

Next-generation Cell-Free DNA Technology

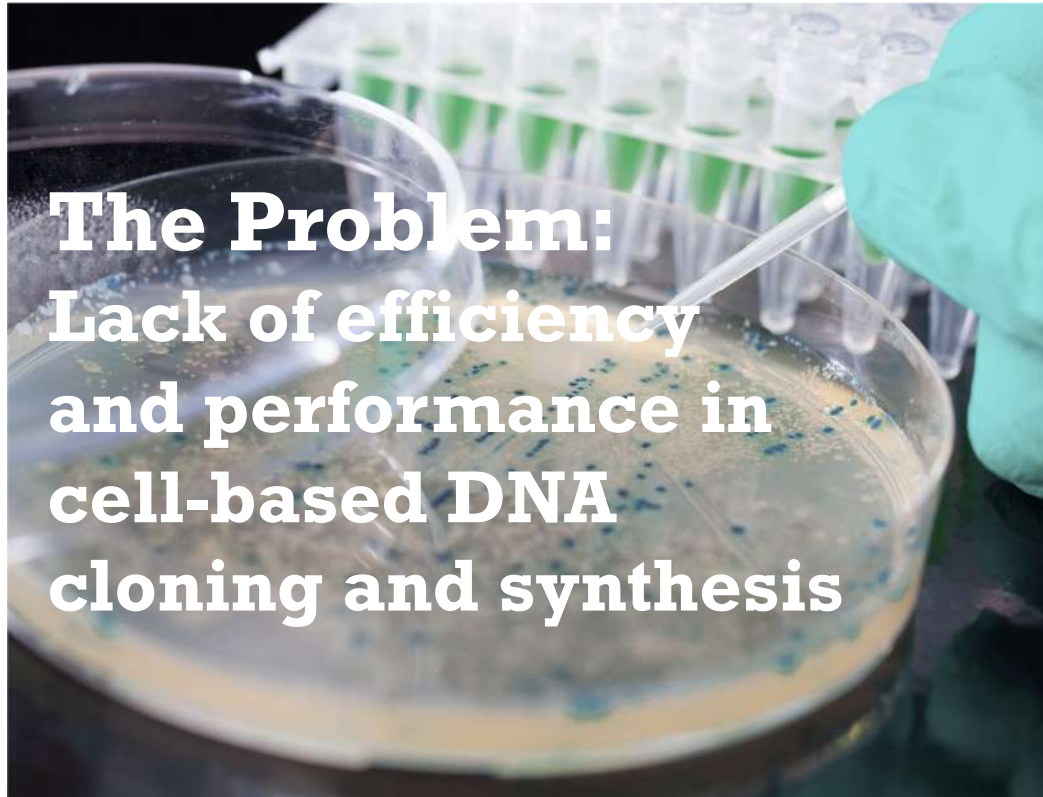
A powerful tool for synthesizing genome-scale
large DNA for Gene Therapy and Synthetic
Biology

Corporate Presentation

Corporate overview



- Providing the next-generation, CELL-FREE DNA technology for gene therapy and synthetic biology
- Founded in December 2018, headquartered in Tokyo
- Team of 16 (of which 5 Ph.D)
- Funding: Series B - \$9M in Q2 2021 (UTEC and ITV)
- Commercial stage - First product (research reagent kit) already on the market globally
- Mission: Accelerating the Bioeconomy through innovative DNA technologies



**The Problem:
Lack of efficiency
and performance in
cell-based DNA
cloning and synthesis**

1. Low throughput

- Cumbersome process
- Long hands-on time requiring overnight incubation
- Not suitable for automation

2. Limitations

- Difficulty in cell-toxic and repeat sequences
- Biosafety issue
- Endotoxin and contamination risk

Our Solution: World's first cell-free synthesis of large circular DNA



1. High throughput

- ✓ Rapid amplification of several hours, 1/10 hands-on time of E. coli cloning
- ✓ Simple, easy and clean process suitable for automation

