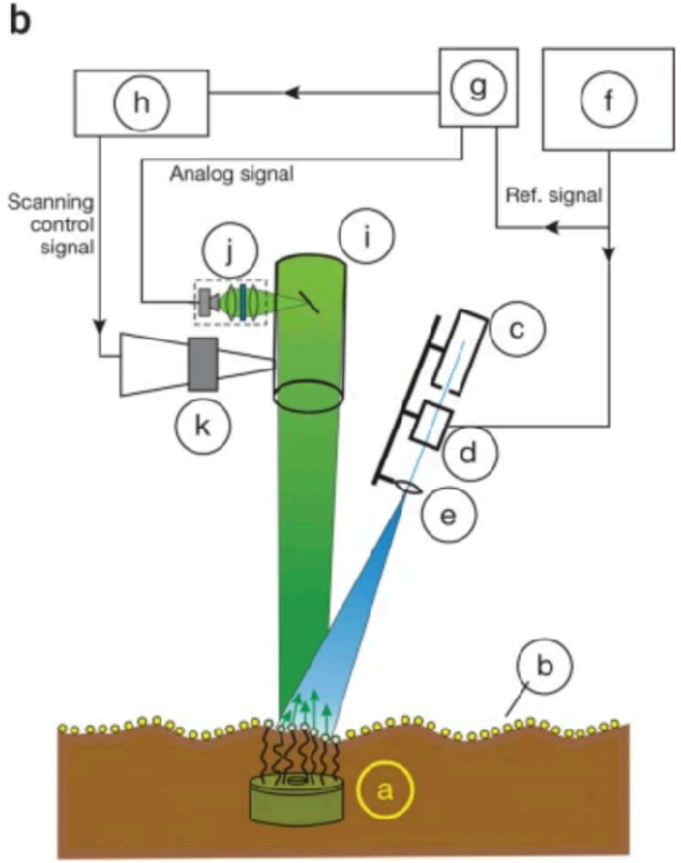
Appl Microbiol Biotechnol (2014) 98:885–895

