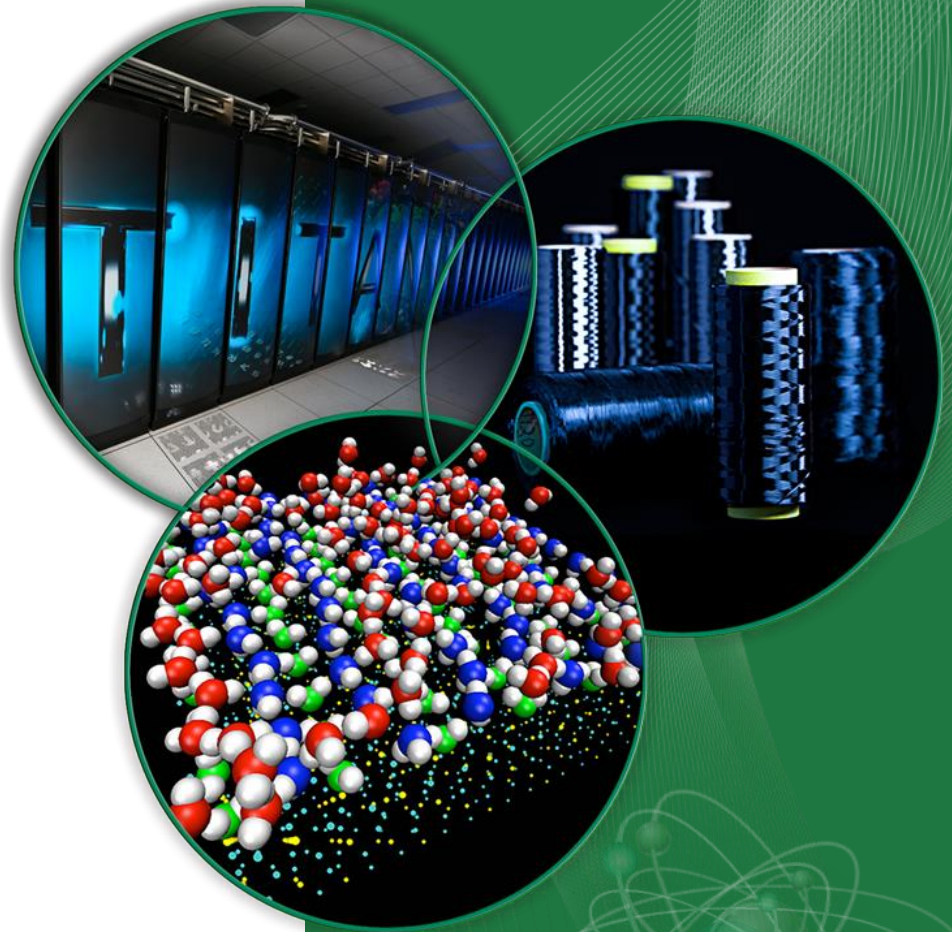


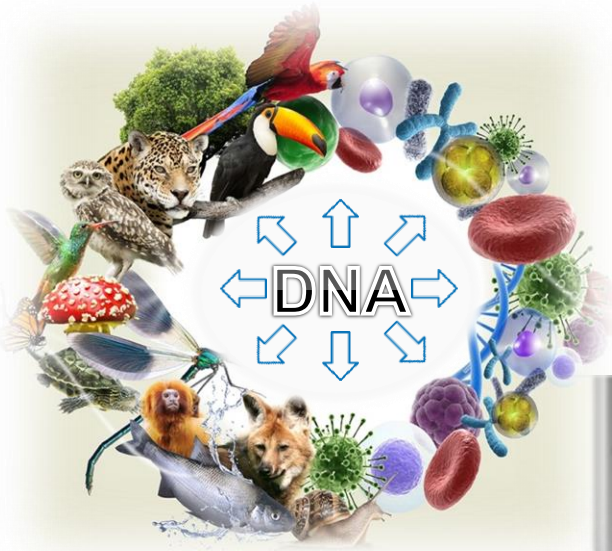
A innovative tool for gene assembly from a universal DNA library