2. Unleash the power of research

- ✓ Applicable to any sequences

3. Save money

- ✓ Save costs by simpler, faster and safer process

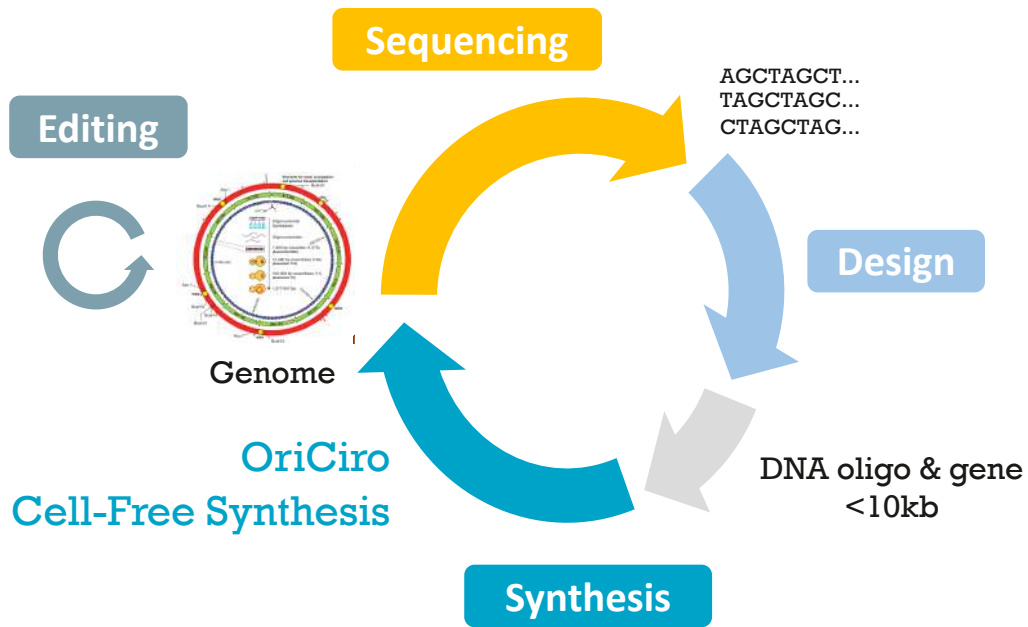
OriCiro cell-free technology provides

- More efficient and capable R&D tool for large DNA assembly and amplification, and
- More flexible, efficient and safer manufacturing method of plasmid DNA

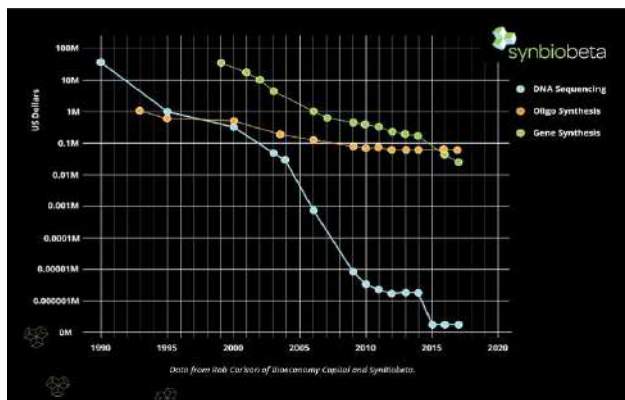
for the applications of

- Gene Therapy
- Vaccines
- Diagnostics, and
- Synthetic Biology

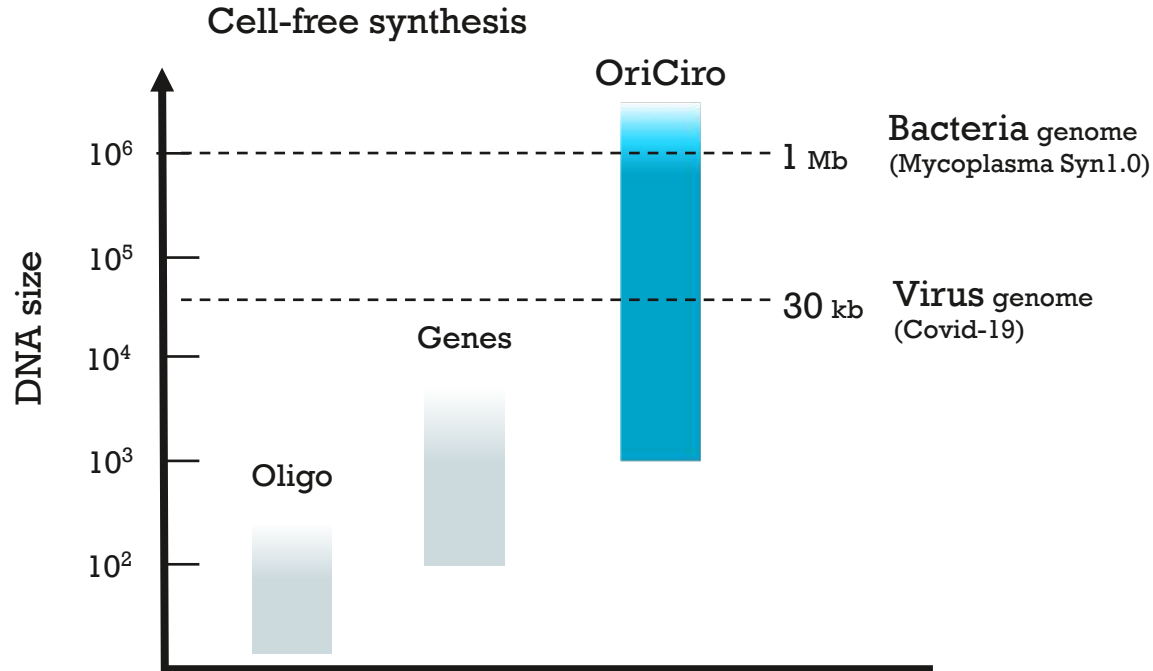
Closing the gap in genome-scale large DNA synthesis



