



INTERNATIONAL
GENETICALLY
ENGINEERED
MACHINE

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INTRODUCTION

- **iGEM competition:**

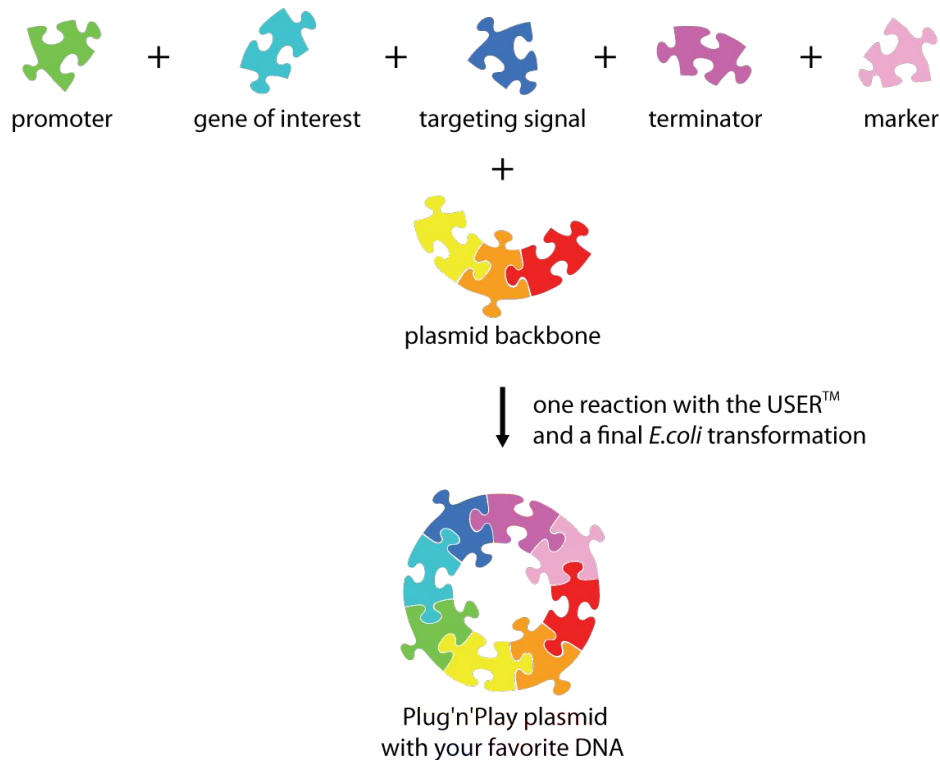
- Annual, worldwide competition in synthetic biology
- Started in 2003
- Initially for undergraduate students
- **Goal:** build simple biological systems from standard, interchangeable parts and operate them in living cells



INTRODUCTION

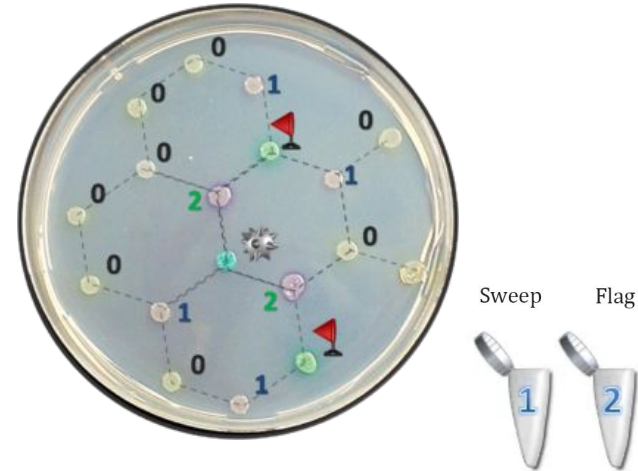
- **BioBricks:**

- Starting point for iGEM projects
- Biological standardized parts such as:
 - Promoters
 - Terminators
 - Reporter elements
 - Plasmid backbones
 - ...
- Building blocks used to construct new biological systems



PREVIOUS IGEN PROJECTS

- **2007 - BioBricks extension**
 - Addressed problem with BioFusion of BioBricks
 - Resulted in the new Freiburg standard
- **2013 - Colisweeper**
 - Minesweeper with bacteria
- **2008 - Biofabricator**
 - Bioprinter used to build other biological materials



IGEM 2016 - PROJECT PROPOSALS

Drylab

DNA computing



Wetlab

Microbial thermometer



DNA COMPUTING: INTRODUCTION

- **What is DNA computing?**
 - Use synthetic DNA molecules as data carrier
 - Computation on the DNA carried out by:
 - Biotechnology laboratory protocols
 - Enzymes
 - DNA itself: self-assembly, hybridisation ...
 - Computation goes on in:
 - In vitro: test tube (watery solution)
 - In vivo (smart medicine)
 - DNA chips with diamond surfaces