Principal Investigator:
Xiaohan Yang

Co-Investigators:
GA Tuskan
HC De Paoli



Challenge to overcome



DNA

Major
Bottle neck:
DNA assembly
(large constructs)

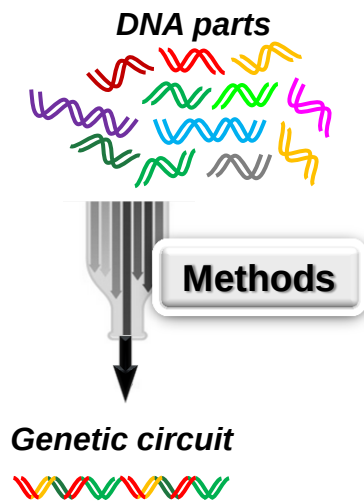
Genetic circuits → RNAs → Proteins → Traits

- Improved yield of crops
- Resistance to pathogens
- Drug/Vaccine production
- Biofuel and Bioproducts

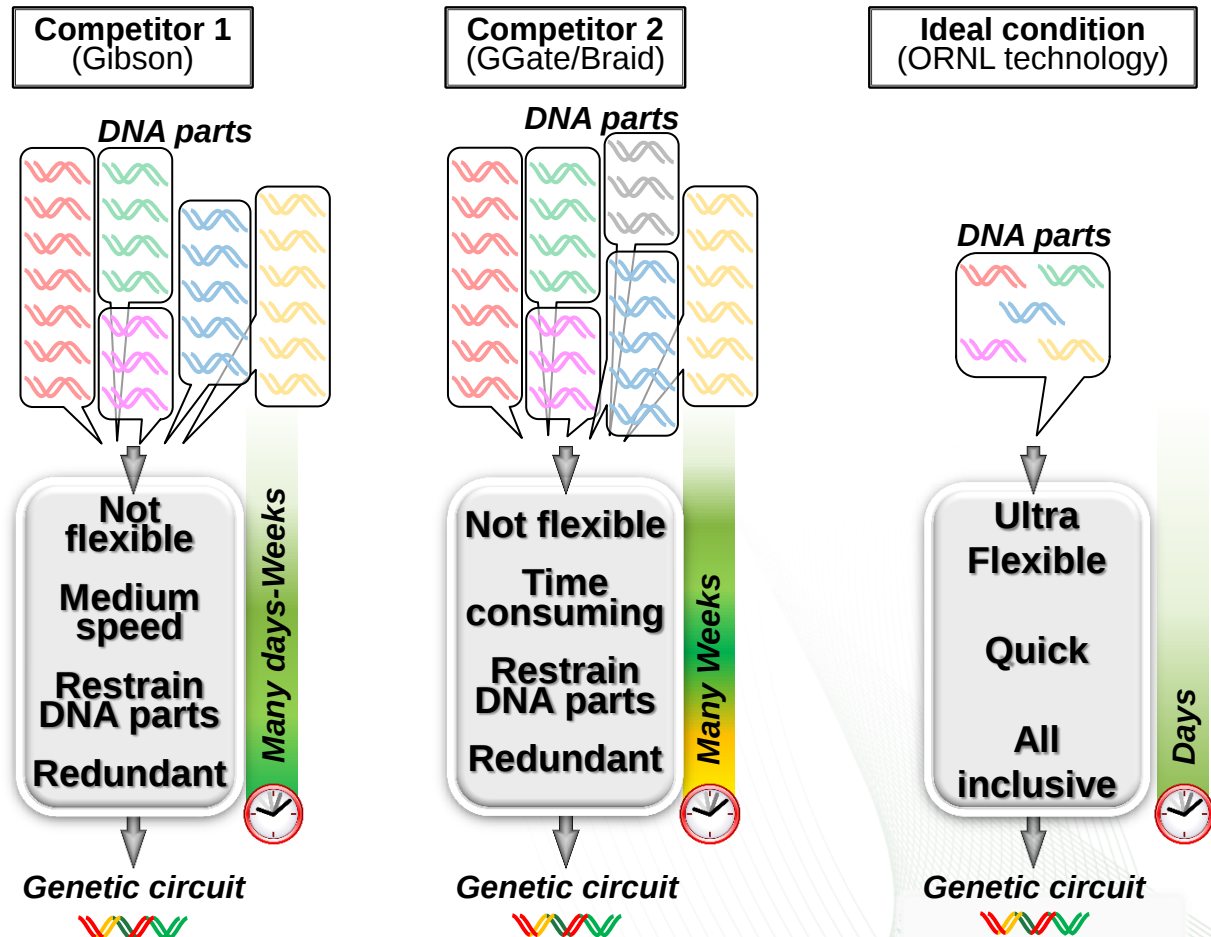


Technology Principle

PRINCIPLE

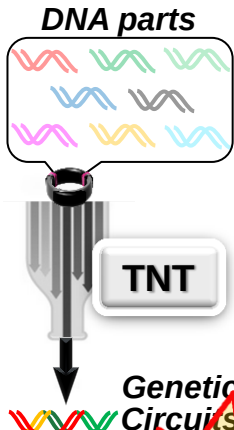


METHODS