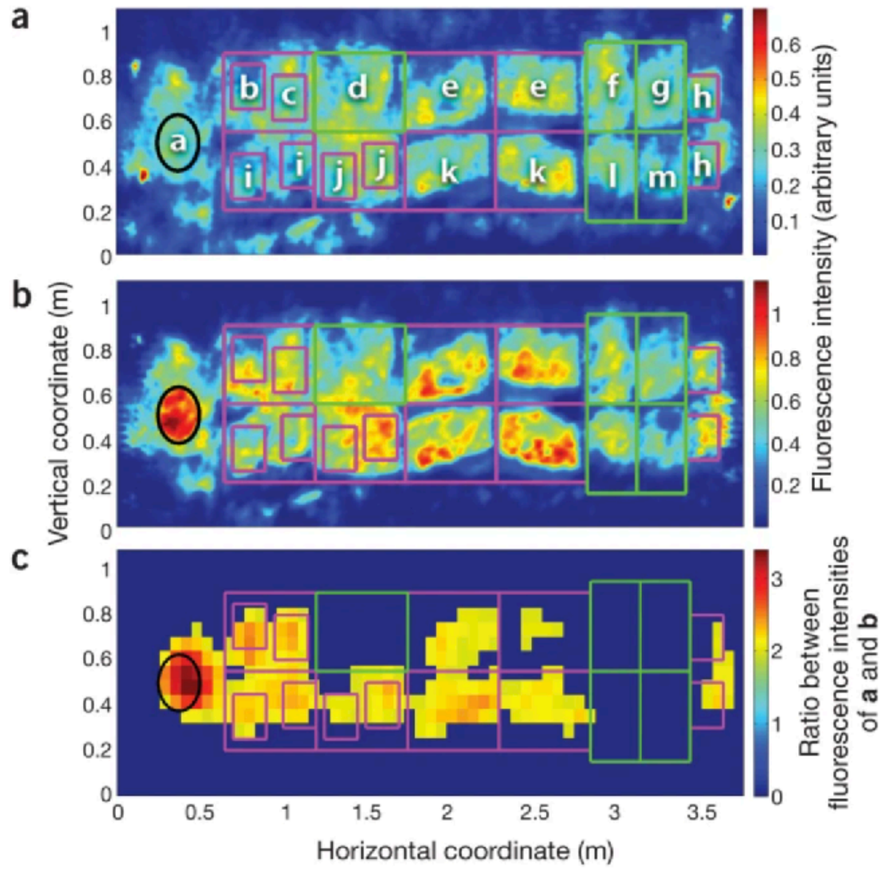
The design

Actuation — remote detection of TNT soil samples





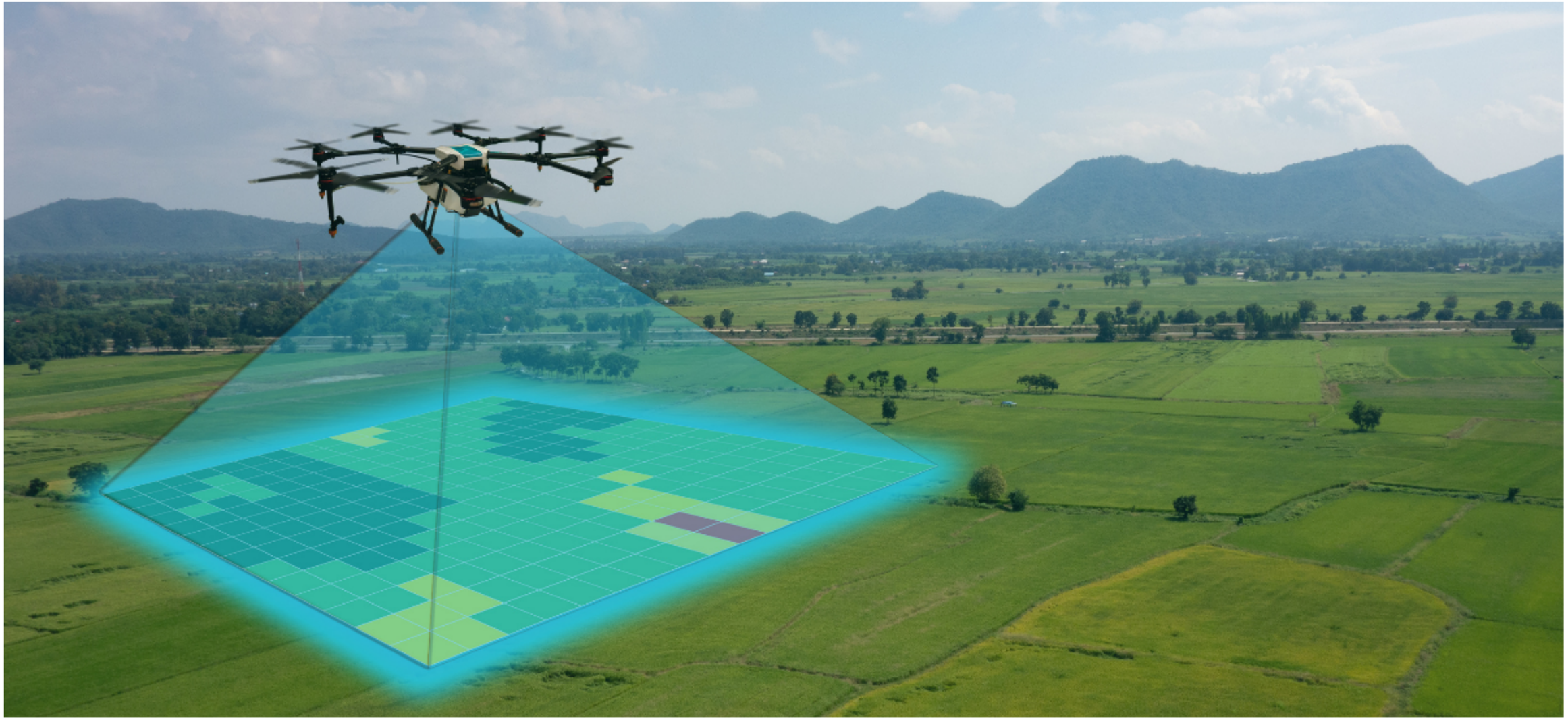




Belkin S. *Nat Biotechnol* **35**, 308–310 (2017).