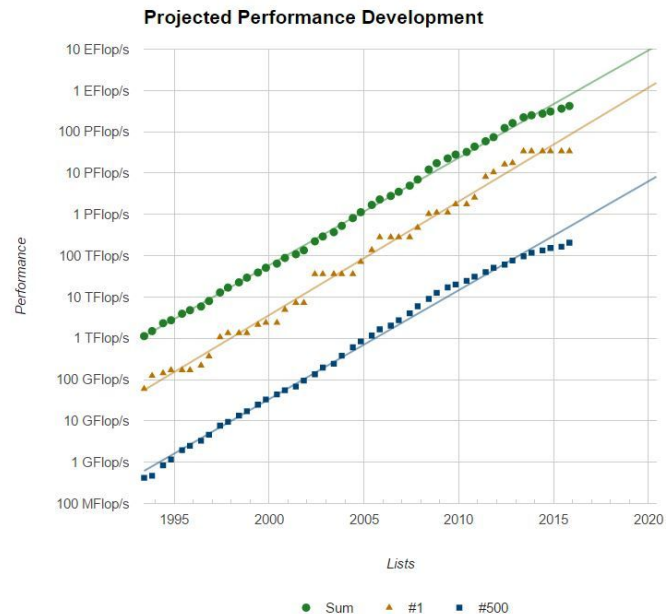
DNA COMPUTING: INTRODUCTION

- **DNA for data storage**

- Enormous capacity: ~ 2200 TB per gram with reliable encode and decode (Goldman et al., Nature 2013)
- Very robust
- Long term (thousands of years)
- Can be easily copied
- Archiving

- **DNA for computation**

- Silicon-based chips reach their limits
- Molecular computation can be performed in other environments (e.g. within a cell)



DNA COMPUTING: (DIS)ADVANTAGES OF DNA

- **Advantages:**

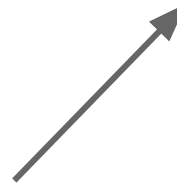
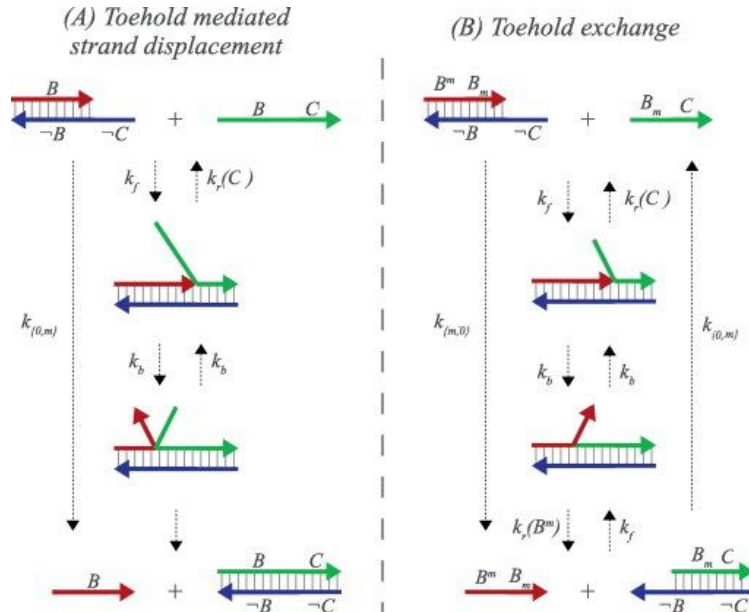
- Small information unit
 - 1 gram of DNA → 2200 TB
- Possibility of an abundance of parallel computations
- Energy efficient: 20 trillion operations per Watt

- **Disadvantages:**

- Slow
- Hard to analyze results
- Not error free
- Expensive: needs constant supply of enzymes and proteins
- Intensive man labour

DNA COMPUTING: BRIEF OVERVIEW

- Hybridization reactions



DNA Hybridization Circuits



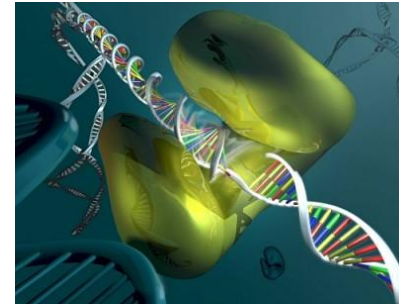
AND, OR, NOT, NAND and NOR gates



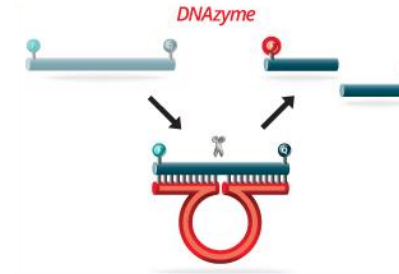
Square root of 4-bit number
(Qian and Winfree)