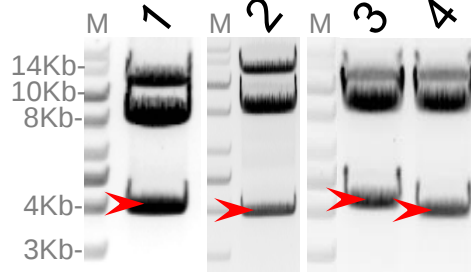
Technology Opportunity

- Preliminary data demonstrating that the technology works:



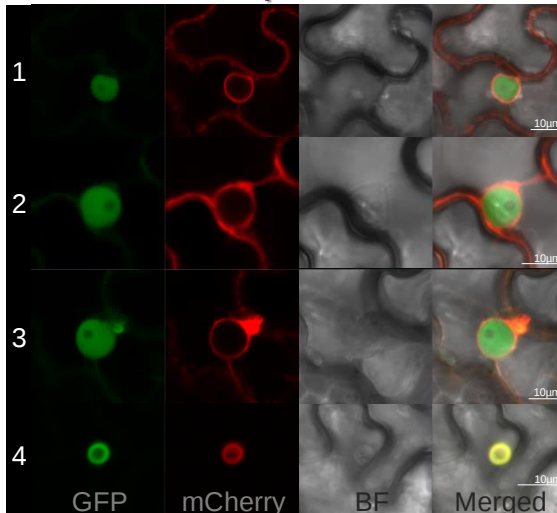
Fast gene assembly

Functional Experiment 1



27 DNA parts into 1 at any combination in 5 days

Functional Experiment 2



mpg DePaoli et al. 2016
SCIENTIFIC REPORTS

Scalable

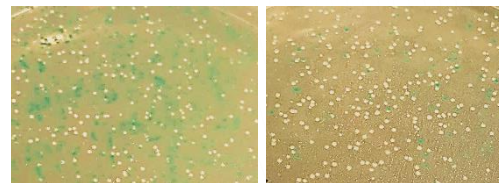
Functional Experiment 3



171 single plus 66 complex assemblies under high throughput setting achieved in 5 days with >94% accuracy

