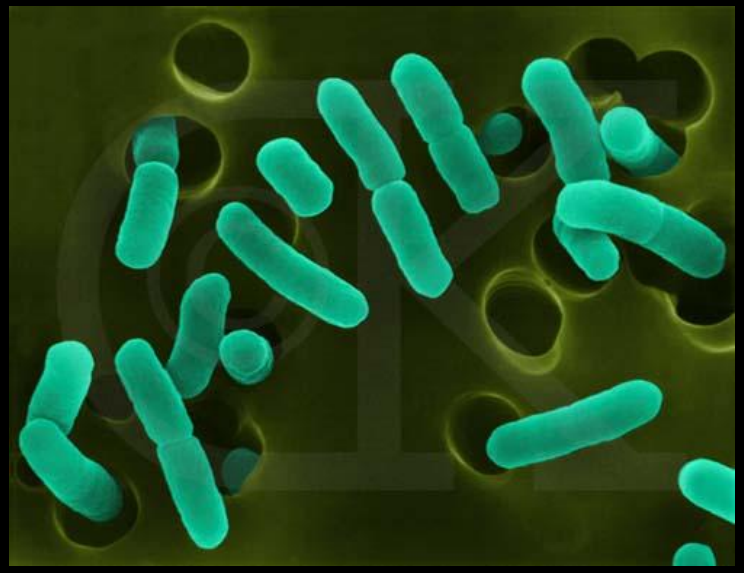
Synthetic Biology:

Topics in health and therapeutics



Images courtesy of http://pathport.vbi.vt.edu/pathinfo/pathogens/E.coli_O157H7.html

Tumor

Killing

Bacteria

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Tumor Killing Bacteria as "Testbed" for Synthetic Biology

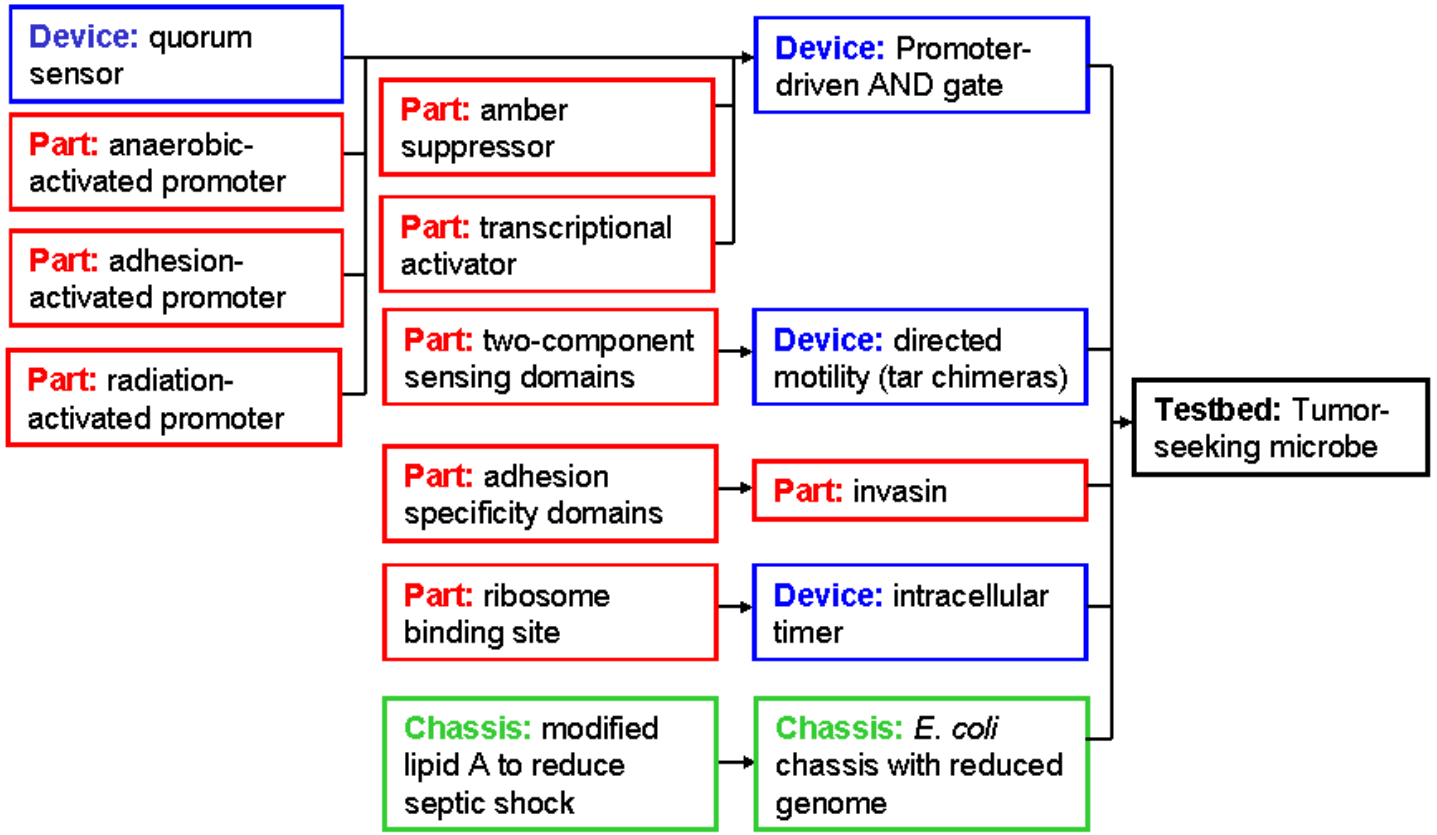
The Goals of the Testbeds are:

- "demonstrate the utility of synthetic biology"
- Integration of thrusts: Parts, Devices, Chassis
- "develop the foundational infrastructure that is needed to make routine the design and construction of any engineered biological system"
- Develop the BioBricks Open Source Registry

The Three Biological Thrusts At Work

Testbed 1:Tumor-Killing Bacteria

Integrating parts, devices, and chassis to complete the testbed

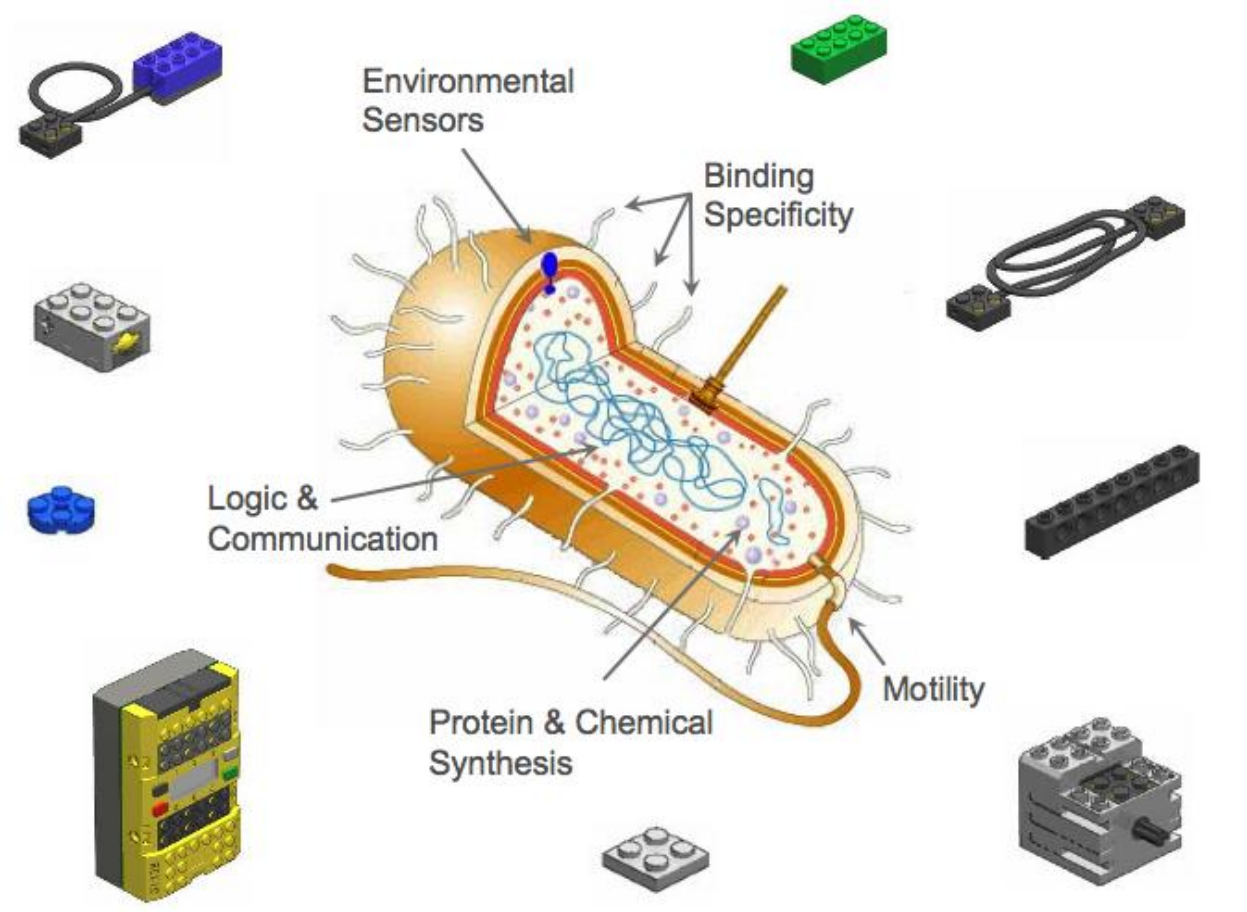


(http://www.synberc.org/testbed_integration.html)

The Utility of Synthetic Biology can be shown through:

- The design of parts, devices, and chassis throughout the entire process