From “reading” to “writing”...

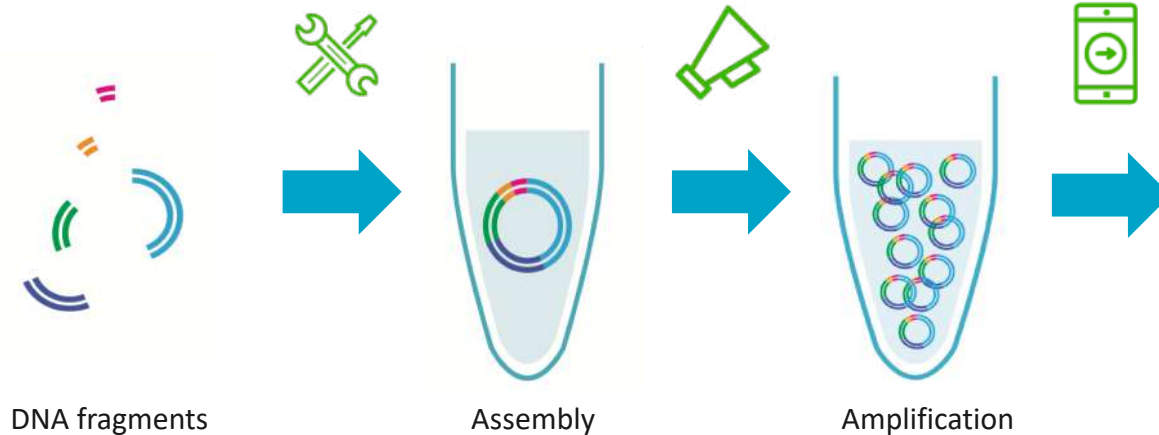


Extending the boundaries in DNA synthesis



Cell-free synthesis and production of large circular DNA

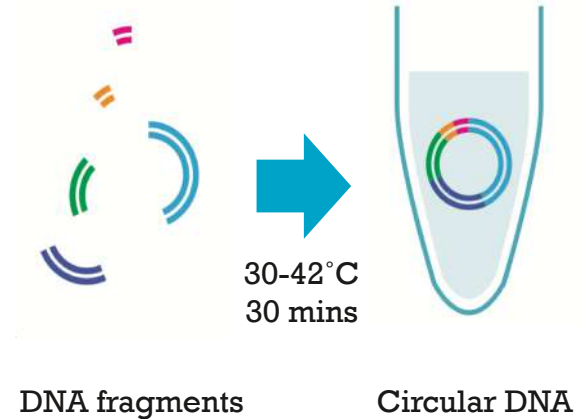
OriCiro[®] Cell-Free Technology



- Plasmid DNA
- DNA, RNA-based vaccines
- Gene therapy
- Microbe, virus & phage genome engineering
- DNA Libraries
- NGS
- Genetic diagnosis
- Artificial chromosome
- Synthetic Biology
- DNA data storage