Future developments

Remote sensing of GFP-positive bacterial colonies with drones



Sustainable development with synthetic biology

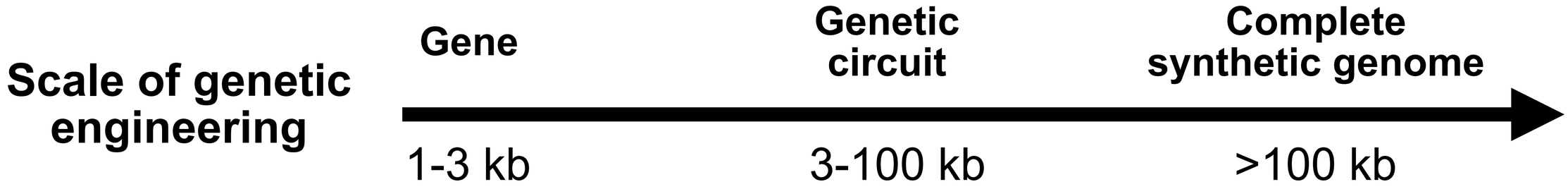
- **Bioremediation**: degrade pollutants such as heavy metals, plastics from contaminated sites
- **Carbon sequestration:** capture and convert greenhouse gases
- **Sustainable material production**: biodegradable plastics, lab-grown leather, bio-based dyes
- **Environmental monitoring**: detect biosensors that detect pollutants in water/soil for real-time data for environmental management and monitoring
- **Gene drives:** spread desirable traits through wild populations
- **Agriculture:** enhance crop resilience to climate change
- **Biomedicine**: cancer therapeutics, disease surveillance
- **Energy**: enhanced production of biofuels

Challenges and considerations

- **Ecosystem risks:** introduction of engineered organisms into the wild can have unintended consequences on ecosystems (biodiversity, food webs)
- **Ethics**: ethical questions about the manipulation of nature and potential for unintended consequence
- **Public perception:** understanding and addressing public concerns about synthetic biology is key for “lab-to-environment” translation

Design choices in synthetic biology

Single gene, gene circuit, or full synthetic genome



Design choices in synthetic biology

Synthetic genomes

BIO-312 Genomic solutions to sustainable development

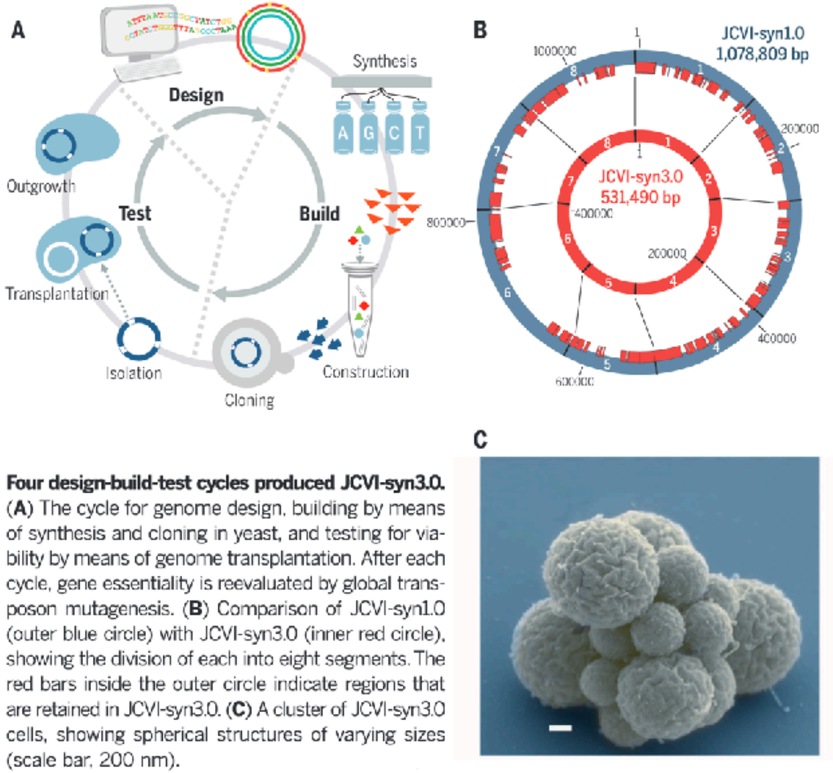
RESEARCH ARTICLE SUMMARY

SYNTHETIC BIOLOGY

Design and synthesis of a minimal bacterial genome

Clyde A. Hutchison III,*† Ray-Yuan Chuang,† Vladimir N. Noskov, Nacyra Assad-Garcia, Thomas J. Deerinck, Mark H. Ellisman, John Gill, Krishna Kannan, Bogumil J. Karas, Li Ma, James F. Pelletier, Zhi-Qing Qi, R. Alexander Richter, Elizabeth A. Strychalski, Lijie Sun, Yo Suzuki, Billyana Tsvetanova, Kim S. Wise, Hamilton O. Smith, John I. Glass, Chuck Merryman, Daniel G. Gibson, J. Craig Venter*

