Cell-free DNA Technology for Synthetic Biology

OriCiro

Version 2.12

Agenda

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Overview

- Focused on cell-free assembly and amplification of large DNA (~1Mbp)
- Providing a next-generation approach of building large DNA unlocking the power of synthetic biology
- Founded in December 2018, located in Tokyo
- Team
  Seiji Hirasaki, CEO
  Masayuki Su'etsugu, Ph.D., CSO, Associate Prof., Rikkyo University
  Atsushi Usami, Ph.D., Outside Director, Partner, UTEC
- Series A: $3.6M from UTEC in March 2019
- Mission: to create a better Bioeconomy with innovative genome technologies

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Problem: Cell-based cloning has been the gold standard for a while...

1973 Cohen and Boyer

2019

1973 Cooper and Mitchell

Wikimedia Commons

2019

by rawpixel on Unsplash

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Solution: Cell-free technology replacing cell-based cloning

- Rapid amplification of large DNA reducing the required time to 1/10 of E. coli cloning
- Simple and easy-to-handle
- Streamlines the research process
- Widens the scope of R&D, allowing for amplification of previously infeasible sequences such as GC rich, repeat and cell toxicity-inducing sequences
- Unlocks the potential of synthetic biology

Technology

Cell-free amplification of large DNA
Cell-free assembly of DNA fragments

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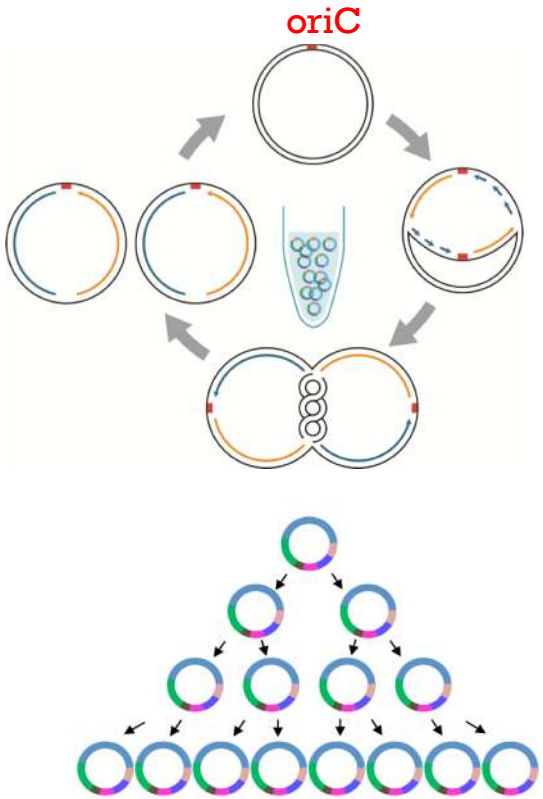
Reconstructing the E. coli genome propagation process in vitro

- Identified 25 proteins essential for E. coli genome propagation and reconstructed the entire propagation process in vitro
- Isothermal incubation at 30℃ for several hours
- Amplification in an exponential manner
- Self-sustaining and repetitive replication process
- Only requirement: circular DNA having *oriC* sequence (0.3kbp)



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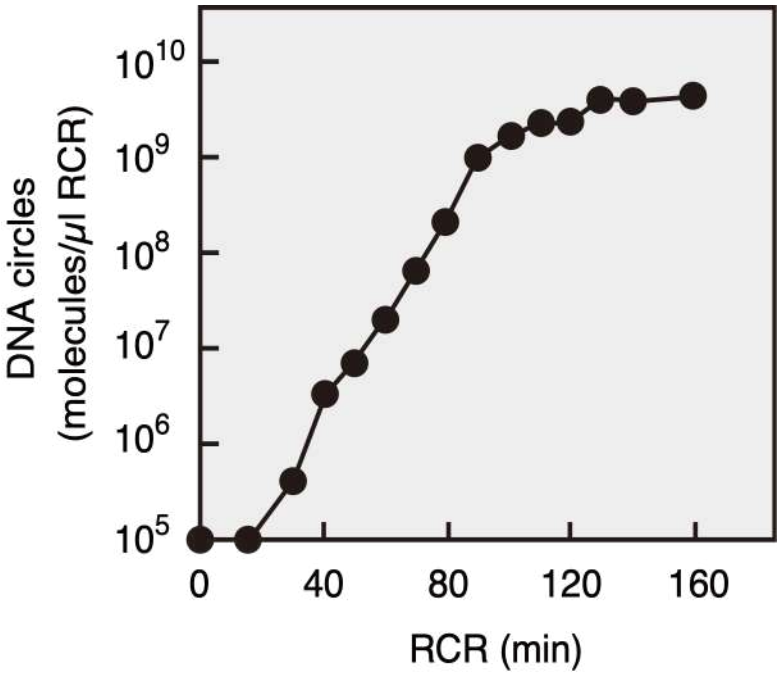
Outstanding performance of amplification by simple process

- ~10^{10}-fold amplification from a single DNA molecule within 3 hours at 30℃ for 10 kbp DNA
- Cell-free, easy-to-handle process
- Applicable to large circular DNA up to 1Mbp
- Applicable to any sequence (no limitations)
- Not recombinant DNA experiment