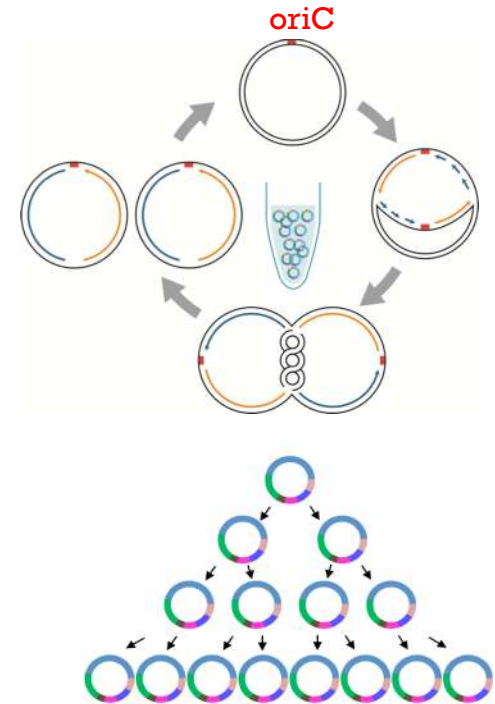
Assembly: One-step assembly of up to 50 DNA fragments

- **One-step assembly** of multiple DNA fragments using homologous overlapping fragment ends (20~60bp)
- Simultaneous assembly of **up to 50** fragments
- Precise assembly by **enzymatic reaction**
- **Simple isothermal process**



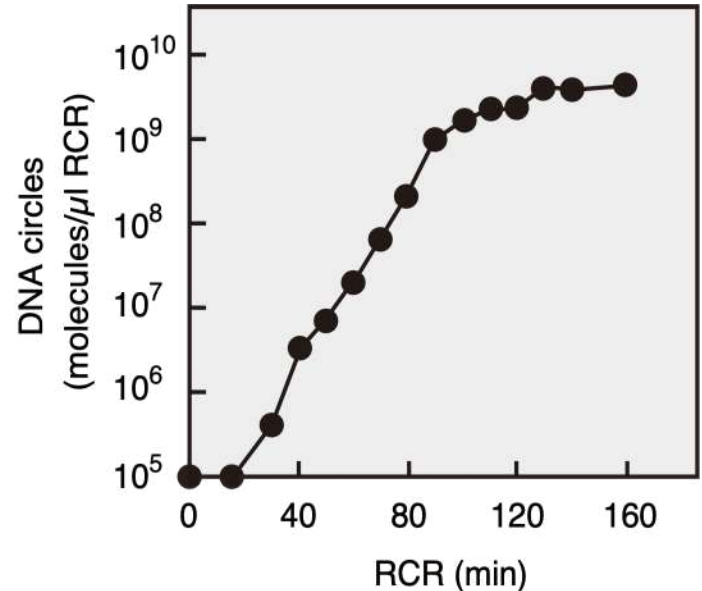
Amplification: Reconstituting E. coli genome propagation

- **Mixture of 26 proteins that reconstitutes the E. coli genome propagation process in vitro** without using E. coli cells
- **Isothermal incubation** at 30°C for several hours
- Rapid amplification in an exponential manner
- Self-sustaining and repetitive replication process
- Typical yield: 100ng/ul
- Only requirement: **circular DNA** having **oriC** sequence (0.3kbp)



Outstanding performance of amplification by simple process

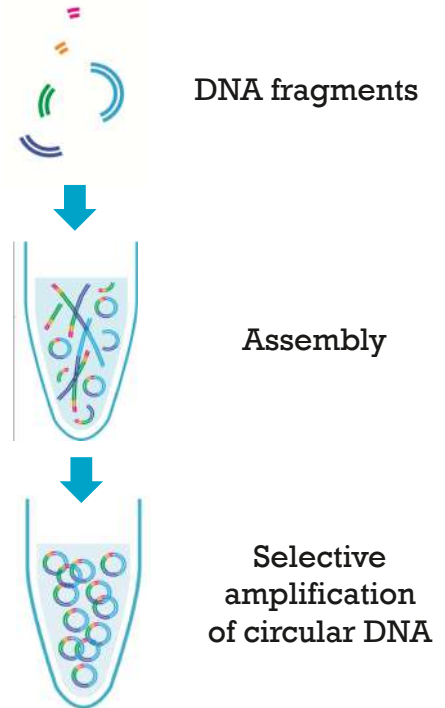
- **~10¹⁰-fold amplification** from a single DNA molecule within 3 hours at 30°C
- Cell-free, **easy-to-handle process**
- Applicable to **large circular DNA** up to 1Mbp
- Applicable to **any sequence** including cell toxic, GC-rich and repeat sequences
- No recombinant DNA experiment