- Registration of these components in the registry for use by other researchers
- Use of "abstraction hierarchy": biology can be engineered like a machine…

Use of Abstraction Hierarchy



Is building a therapeutic microbe really like designing a machine?

Top-down? Bottom-up?

Start with an organism that possesses the desired qualities

Edit or "tweak" by removing or adding genes

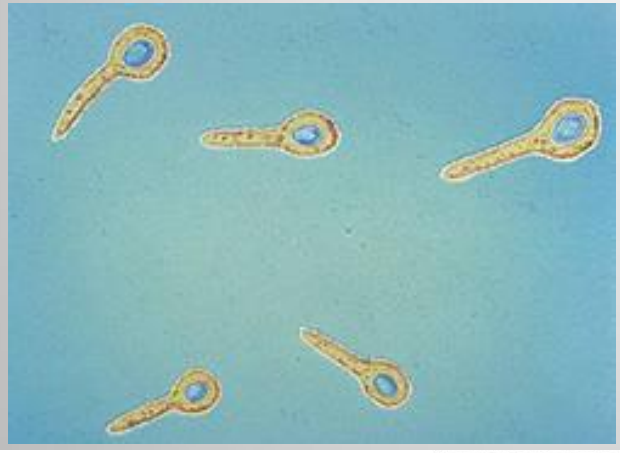
Add genes that will provide desired behaviors in a controlled manner

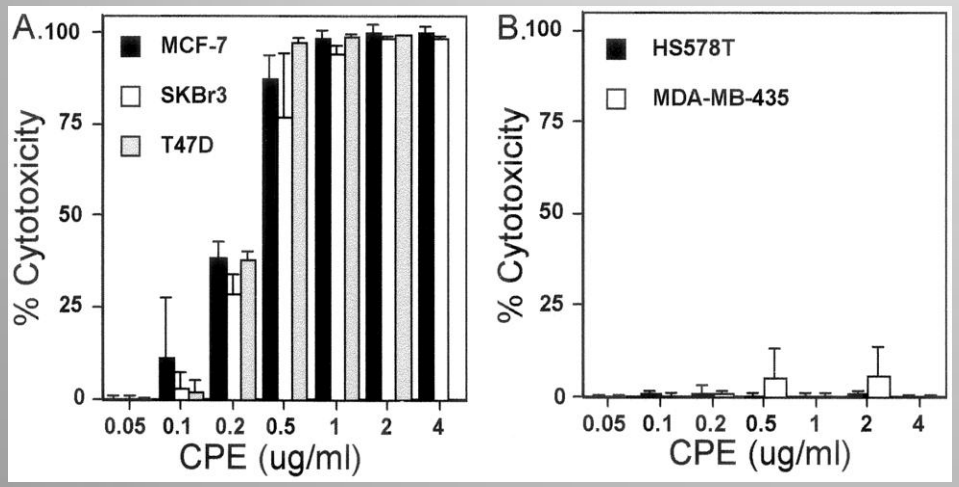
Start with a well-characterized, model organism