25 MARCH 2016 • VOL 351 ISSUE 6280



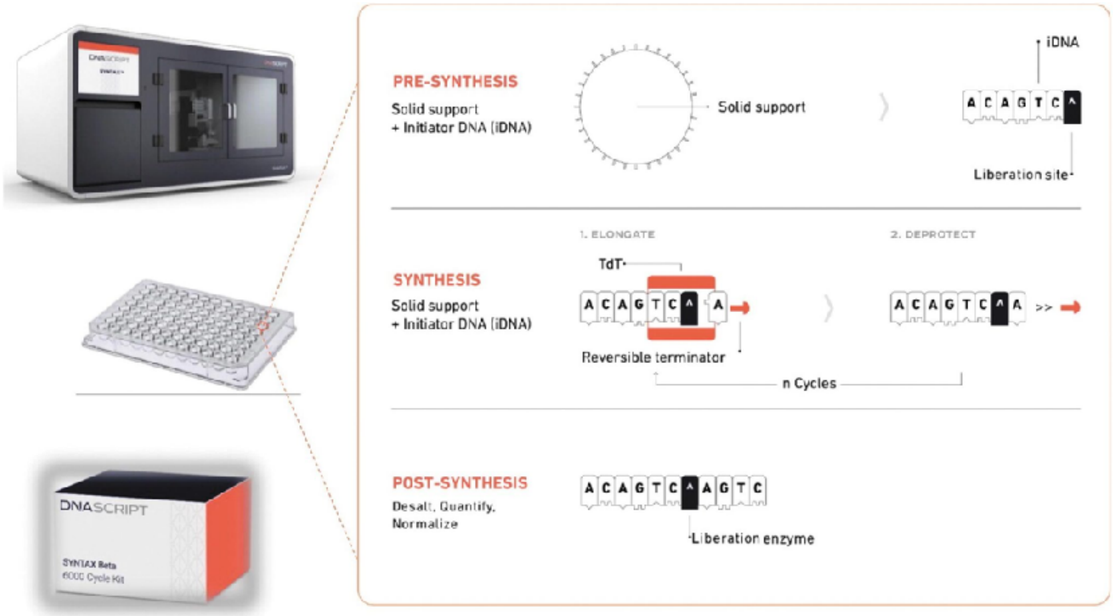
Four design-build-test cycles produced JCVI-syn3.0. (**A**) The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis. (**B**) Comparison of JCVI-syn1.0 (outer blue circle) with JCVI-syn3.0 (inner red circle), showing the division of each into eight segments. The red bars inside the outer circle indicate regions that are retained in JCVI-syn3.0. (**C**) A cluster of JCVI-syn3.0 cells, showing spherical structures of varying sizes (scale bar, 200 nm).