Su'etsugu et al., Nucleic Acids Research, 2017, Vol 45, No. 20 11525-11534

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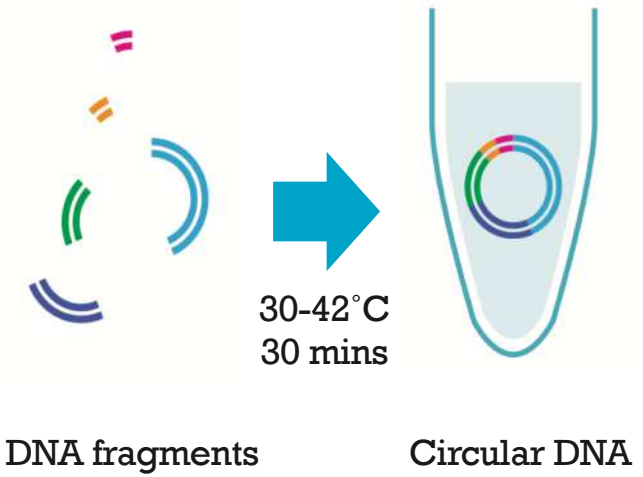
Distinguished advantages of our amplification technology

	PCR	E. coli cloning	OriCiro Technology
DNA size	+ <10kbp	++ <300kbp	+++ <1Mbp
Operation	Thermocycler	Cumbersome process Several days requiring techniques	Very simple process Several hours of isothermal incubation
Biosafety	Cell-free	Recombinant DNA experiment	Cell-free
Fidelity	+ 10^{-4}~10^{-6} error/bp	+++ 10^{-10} error/bp	++ 10^{-8} error/bp *
Sequence applicability	Not applicable to GC rich and repeat sequences	Not applicable to sequences that cause cell toxicity	Applicable to any sequence
Product	Linear DNA	Circular DNA	Circular DNA

* Will be improved to match that of E.coli cloning soon as the error-correction mechanism be incorporated.

Assembly: One-step assembly of up to 50 DNA fragments

- One-step assembly of multiple DNA fragments using homologous sequences of the fragment ends
- Simultaneous assembly of up to 50 fragments
- Enzymatic reaction
- No thermocycler required



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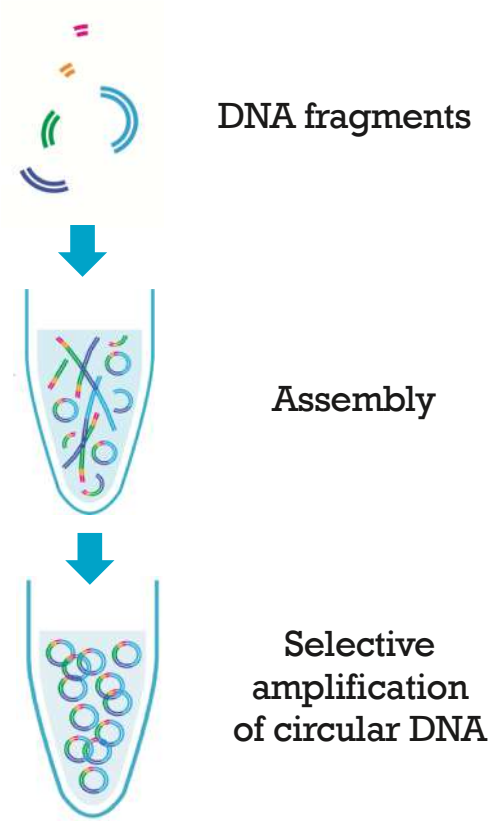
Combination: Efficient synthesis of genome-scale DNA

- Synergetic effect by combining assembly and amplification processes
- Unnecessary (linear) DNA is not amplified because only circular DNA is amplified
- No need of purification unlike in E. coli cloning
- Enabling cell-free synthesis of genome-scale DNA



IP: Covering a broad area of applications with long patent life

	Application/ Publication No.	Expires	Countries	Status
Amplification	WO2016080424	2035	US, EP, JP, CN	US: Granted (10301672) JP: Granted (6262877) EP, CN: Under review
	WO2017199991	2037	US, EP, JP, AU, CA, CN, IL, IN, KR, RU, SG	National phase
	WO2018159669	2038	WIPO	PCT application filed
Assembly	WO2019009361	2038	WIPO	PCT application filed
Editing	PCT/JP2019/29793	2038	WIPO	PCT application filed yet-to-be published

Wide range of potential applications

- Any research process currently using E. coli cloning
- Plasmid DNA development and manufacturing
- Modifications of microbe and plant genomes
- Building of artificial genomes from DNA oligos
- Sample preparation for the next generation sequencing
- New approaches to genetic diagnosis and gene therapy
- DNA data storage
- DNA as an advanced material

Products and Services

- Reagent kits for large DNA amplification and assembly for academia research
- Technology licensing to CDMOs and gene synthesis companies for plasmid DNA development and manufacturing
- Custom-made libraries of large DNA
- Co-development

Future Partnership

- Distribution of the reagent kits
- Plasmid DNA development and manufacturing (CDMOs and gene synthesis companies)
- Co-development based on our proprietary technology
- Investors for Series B financing

Conclusion

- OriCiro offers cutting-edge, cell-free assembly and amplification technologies of large DNA
- Increases the efficiency and widens the scope of partners' R&D dramatically
- Unlocks the potential of synthetic biology
- Seeks partnership opportunities