Example

How is cancer therapy research on *Clostridium* a "top-down" approach?

Clostridium Genus





- Gram +, bacillus, sporulating, obligate anaerobes
- Only a few species are pathogenic to humans.
- Many release (entero)toxins capable of lysing, or "popping", eukaryotic cells.

Images courtesy of Kominsky, Scott L., et al. "*Clostridium perfringens* enterotoxin elicits rapid and specific cytolysis of breast carcinoma cells mediated through tight junction proteins claudin 3 and 4". *American Journal of Pathology*. 4(2004): 1627-1633.

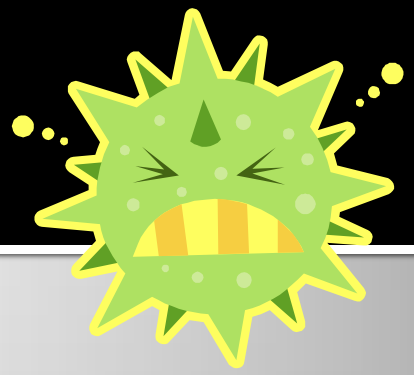
And http://textbookofbacteriology.net/clostridia.html

Important Considerations

- Growth control and safety
- Immune evasion
- Therapeutics

… desired behaviors?

Growth, Control, and Safety



Desired behavior: ***growth is kept in-check***

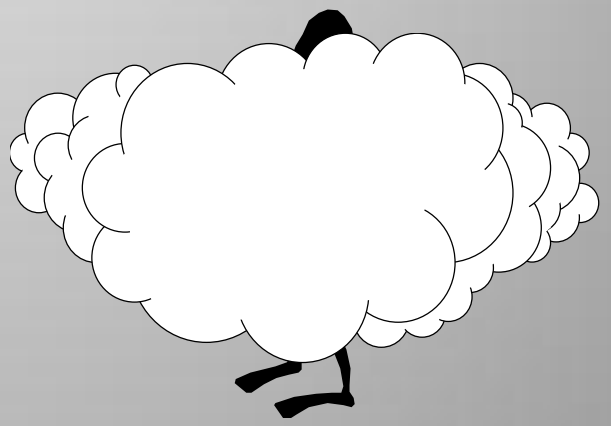
- *Clostridium* spores ONLY germinate in low oxygen environments (e.g. tumors).
- *E. coli* K12 will be engineered to lack ion transport systems.

Immune Evasion

Desired behavior: ***avoid detection by the immune system***

- *Clostridium* was not cleared by the immune system in tests.

- *E. coli* K12 will have modified LPS and addition of O-antigen.



Therapeutics

Desired behavior: ***ability to kill tumor cells efficiently***

- *Clostridium* naturally releases enterotoxins capable of lysing tumor cells.
- Recombinant strains: enhanced killing.
- *E. coli* must be programmed to kill.

Clostridium	*E. coli* K12
Injection	Injection
Growth control: **innate**	Growth control: **programmed**
Immune evasion: **innate**	Immune evasion: **programmed**
Therapeutics: **innate and programmed**	Therapeutics: **programmed**

Feasibility of Bottom-Up Method:

- Theory differs from practice
- Time in lab often spent synchronizing components
- BioBricks Registry does not account for dynamic nature
  of living systems
- Will the Trial and Error processes be shared as well?