Chemical synthesis of a 531 kb minimal genome with 473 genes

Design choices in synthetic biology

DNA synthesis for genetic circuit designs

DNA printer based on enzymatic DNA synthesis

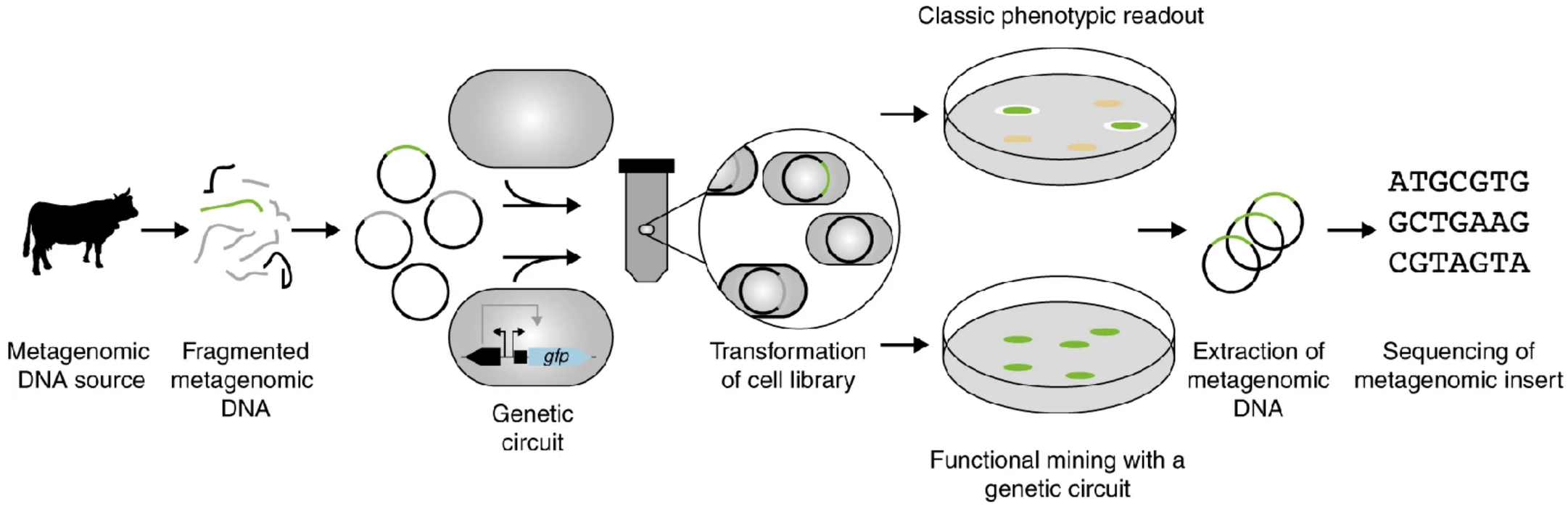


DNA synthesis as-a-service (up to 8 kb)

	Standard	Plus
Length		
Linear DNA	200 - 8,000 bp	8,001 - 12,000 bp
Plasmid DNA (E.coli compatible, containing one ORI and at least one resistance gene)	2,900 - 8,000 bp	8,001 - 20,000 bp
Complexities		
Global GC Content	35 - 65%	30 - 35%, 65 - 70%
Local GC Content (within a 100 bp window)	25 - 75%	20 - 25%, 75 - 82%
Internal Homopolymers	≤ 15 bp for A/T ≤ 10 bp for G/C	≤ 20 bp for A/T ≤ 16 bp for G/C
Short Repeats	≤ 20 bp for 2 - 4 bp repeats ≤ 28 bp for 5 - 8 bp repeats	
Long Repeats	≤ 220 bp for 9 - 72 bp repeats	
Accuracy	Linear DNA: >90% sequence-correct molecules (Error rate: <1:1120,000 bp at 12 kb) Plasmid DNA: Clonal	