Reliable

Functional Experiment 4



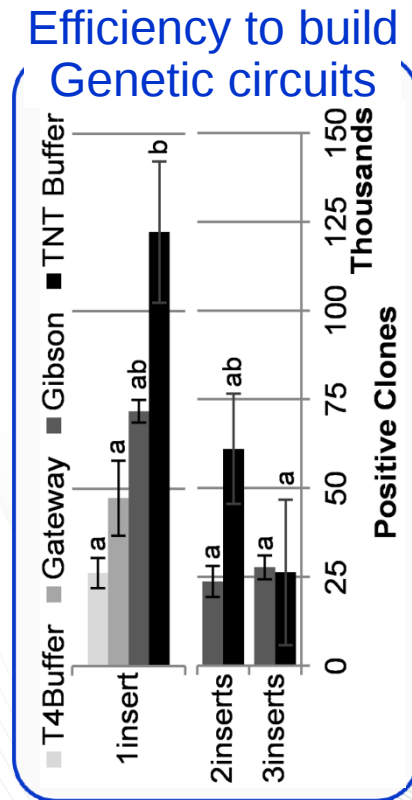
T4 Ligase Buffer

ORNL Buffer

Up to 8-fold higher efficiency

Technology Leadership

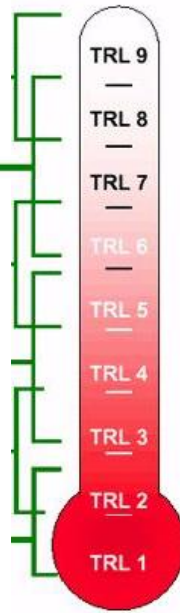
- Why is this technology unique?
 - First to be ORF compatible
 - First to be flexible
 - First to allow common and sharable platform
 - First 'restriction enzyme-dependent' method to overcome domestication
- Performance indicators that show this new technology works better than the existing ones:
 - Flexibility and ORF compatibility reduce library size, time for building large genetic circuits and, combined, reach goals faster.
 - Reactions are as much efficient or better than competitors through unique TNT Buffer.



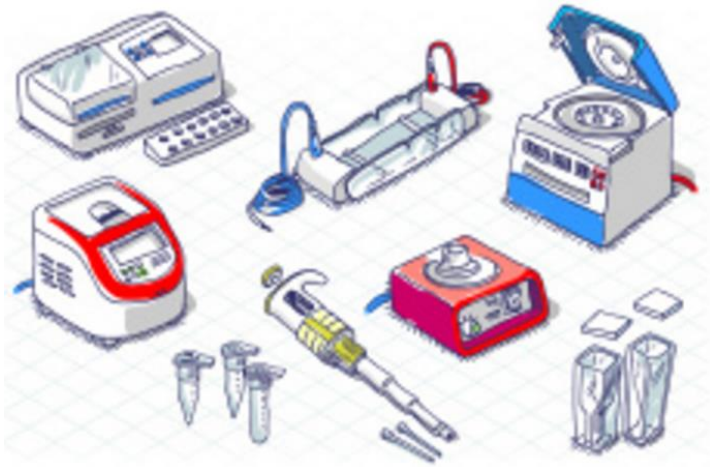
Technology Opportunity

- Current stage – System prototype demonstration (***beta-tested*** and qualified *in different organisms*)
- For a commercial product/service (cloning kit), TRL Level is currently 7.
- Additional research made it inclusive (for other systems: yeast and mammals) and robust with ***demonstration in an operational environment.***
- Prospect
 - Cloning kit offered by molecular biology providers;
 - Gene synthesis service, specially for recurring customers