Current Common Treatment Methods

- Chemotherapy:

  the use of chemical agents to kill cancer cells and prevent them from growing

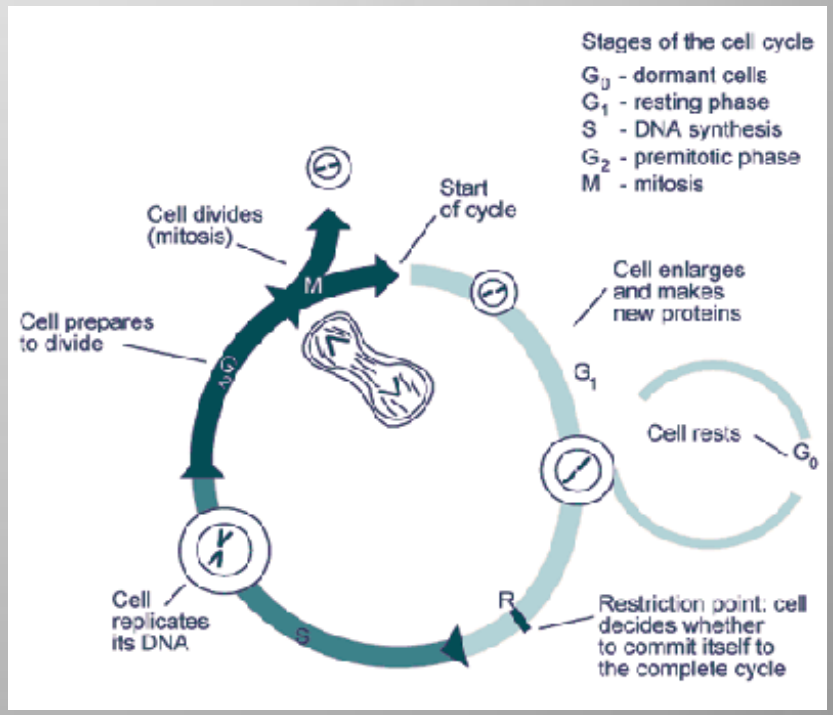
- Radiotherapy:

  medical use of x-rays to fight and control malignant cells in cancer treatment

Chemotherapy

Advantages

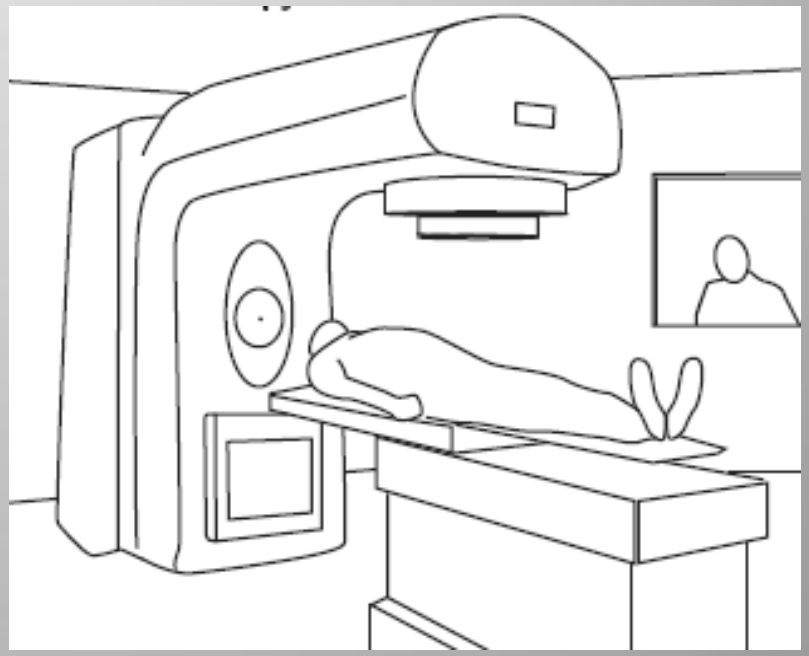
Disadvantages



Radiotherapy

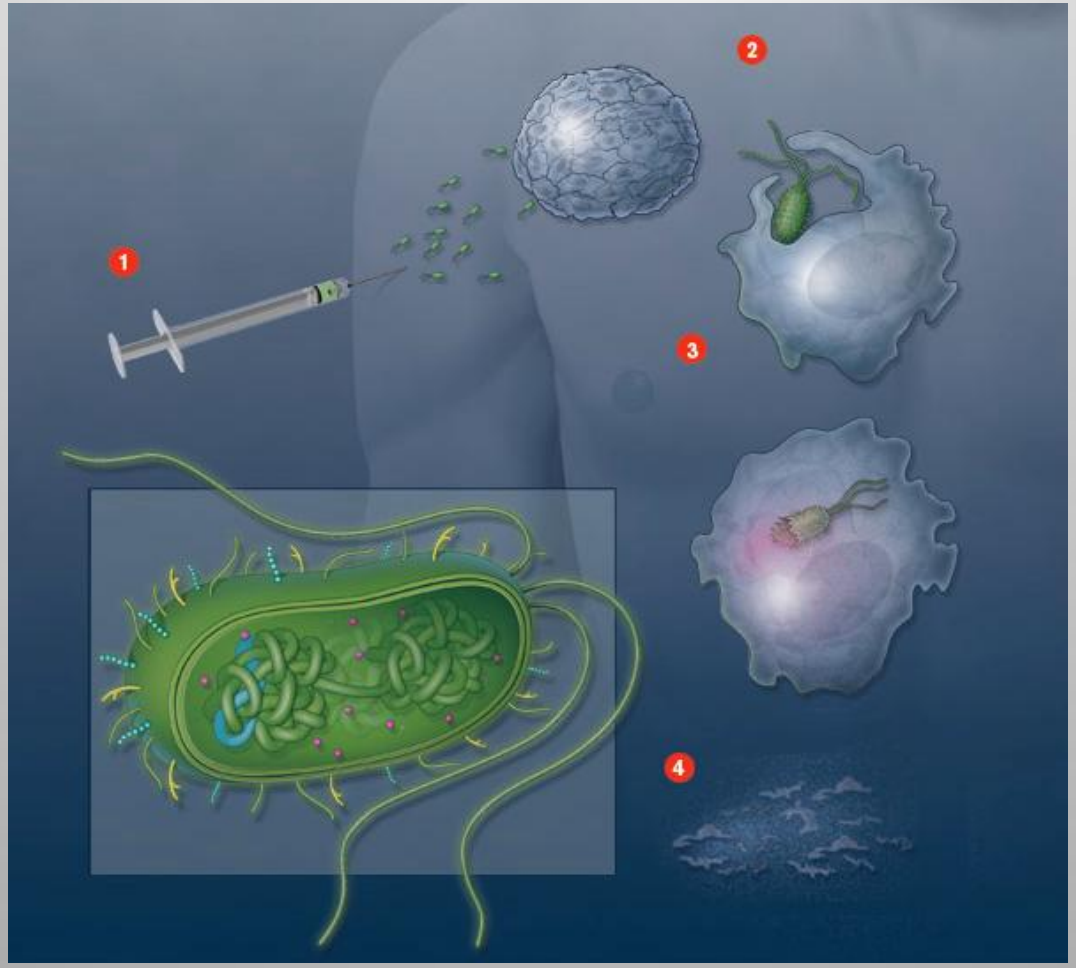
Advantages

Disadvantages



Why is TKB so appealing?

Less invasive.
More direct and site-specific.