Design choices in synthetic biology

Environmental DNA for discovery of genetic circuits

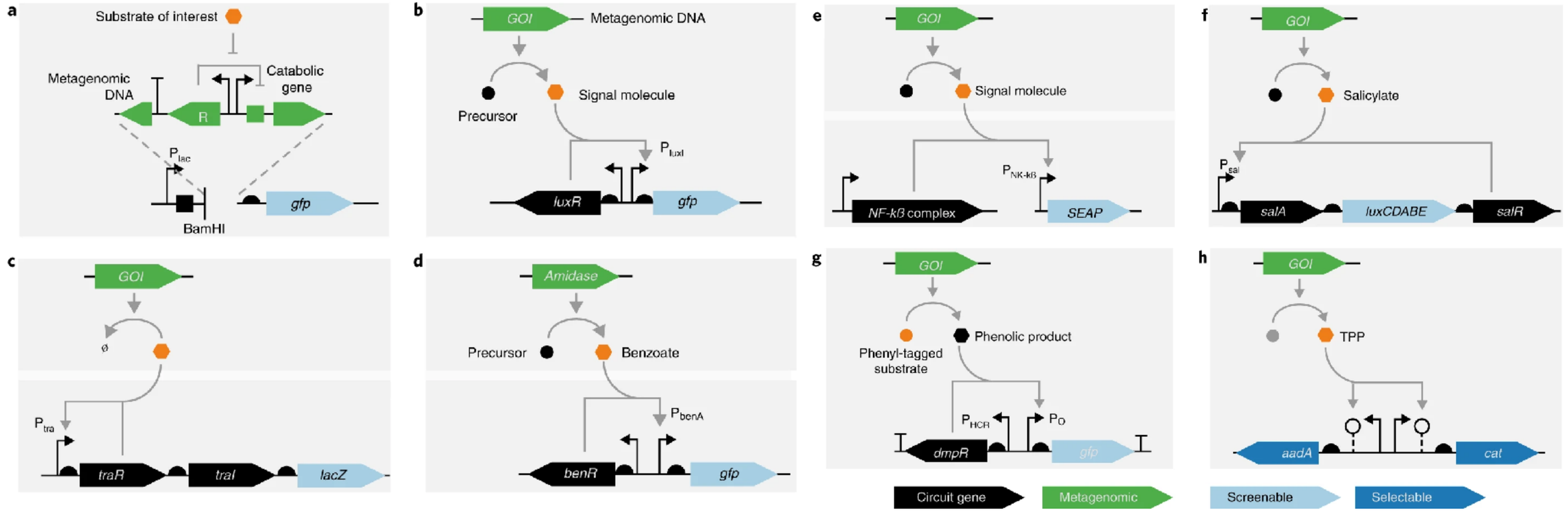


Discovery of DNA and circuits in the range of 5 to 150 kb

van der Helm, E., Genee, H.J. & Sommer, M.O.A. *Nat Chem Biol* **14**, 752–759 (2018).

Design choices in synthetic biology

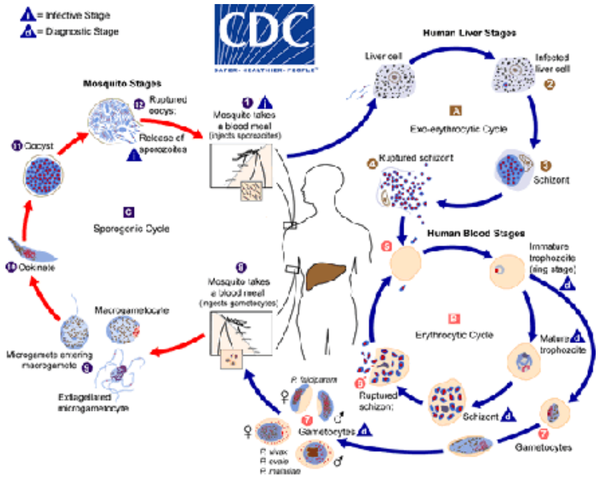
Genetic circuits to process eDNA libraries for various screens



van der Helm, E., Genee, H.J. & Sommer, M.O.A. *Nat Chem Biol* **14**, 752–759 (2018).

Malaria

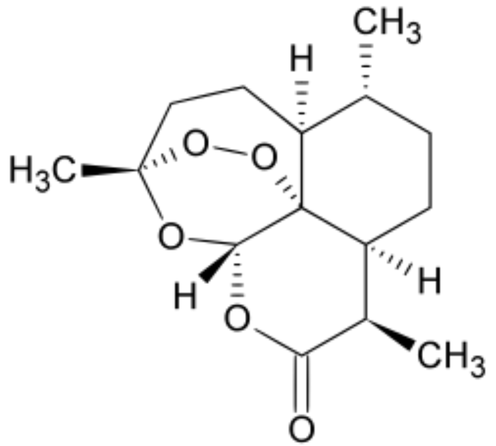
- A life-threading disease caused by *Plasmodium* parasites (eg, *P. falciparum*) that spreads to humans via bite of an infected female *Anopheles* mosquito
- About 260 million malaria cases worldwide and 600,000 deaths in 83 countries (mostly in the African region and children under 5)
- Risk factors of malaria infection and death include tropical climate (environmental), poverty (socio-economic), and limited healthcare (socio-economic)
- Prevention & treatment: bed nets, antimalarial therapy