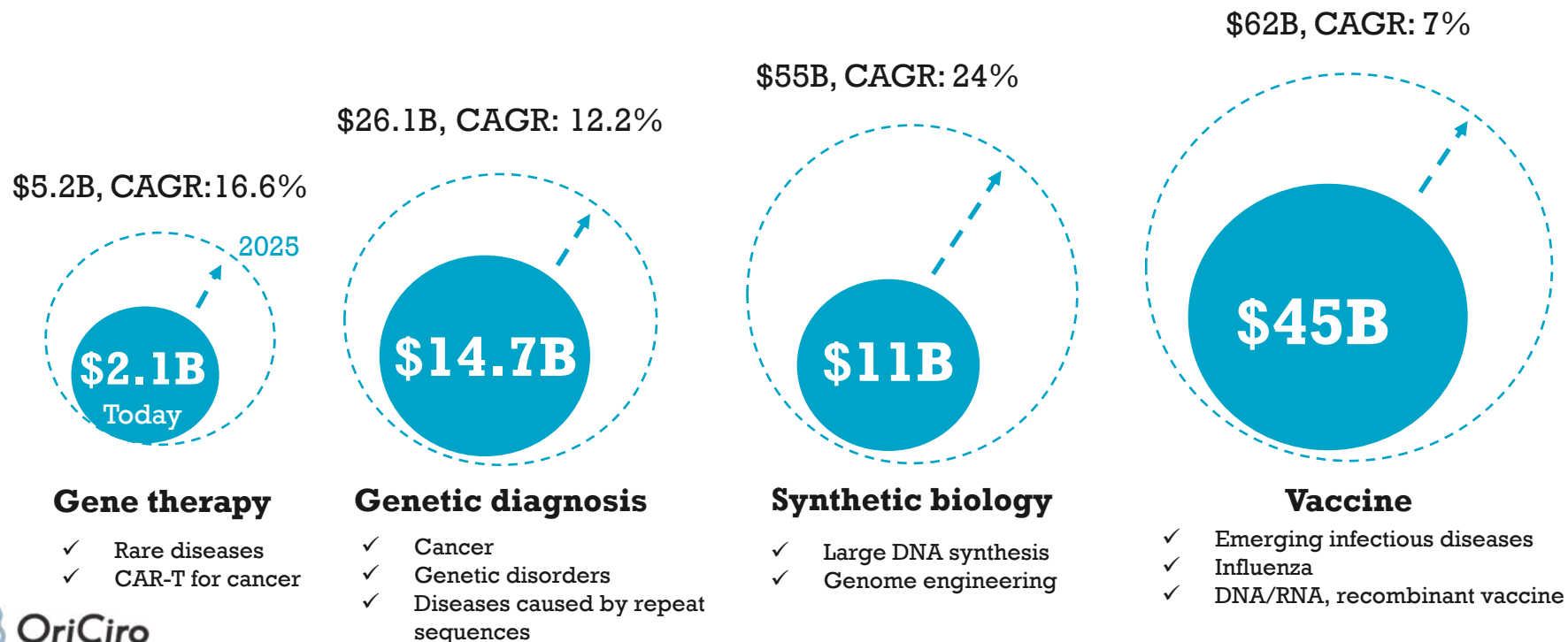
Su'etsugu et al., Nucleic Acids Research, 2017, Vol 45, No. 20 11525-11534

Efficient building of genome-scale DNA

- **Synergetic effect** by combining assembly and amplification processes
- **Selective amplification** of circular DNA: assembly intermediates (linear DNA) are not amplified
- No need of E. coli transformation and culture
- Enabling cell-free building of genome-scale DNA