DNA COMPUTING: BRIEF OVERVIEW

- **Enzyme reactions**
 - Restriction enzymes
 - Nicking enzymes
 - Ligase enzymes
 - Polymerase enzymes

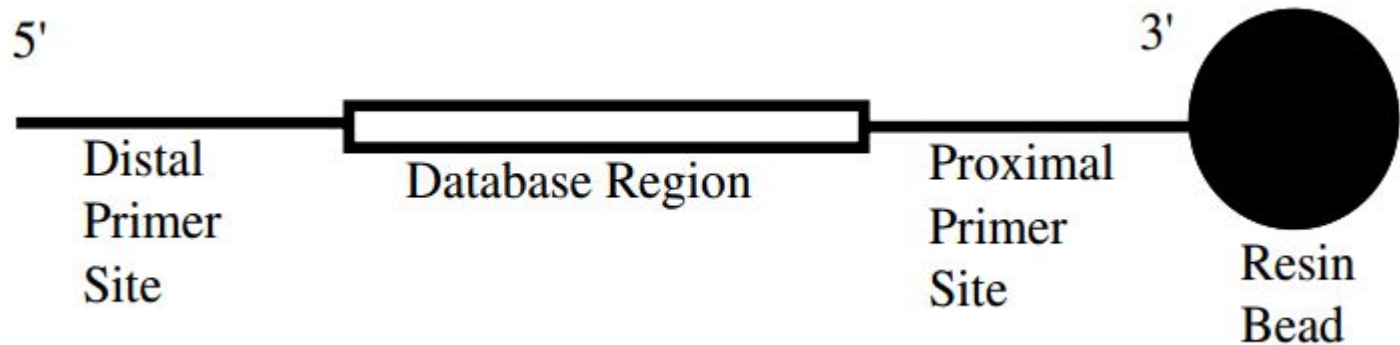


- **DNAzyme reactions**
 - DNA-based sequences that possess enzymatic activities



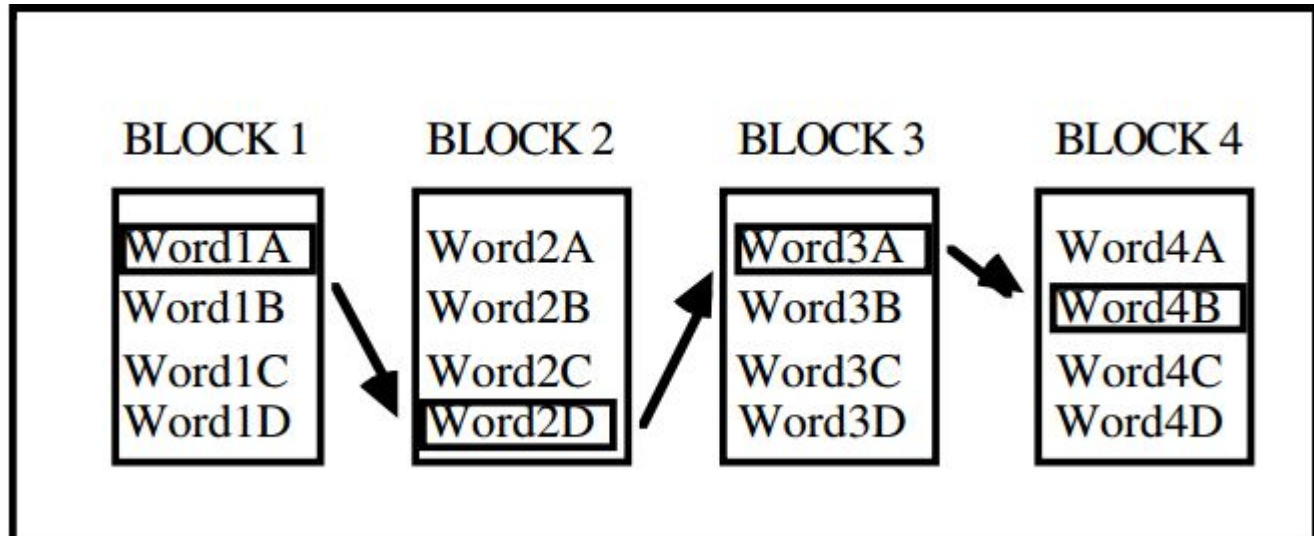
DNA DATABASE - EXAMPLE

- **Database entries:**
 - Resin bead
 - Proximal/Distal primers
 - Database region