Antimalaria medication

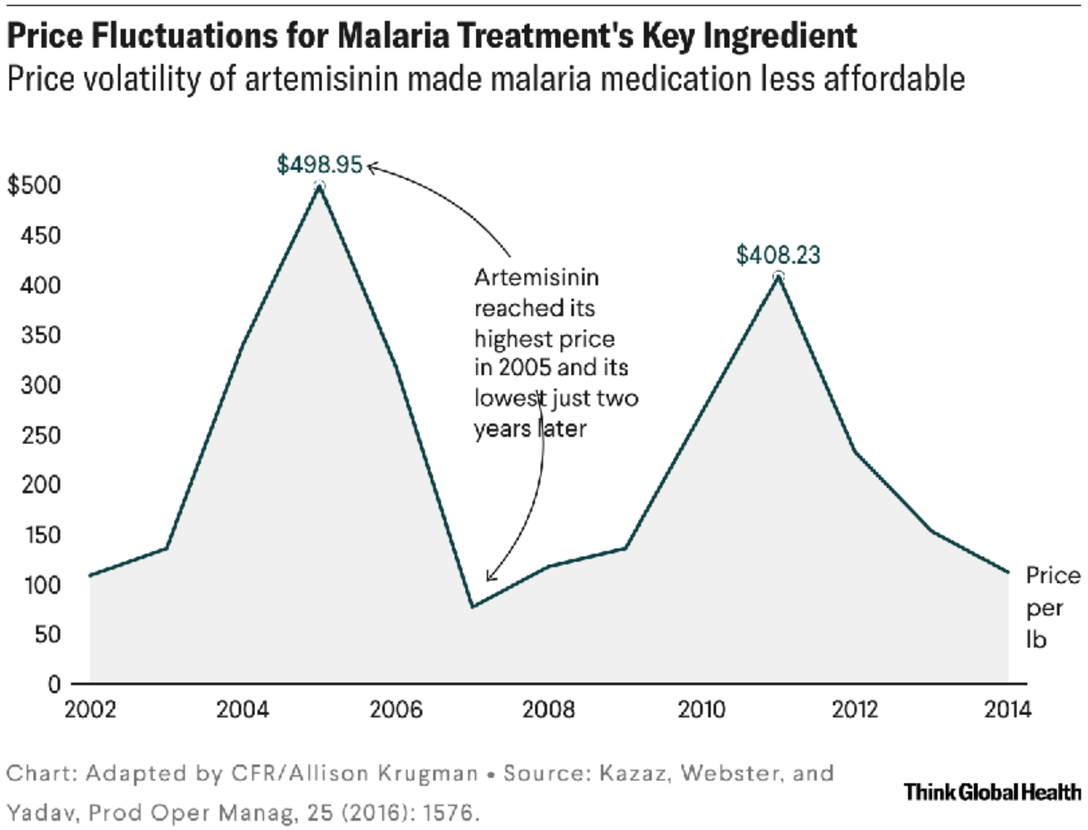
Artemisinin-based Combination Therapy (ACT)

- Artemisinin is a natural compound extracted from the plant *Artemisia annua* (sweet wormwood) that was originally described as an anti-malarial treatment by the Chinese physician Ge Hong in the 4th century
- Artemisinin (re)discovered by Tu Youyou in the 1970s as part of the Chinese military project “Project 523”. Ge Hong’s original 4th century extraction method was key to obtain the active compound from the A. annua plant (fresh *vs* dried leaves + extraction at low temperatures)
- Tu Youyou was awarded with the Nobel prize in 2015 for her “Discovery of Artemisinin: A Gift from Traditional Chinese Medicine to the World”
- Artemisinin kills Plasmodium parasites in the blood stage of injection and acts rapidly via production of free radicals that damage parasite proteins
- Used worldwide in Artemisinin-based Combination Therapies (ACT) and is now the standard first-line treatment for *P. falciparum* malaria



Artemisinin

- Strong prize fluctuations for artemisinin ($100-500)
- Artemisinin can be only extracted from the *Artemisia annua* plant
- Crop yields vary based on whether and farming conditions (bad harvest = lower supply = higher price)
- Oversupply and undersupply creates year-to-year uncertainties for farmers, manufacturers, and health programs
- Long production cycle (6-8 months) creates lag in Artemisinin availability



Semi-synthetic artemisinin (SSA)