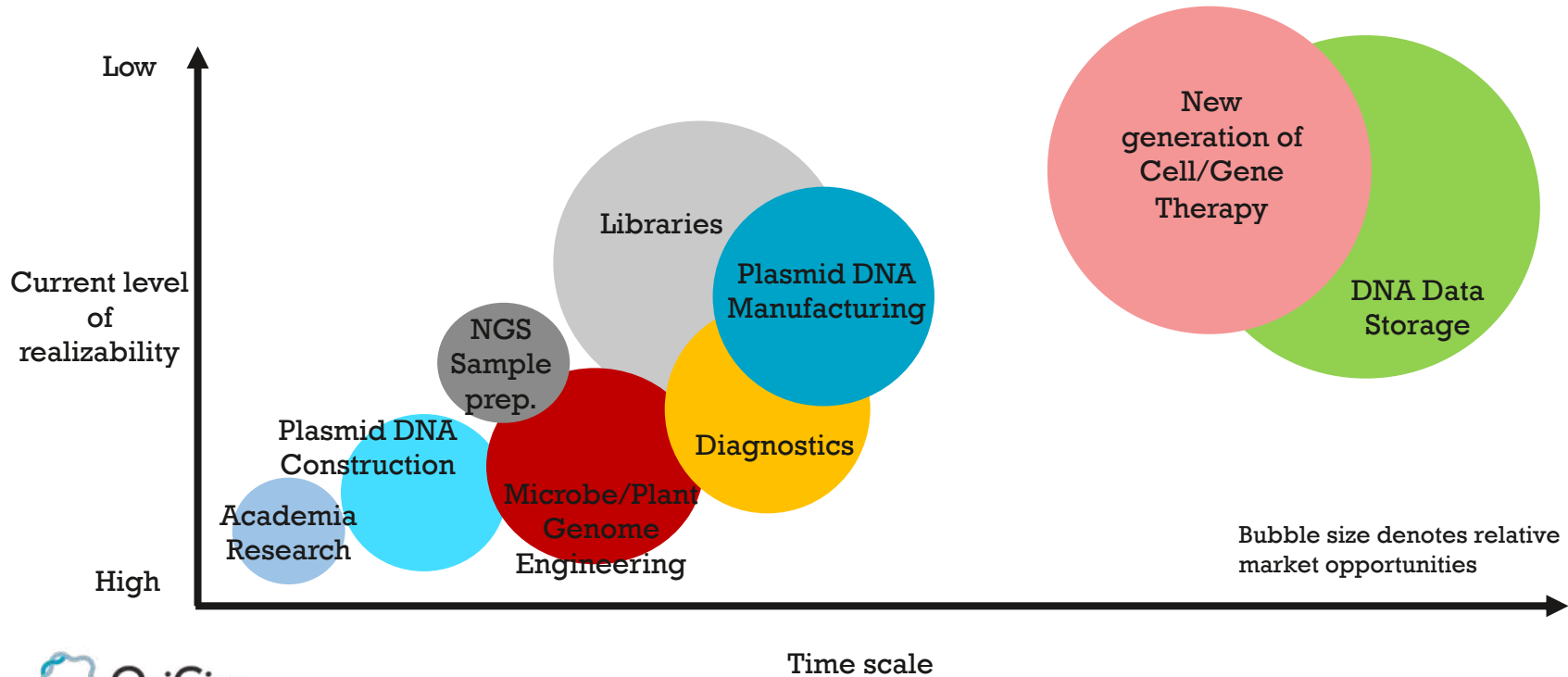


Significant opportunity in growing target markets



Sources: Global Market Insights, Markets and Markets, Grand View Research, company internal analysis

Wide range of potential applications



Products and Services



Reagent kits for academia and non-profit research



Research program, technology licensing and CRO services for corporate customers



Plasmid DNA manufacturing



Biopharma application (drug discovery)

Products and Services – Research reagent kit



- “OriCiro Cell-Free Cloning System”
 - Ready-to-use enzyme mixture for research use
 - Contains enzyme mixture for 5 assembly and 10 amplification reactions
- For academia and non-profit research
- List price: USD550/kit
- Available for purchase through e-commerce (www.ec.oriciro.com) and distributors



Products and Services – Corporate customers



- “OriCiro Cell-Free PASS” for research purpose
 - Program of accessing OriCiro cell-free technology for research purpose
 - Option of three packages: 5/10/20 reagent kits
 - Includes technical support
- Technology licensing for uses of manufacturing and other commercial purposes
- CRO services
 - Provides on-demand research services addressing the specific needs of customers

Products and Services – pDNA manufacturing



- Provides plasmid DNA for pharmaceuticals manufactured by OriCiro cell-free technology
- Manufacturing process development in progress: service launch targeted in 2022
- Working on scaling up and GMP compliance
- Open to the discussion of technology licensing of the manufacturing process for customers' in-house manufacturing

Next steps



- Enzyme mixture production scaling up
- Introduce robust QA/QC system, ISO9001 accreditation in Q3 2021



- Focused access to potential corporate customers



- Process development of DNA amplification scaling up (100mg per batch)
- Introducing GMP compliant system



- Internal R&D for biopharma application (drug discovery)
- Focused access to potential co-development partners