System/Subsystem
Development



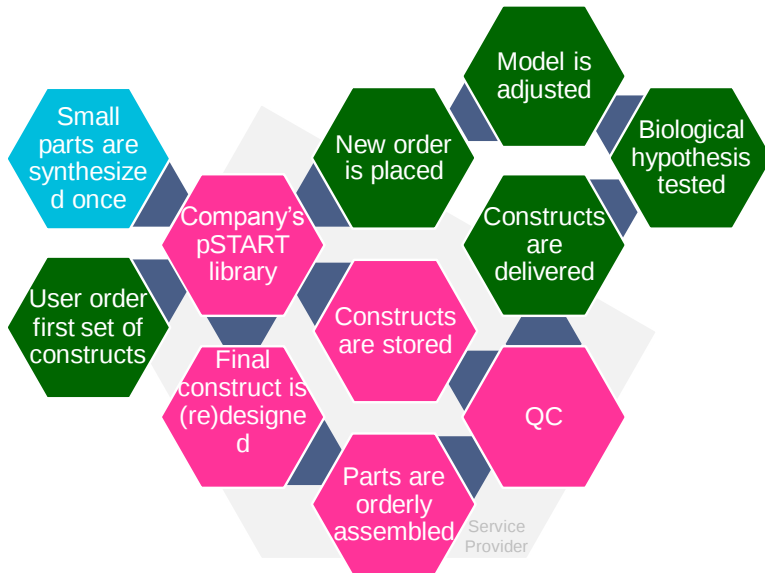
Applications – Target Customers – Current Practice



Kit for synthetic biology

Molecular biology companies may offer ORNL Technology as a “do yourself” kit. We have developed Plant, Microbes and Mammalian kits. Materials include pSTART and organism-specific set of assembling vectors (total of 16), 5X Activation Buffer, 5X one-pot high efficiency Buffer, Enzymes and TFO1-TFO2 Buffers (for cloning of non-domesticated parts). Kit size is expected to be small, medium and large, for 50, 250 and 1000 reactions, respectively.

Applications – Target Customers – Current Practice



A platform for gene synthesis

Users submit digital sequences of desired constructs, company order/PCR oligos/small parts once, build library, applies combinatorial assembly for orderly joining multigene constructs,

delivers quality material that is also stored, and provide potential refactoring of parts upon user's request.

Competitive Differentiation

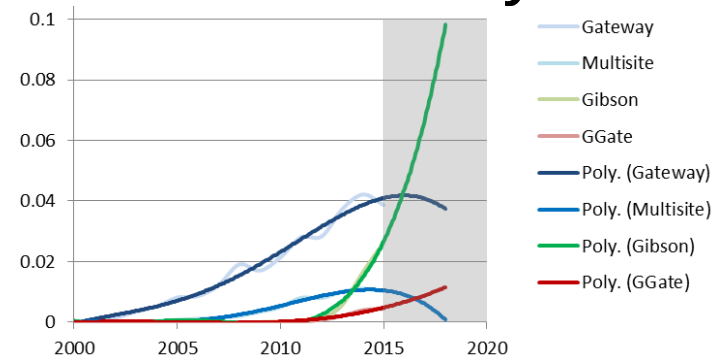
Technology Feature (Principles for Genetic Circuit)	ORNL Technology (TNT)	Competitor 1 (Gibson)	Competitor 2 (GGate/2.0)	Competitor 3 (Gateway)	Competitor 4 R.E.
Cloning Flexibility					
Universality					
Speed (ultra-fast)					
ORF ready					
Optimized reaction buffer					
Handle repetitive DNA					
Fits multiple organisms					

Market Opportunity

- Size of the available market (**2015**):
 - **Category 1** - Basic cloning and genetic circuits: **\$2.9bi** (biotechnology reagents, R&D):
 - **Category 2** - Biotech industries adopting the technology: **\$25.3bi** (agricultural) and **\$24.1bi** (pharmaceutical and therapeutic ,i.e., biomarkers).

- What fraction of the available market is likely to be captured?

- “Good” fraction (**Category 1**)
- “*Small*” fraction (**Category 2**)



- How is the total market expected to grow (**2018**)?

1.9 to **\$5.6bi**; 25.3 to **\$34.7bi**; 24.1 to **\$35.3bi**

Intellectual Property Available For Licensing

TNT-Cloning System, U.S. Patent Application 14/789,112 filed on July 1, 2015.

Inventors: Gerald A. Tuskan, Xiaohan Yang, and Henrique Cestari De Paoli

Published Patent Application Available:

<http://appft1.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PG01&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&s1=20160002644.PGNR>.

UTB ID 201403356

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