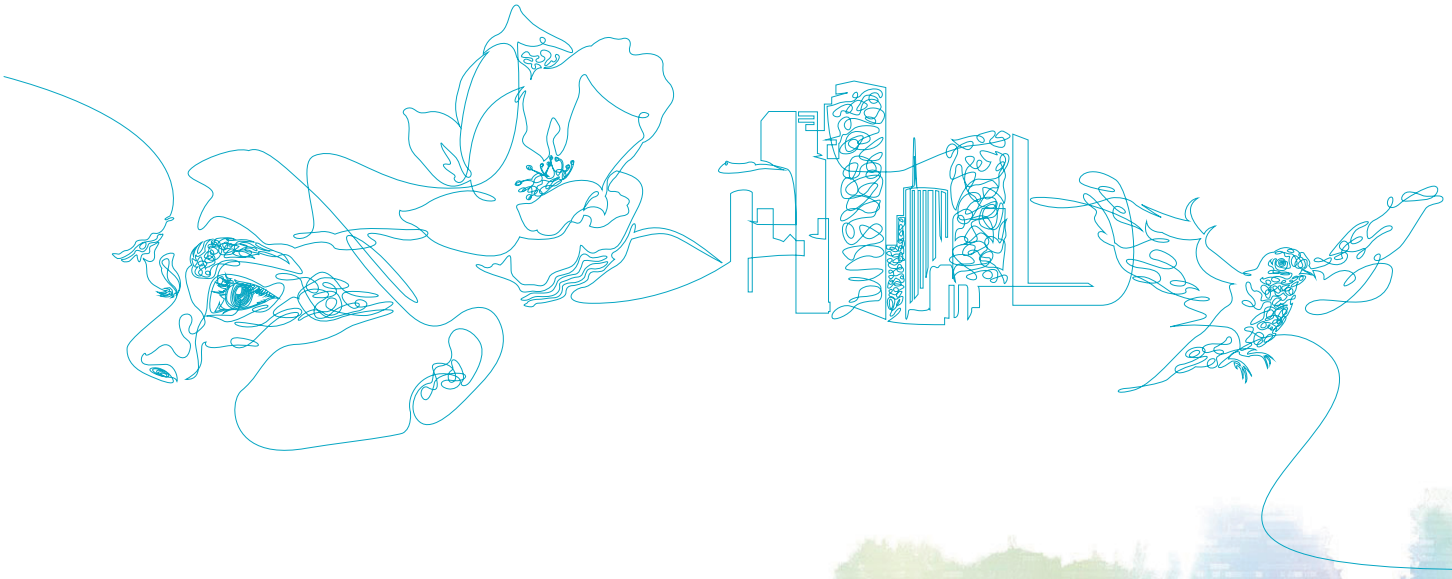
OriCiro

Innovative and Best-In-Breed DNA Technology Overcomes Challenges in the Development of Vaccine, Cell and Gene Therapies

White paper



1. Introduction

The world needs great science more than ever! Over the last year, the world has been significantly impacted by the COVID-19 pandemic and with it we have witnessed an unprecedented global and industry-wide development campaign to combat its spread in record time. The mRNA vaccines produced by Pfizer/BioNTech and Moderna as well as the up-and-coming therapeutic antibodies for COVID-19 are prime examples of the rapid evolution of cutting-edge drug development approaches and our ever-increasing reliance on genetic materials and their engineering technologies.

The global effects that have been brought on by the COVID-19 pandemic underscores the criticality of being able to get drugs to developed and to the market as quickly as possible, while not compromising standard quality and safety criteria.

Alongside the acceleration of vaccine development seen in response to the COVID-19 pandemic, cell and gene therapies have shown huge advances over the past decade with several methods already in mainstream use (gene editing, CAR-T, and AAV therapies). A look at the history and potential future of therapeutics in these areas further highlights the importance of genetic engineering in the drug development process.

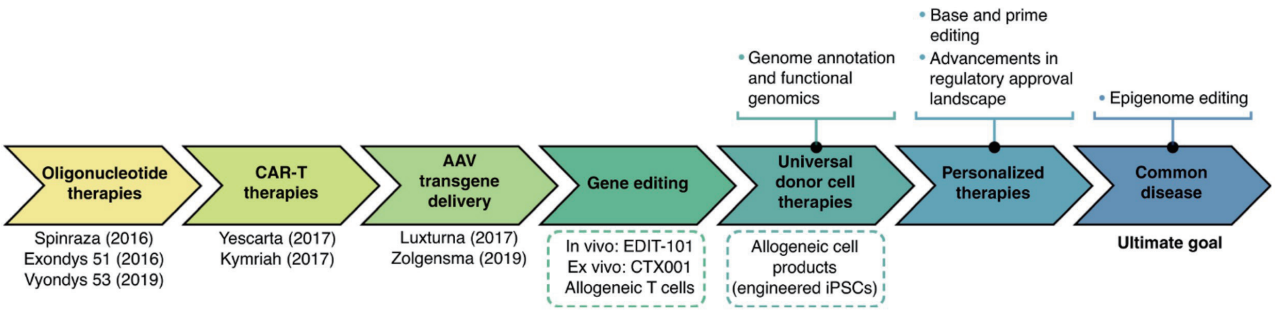


Figure 1 Evolution of Therapeutics

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The exponential rise in novel biotechnological approaches and the validation of increasingly complex treatment options are furthering the demand and necessity of genetic manipulation especially for large, genome-scale DNA synthesis to support use cases in synthetic biology and gene editing.