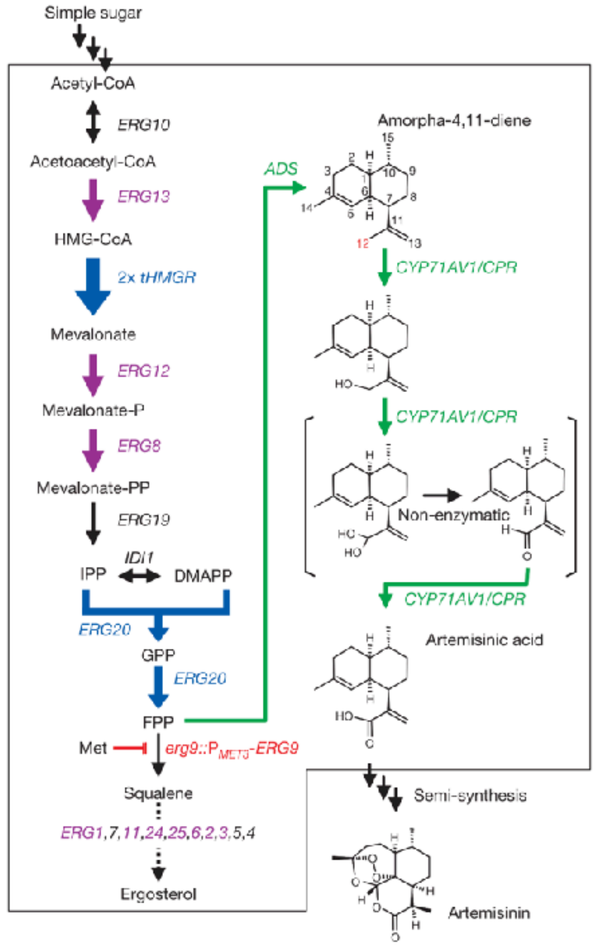
From plant to engineered yeast

- **Goal:** Laboratory-produced version of artemisinin to address fluctuations in natural artemisinin supply.

- **Step 1:** Genetic engineering of the mevalonate pathway in yeast to enhance conversion of sugar into FPP, an intermediate of sterol biosynthesis, and introduction of *A. annua* plant enzymes to convert FPP into **artemisinic acid** (=a precursor of arteminisin) in a 3-step oxidation process.

- **Step 2:** Artemisinic acid is transported out of yeast cells, retained on the outside of engineered yeast, cells are purified, and chemically converted to **artemisinin**.

- Fast production (4-5 days) with consistent yield & purity and highly scalable with industrial fermentation



Nature Vol 440|13 April 2006|doi:10.1038/nature04640

The journey of semi-synthetic artemisinin

- **Origin & promise:**
  - Melinda & Bill Gates Foundation sponsored research on SSA with $64 million 2005
  - Sanofi licensed the engineered yeast from UC Berkeley in 2008, with the aim to provide a stable, scalable, and affordable alternative to plant-based artemisinin (“no profit, no loss” model)
  - Sanofi produced by 2014 semi-synthetic artemisinin to treat 40 million people with malaria

The journey of semi-synthetic artemisinin

- **Real world challenges**:
  - Sanofi stopped production in 2015 due to lower prices of natural artemisinin ($250/kg *vs* $400/kg)
  - ACT manufacturers and competitors were reluctant to buy semi-synthetic artemisinin from Sanofi
  - Demand flattened due to better diagnostics of malaria making artemisinin combination therapy unnecessary
  - Sanofi sold the SSA plant to Huvepharma
  - Gates Foundation issued in 2017 new grants for a “truly sustainable low cost supply of semi-synthetic artemisinin” and $100/kg or less

The journey of semi-synthetic artemisinin

- **Novel innovations**
  - Optimize yeast fermentation process
  - Engineering *E. coli* to produce artemisinin acid
  - Use of *A. annua* plant cells for fermentation
- **Best of both worlds?**
  - Use crude plant extracts for natural product synthesis using novel bioreactor with more efficient yields
  - Collaboration between plant farming industry and biotech

Lessons learned from SSA for synthetic biology in sustainable development

- Research into semi-synthetic artemisinin crossed several boundaries: military, private non-profit foundations, university, for-profit pharmaceutical companies
- Hundreds of compounds have been screened in the past for anti-malarial activity yet only a plant extract revealed one of the most potent treatments against malaria (—> eDNA and metagenomics-based synthetic biology)
- Is synthetic biology the ultimate solution towards sustainable development or equally fragile like the farming industry?
- Artemisinin-resistant *P. falciparum* has emerged with the rise of ACT therapy
- Novel plant-based anti-malarial compounds are being developed (eg, neem tree from tropical areas) which reemphasis the need for conservation of habitats for future healthcare

Design a biosensor for real-world impact

- **Goal**: design a biosensor that targets one of the UN SGD missions
- **Questions:**
  - What will the sensor detect (toxin, pathogen, pollution, hormone)?
  - Where will it be used (environment, healthcare, agriculture, industry)?
  - How will you design it (sensor, processing unit, actuator)?
  - How will you it be used in the wild (deployment, specialized?)
  - Are there any ethical considerations (risk of misuse, who benefits, who doesn't)?
  - Is it economically feasible (affordable, who would fund it, who would pay for it)
- **3-minute pitch**