Leadership team



Seiji Hirasaki, MSc
CEO and Co-founder



Masayuki Su'etsugu, Ph.D
CSO and Co-founder



Shigemasa Sasaki
COO and CBO



Nasir Kato Bashiruddin, Ph.D
CTO



Atsushi Usami, Ph.D
External Director, Partner of UTEC



Milestones

	Event
July 2020	Product launch of the reagent kit in Japan ✓
October 2020	Global launch of the product ✓
Q2 2021	Series B ✓
H2 2021	Establish US subsidiary
	Enzyme production scaling up (X10)
	ISO9001 accreditation ✓
2022	Launch plasmid DNA manufacturing services
	Launch drug discovery services

Highlights

Disruptive Technology

- Offers next-gen, cell-free large DNA synthesis and manufacturing technology giving advantaged market positions over current cell-based approaches

Value to Customers

- Increases efficiency and widens the scope of customers' R&D
- Provides more flexible and cost-effective plasmid DNA manufacturing with better safety profile

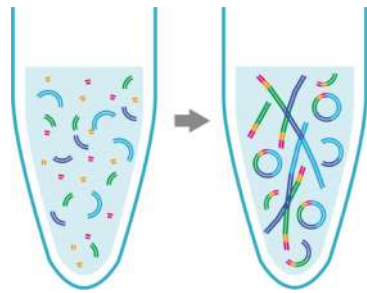
High Potential of Growth

- Addresses high-growth markets including vaccine, gene therapy and synthetic biology
- Wide range of potential applications

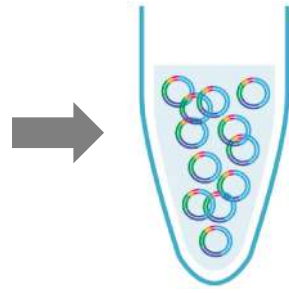
Appendix

Example: Cell-free process accelerates vaccine development

Cell-free Large DNA Synthesis

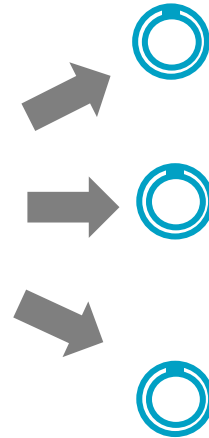


Assembling fragments to build large DNA



Amplification

Plasmid virus genome



DNA vaccine



mRNA vaccine



Cell

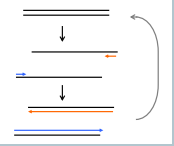
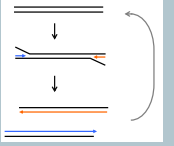
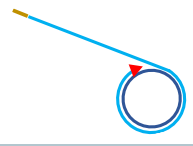
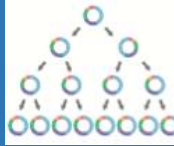
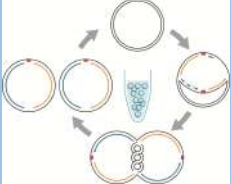


Recombinant vaccine

Distinguished advantages of OriCiro amplification

	PCR	E. coli cloning	OriCiro™ Cloning
DNA size	+ <10kbp	++ <50kbp	+++ <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	+ $10^{-4} \sim 10^{-6}$ error/bp	+++ 10^{-10} error/bp	++ 10^{-8} error/bp *
Sequence applicability	Not applicable to GC rich and repeat sequences	Not applicable to cell- toxic sequences	Applicable to any sequence
Product	Linear DNA	Circular DNA	Circular DNA

Comparison of Cell-free DNA Amplification Technology (1)