DNA DATABASE - EXAMPLE

- **Database region:**
 - Word - Block design



DNA DATABASE - EXAMPLE

- **Generating words:**
 - No G's - decrease chances of secondary structure formation
 - Melting temperature
 - Hamming distance
 - Frameshift

DNA DATABASE - EXAMPLE

- **Querying**

- Synthesise query-probe with fluorescent marker
 - Complementary with desired database entry
- Mix probe with database library
 - Hybridization
- Separate beads (database entries) which hybridised with query
 - FACS (Fluorescence Activated Cell Sorting)
- Sequence separated sequences

DNA DATABASE - EXAMPLE

- **Translation to real-life database: semantic web**
 - Database entries: triples
 - E.g. Martijn - has_favorite_beer - Karmeliet
 - Coded into DNA-sequence
 - Query:
 - What is Martijn's favorite beer?
 - Query : Martijn - has_favorite_beer
 - Hybridisation -> separation -> sequencing
 - Sequenced database entry will read "Martijn - has_favorite_beer - Karmeliet"
 - → Martijn's favorite beer = Karmeliet

DNA COMPUTING: OUR PROJECT PROPOSAL

Simulation of a database encoded in DNA that can be queried by using DNA computing techniques: DNA origami & DNA strand displacement

NOW: digital information is stored in DNA but the entire DB need to be sequenced in order to retrieve *some* information.

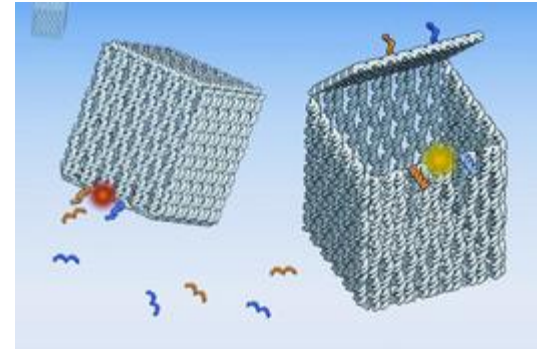
GOAL: design a database system in which sequence selective capture is possible

HOW:	Database	= collection of DNA molecules (in vitro: in solution or on chip - in vivo)
	Query	= programmed DNA origami & DNA strand displacement reactions

DNA COMPUTING: OUR PROJECT PROPOSAL

DNA origami

- The nanoscale folding of DNA to create two- and three-dimensional shapes at the nanoscale.
- Can be designed using the cadnano software (MIT)
- Can recognize a DNA element and change its conformation as a consequence of hybridization
- The conformational change can lead to the release of a molecule, for example a drug, or possibly a subquery sequence - this can be modelled.
- DNA origami can interact with each other



DNA strand displacement cascade

- Can be simulated in visual DSD (Microsoft). DSD is a programming language for DNA strand displacement reactions.
- Is already used in the context of DNA sequence detection (for example biosensors)

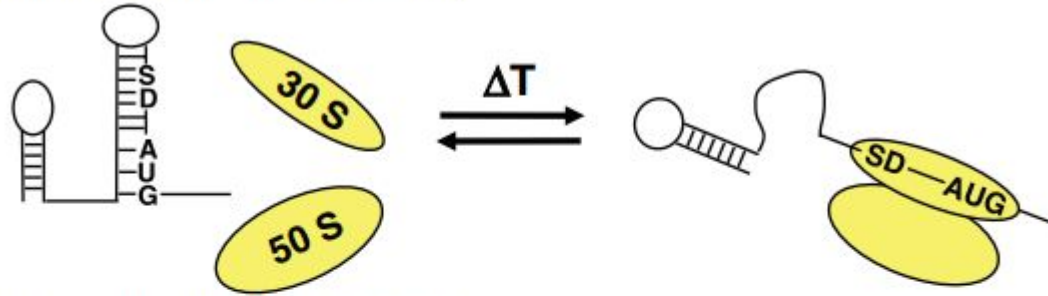
WETLAB - THE MICROBIAL THERMOMETER

- Temperature = important parameter !
- Microbes developed protective mechanisms
- RNA thermometers (riboswitches)