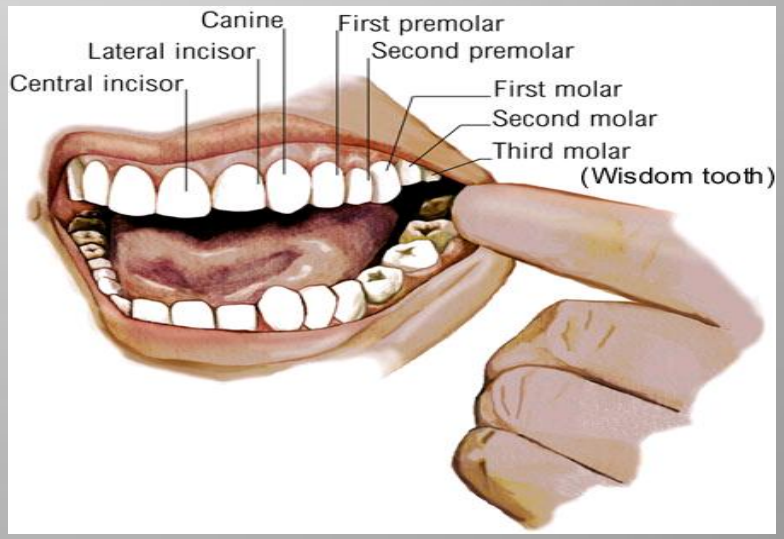


Bacteria as a Therapeutic Device

- Western medicinal treatment strategies
  - Pharmacopoeia of small molecules
- Previous uses of bacteria in medicine
  - Bladder Cancer and Bacillus Calmette-Guerin (BCG)

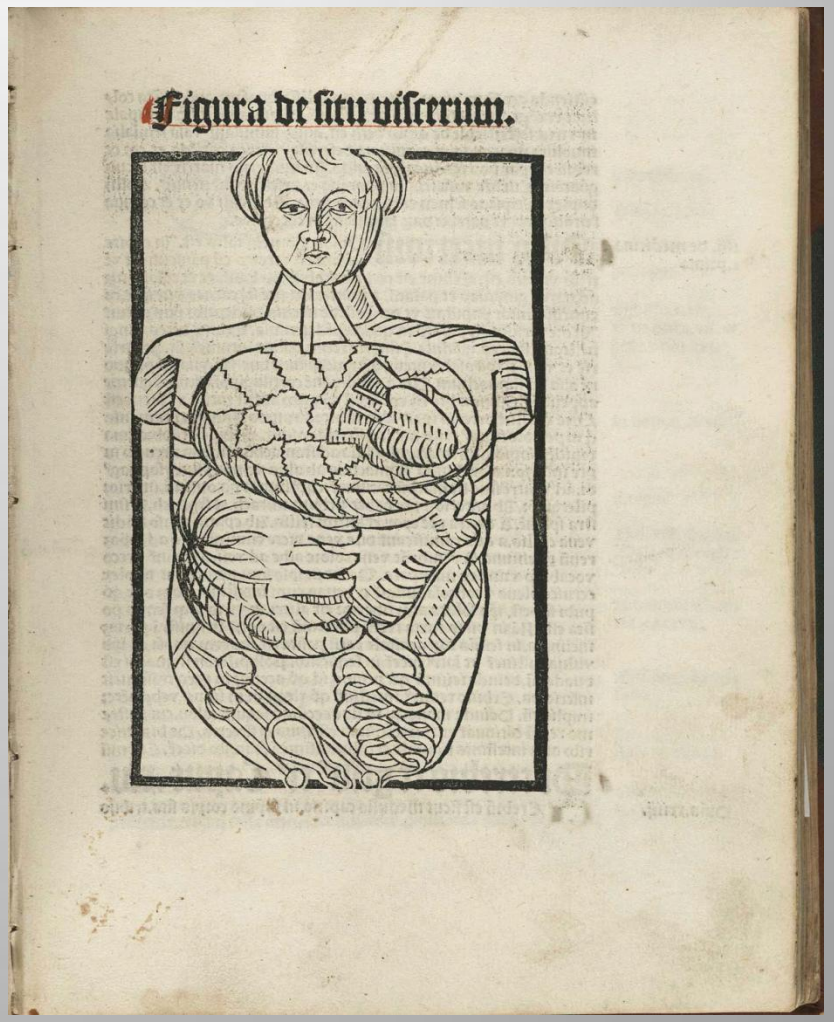
Bacterial modification for medicinal use

- Oragenics and Osel:
- Dental cavity protection, UTI treatment - a lifetime with microbes
  - Resistance? Mutation?
  - Safety Concerns



Bacterial modification for medicinal use, cont.

- Irritable Bowel Syndrome
  - Selective secretion of cytokines
  - Some recombinant gene technology used
  - Modification of existing bio-systems



Synthesized Bacteria and Cancer

- Differences between TKB and preexisting bacterial therapies
- Direction of approach (top-down, bottom-up)
  - *Listeria monocytogenes*, eliciting of innate immune response (Cerus Inc., Concord)
  - *Salmonella typhimurium*, inherent localization with added toxicity (Vion Pharmaceuticals, New Haven)

Bacteria

- Problems as Anderson and Synthetic Bio see them
  - Safety concerns
  - Problems of technical mastery
- FDA Concerns
  - Safety, as well
  - Cost benefit analysis
  - Cancer ideal, potential benefit high and cost low

Safety Issues?