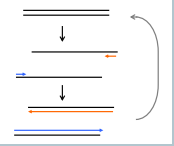
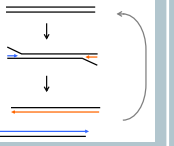
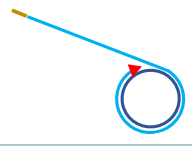
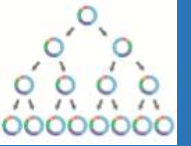
	PCR 1983- 	INAAT (Isothermal Nucleic Acid Amplification Technology) 1991- 	RCA-based (Rolling Circle Amplification) 1995- 	OriCiro 2017- 
Application	<ul style="list-style-type: none"> • Diagnostics • DNA synthesis (small scale, gene size) • NGS sample prep 	<ul style="list-style-type: none"> • Diagnostics 	<ul style="list-style-type: none"> • Diagnostics • DNA synthesis (plasmid size) • NGS sample prep 	<ul style="list-style-type: none"> • Diagnostics • DNA synthesis (large scale, plasmid/genome size) • NGS sample prep
Market size	\$7B in 2016* ¹	\$1.6B in 2016* ²	\$1.7B in 2017 for WGA(estimated* ³)	
Technology	<ul style="list-style-type: none"> • Traditional PCR, Quantitative PCR: Thermo Fisher Scientific, LGC, Takara Bio, Bio-Rad, Agilent 	<ul style="list-style-type: none"> • NASBA, HDA, LAMP, SDA, SPIA, NEAR, TMA, RPA, SMAP2 (DNA detection) Alere, bioMerieux, BD, Eiken Chemical, Hologic, Lucigen, Meridian Bioscience, Ustar Biotechnologies, Quidel, OptiGene 	<ul style="list-style-type: none"> • WGA/MDA (whole genome amplification for NGS): GE Healthcare, Expedeon, Illumina, Sigma-Aldrich, Thermo Fisher Scientific, Active Motif 	<ul style="list-style-type: none"> • Reconstitution of Replication Cycle Reaction (RCR): OriCiro Genomics, Inc.
Key player	<ul style="list-style-type: none"> • PCR-based gene synthesis: IDT, Twist bioscience, GenScript, Genewiz 		<ul style="list-style-type: none"> • dbDNA (DNA manufacturing) : Touchlight • RapidAMP (DNA synthesis) : SGI-DNA 	

*1 <https://www.alliedmarketresearch.com/polymerase-chain-reaction-technologies-market>

*2 <https://www.grandviewresearch.com/industry-analysis/isothermal-nucleic-acid-amplification-technology-inaat-market>

*3 <https://www.futuremarketinsights.com/press-release/whole-genome-amplification-market>

Comparison of Cell-free DNA Amplification Technology (2)

	PCR 1983- 	INAAT (Isothermal Nucleic Acid Amplification Technology) 1991- 	RCA-based (Rolling Circle Amplification) 1995- 	OriCiro 2017- 
DNA size	++ <10 kb	+ <1 kb	+++ 70 kb*4	+++++ 1k - 1Mb
Process	Primer, DNA Polymerase, Thermocycle	Primer, DNA Polymerase, +α (Enzyme etc.), Isothermal	Primer, Phi29 DNA Polymerase, Isothermal	oriC sequence, 26 replication proteins, Isothermal
Fidelity	+ 10 ⁻⁴ ~10 ⁻⁶ error/bp	+ 10 ⁻⁵ error/bp	++ 10 ⁻⁶ error/bp*4	+++ 10 ⁻⁸ error/bp
Replication Speed	20 - 70 bases/sec	20 - 70 bases/sec	~50 bases/sec	~1,000 bases/sec
Sequence difficulty	Difficulty in GC rich and repeat sequences	Difficulty in GC rich and repeat sequences	Primer bias, slippage of repeat	No limitations
Amplified Product	Linear DNA	Linear DNA	Linear DNA	Monomer supercoiled circular DNA



*4 Processivity and fidelity of Phi29 DNA polymerase

Advantages of OriCiro Cell-Free pDNA manufacturing

		OriCiro Cell-Free	E. coli fermentation
Plasmid construct	Selection system	Not required	Required
	DNA sequence	No limitations	Difficulty in some sequences
Process	Growth	Simple (isothermal incubation)	Cumbersome (cell-bank generation, culture media, cell harvest and lysis)
	Purification	Simple	Multiple step (filtration, chromatography)
	Time per cycle	One ~ several days	~ several weeks
Safety	Risk of endotoxin/ contamination	<<	
Cost	Material	=	
	Facility investment	<<	
	Labor	<<	



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

	Publication No.	Title	Expires	Countries	Status
Amplification	WO2016080424	Method of amplifying circular DNA	2035	US, EP, JP, CN	US: Granted (10301672) JP: Granted (6262877) EP: Granted (3222717) CN: Under review
	WO2017199991	Method for amplifying cyclic DNA	2037	US, EP, JP, AU, BR, CA, CN, IL, IN, KR, RU, SG	RU: Granted (2748736) National phase
	WO2018159669	Method for replicating or amplifying circular DNA	2038	US, EP, JP, AU, BR, CA, CN, IL, IN, KR, RU, SG	National phase
Assembly	WO2019009361	DNA production method and DNA fragment joint kit	2038	WIPO	JP: Granted (6701450) KR: Granted National phase
Editing	WO2020027110	Method for editing DNA in cell-free system	2038	WIPO	PCT application filed