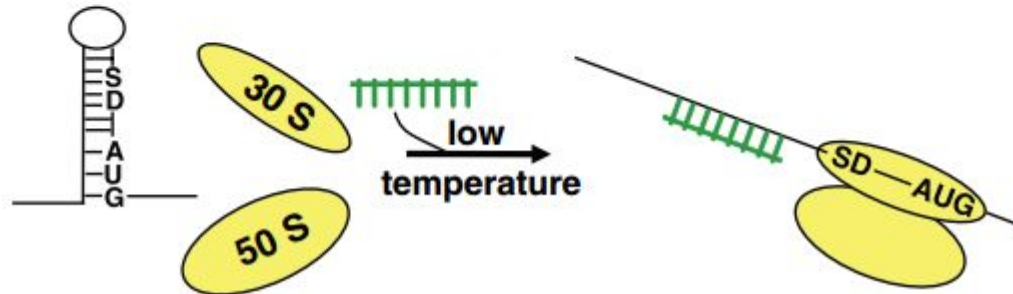


WETLAB - THE MICROBIAL THERMOMETER

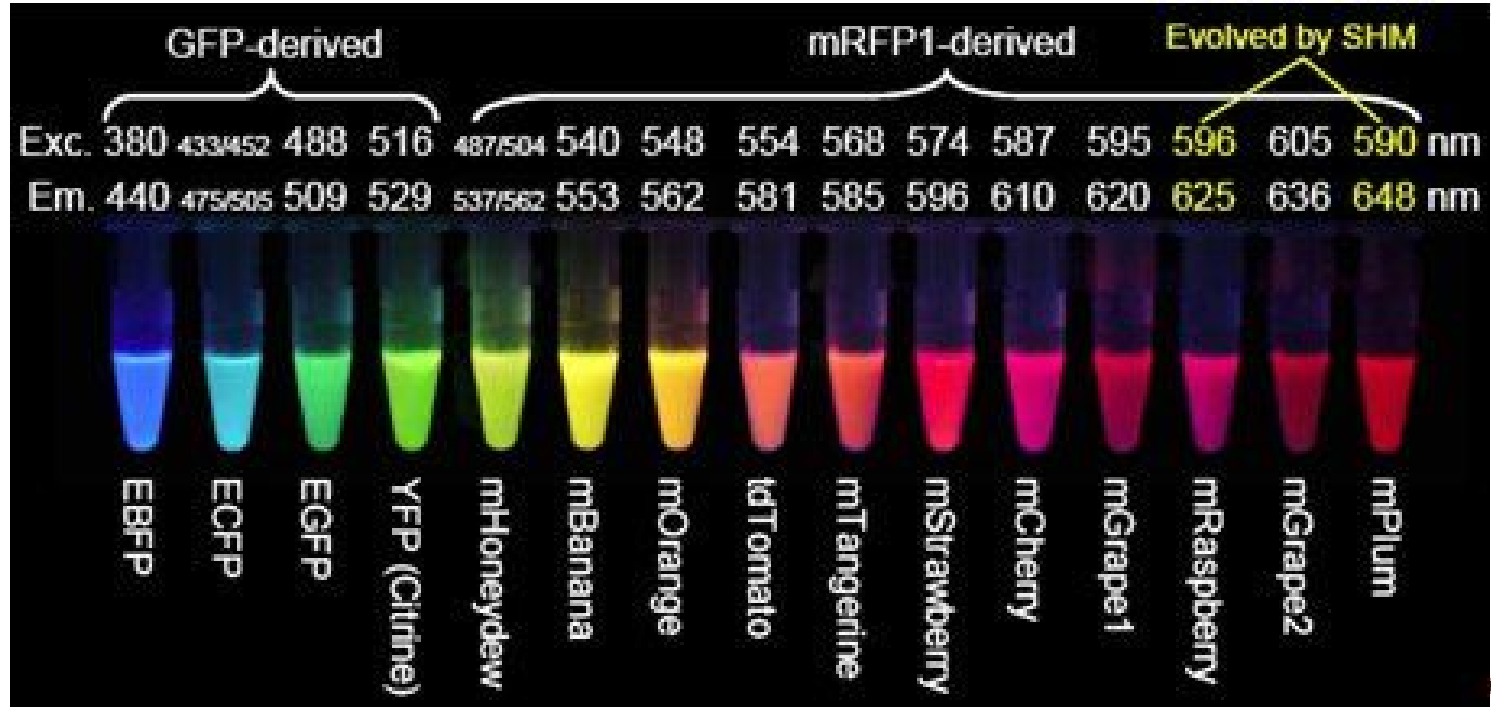
I. Translation initiation (*cis*-acting)



II. Antisense binding (*trans*-acting)



WETLAB - THE MICROBIAL THERMOMETER



WETLAB - THE MICROBIAL THERMOMETER