- FDA, regulatory bodies, involved scientist
  - Only real concern
- Beyond safety, human practices
  - Dual use, who gets access, will this allow flourishing?

Safety, Security, and Preparedness Issues: Dual Use

Dual Use: Alternate functions of the project (its parts, devices, chassis) that pose threatening consequences.

Primary dual use concerns for the TKB project :

- Designed to evade the immune system
- Designed to kill cells
- Designed to be modular and easily augmentable for other synthetic biology projects

Safety, Security, and Preparedness Issues: BioBricks

The BioBricks registry is still in its early stages of development, but the threat it poses is very real.

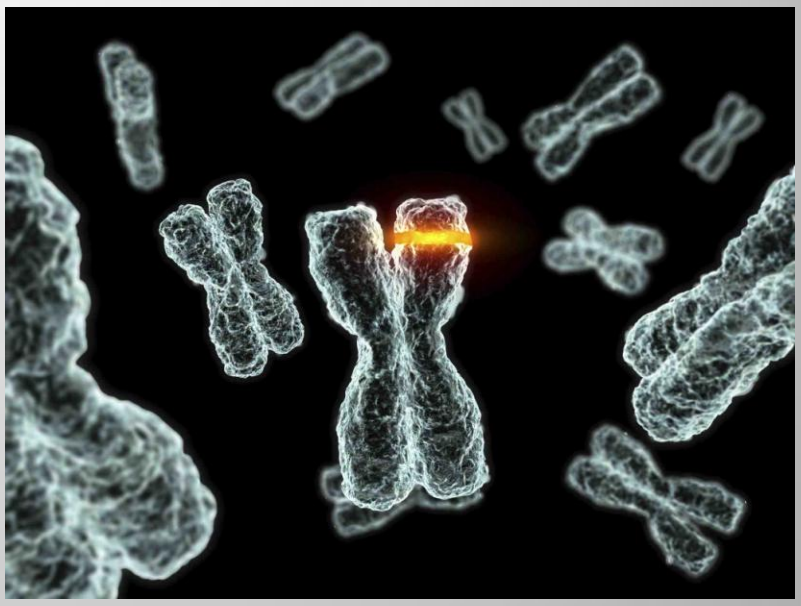
The possibility of "dangerous" parts being shared in an open source environment could be extremely enticing for "rouge scientists" to make projects such as TKB into more virulent and harmful pathogens for terrorist use.

The solution is still widely debated:

- Absolute open source? (Tom Knight)
- or limited use of the registry? (Chris Ande

Safety, Security, and Preparedness Issues: Mutation

Is there a possibility for DNA mutations to affect the Programmed functions of TKB?



The current statistic thrown around is a 10^-6 chance per base pair of mutation (A very real possibility).

Solution: Integrate multiple "fail-safe" devices into the project that will terminate the TKB cell not only after it reaches the tumor cell, but also if a mutation is present.

Human Practice Issue: Timeframe of the Project

Currently the TKB project is at the crux of passing from one stage of development to the next (from chassis development to animal testing stages). The schedule for the litigation to clear up and animal testing to begin is mid-January.

Even with this progress, the project is still in early stages of development, and a definite timeline is still unavailable.

Human Practice Issue: Interest and Funding

Even though the project is still in such early developmental stages, why call it "Tumor Killing Bacteria" versus "fundamental technologies development"?

In order to establish interest both from funding sources (i.e. federal or venture capitalists) as well as from prospective researchers entering the field.

Human Practice Issue: Distribution

The access to treatment of cancer from a completed TKB project will be significantly limited by the cost of the cells once they are ready for distribution.

The cost of the cells will be a factor not of producing the drug, but of both the licensing fees and the cost of clinical trials for FDA approval, as well as the profit margin the licensed companies wish to achieve.

Thank you

Any Questions?