2. Problem Statement

When working toward the development of the various aforementioned treatment modalities, the industry still relies heavily on PCR and cell-based cloning for the manipulation of genetic information at early and late stages of development. Both are decades old methodologies and are deeply rooted in effectively all aspects of biological research and drug development. As paradigmatic as PCR and cell-based cloning are, they are not without their limitations and drawbacks and more efficient and flexible alternatives could greatly accelerate development timelines at a variety of steps in the drug development process and for a wide variety of modalities and indications.

PCR provides an efficient method of synthesizing linear DNA in vitro, but is limited in DNA strand length and sequence composition and can lead to sequence biases introduced during each

amplification cycle. Cell-based cloning is often used when trying to amplify larger sequences, however, this approach also comes with various challenges from time-consuming cloning and unpredictable cytotoxicity issues at the bench and iterative experimentation for fine-tuning conditions for viable cell banks for manufacture. In the context of manufacturing DNA, once the vehicle for amplification has been established, the appropriate filtration process to isolate the target genetic material still needs to be developed separately. Further, both PCR and cell-based cloning require hands-on intervention and do not lend themselves easily to automation given the time, materials, and process optimization required to produce the amplified product cost-effectively.

There will continue to be enhancements to these technologies, but it makes sense to think outside the box if we are to accelerate the research, discovery, and the manufacturing lifecycle for a new era of drug development.

3. An Overview of DNA Amplification

Since the discovery of DNA by Miescher in 1870, the interest and complexity of applications for DNA has come to dominate all aspects of biomedical research.

The advent of polymerase chain reactions (PCR) in 1983 further accelerated the field of molecular biology, giving scientists the ability to amplify target DNA in vitro, dramatically reducing the time required to generate genetic material. As an essential technology for genetic engineering, scientists have been able to sequence and understand the role DNA plays in various biological systems. This, in turn has resulted in the development of a multitude of diagnostics and therapeutics.

As critical as PCR has become to genetic engineering, nearly three decades since its introduction, there remain challenges that must be overcome depending on the sequence to be amplified. Sequences with high GC-content are often difficult to amplify due to the strong bonds that form between these bases, while longer sequences generally require added experimentation to optimize cycle temperature conditions and risk creation of unwanted DNA products. PCR also has inherent non-negligible error rates that will continue to propagate during every amplification cycle that takes place. The required thermal cycling processes and the challenges highlighted also result in an inability to effectively scale this process.

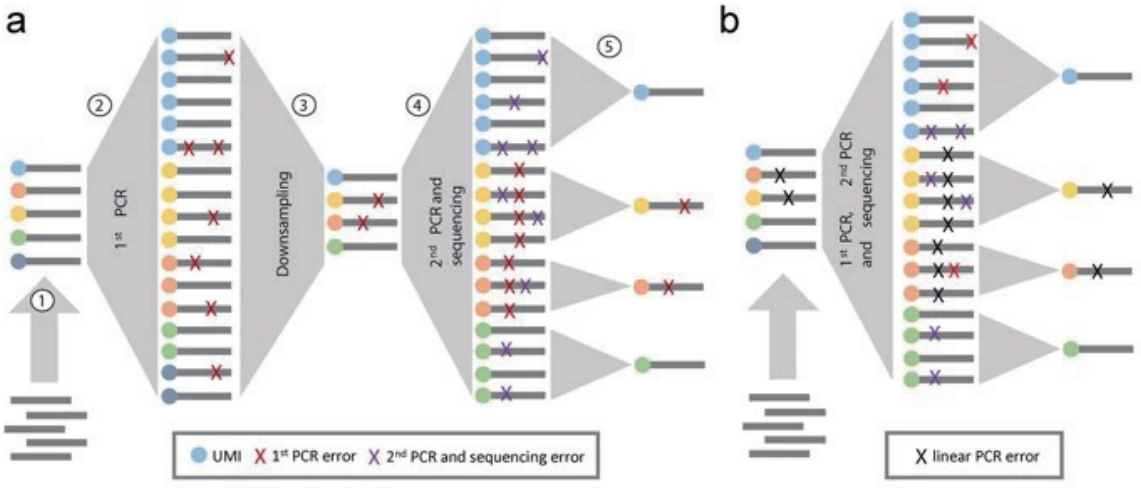

Fig. 2 highlights the impact to errors during PCR cycles.
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This is not to discount the significant impact and importance of this technique. As noted earlier, the advent of PCR has been key in the development of countless technologies including genotyping, paternity testing, forensics, detection of mutations, sequencing, etc.

Where PCR has not been viable, scientists have often used cell-based cloning strategies. Cell-based cloning techniques have been developed over the last 50 years and generally result in more accurate DNA amplification and the ability to handle longer lengths (up to 1 Mbp using chromosomal vectors). Numerous variations of cell-based cloning have been developed, though they generally follow a standard approach.

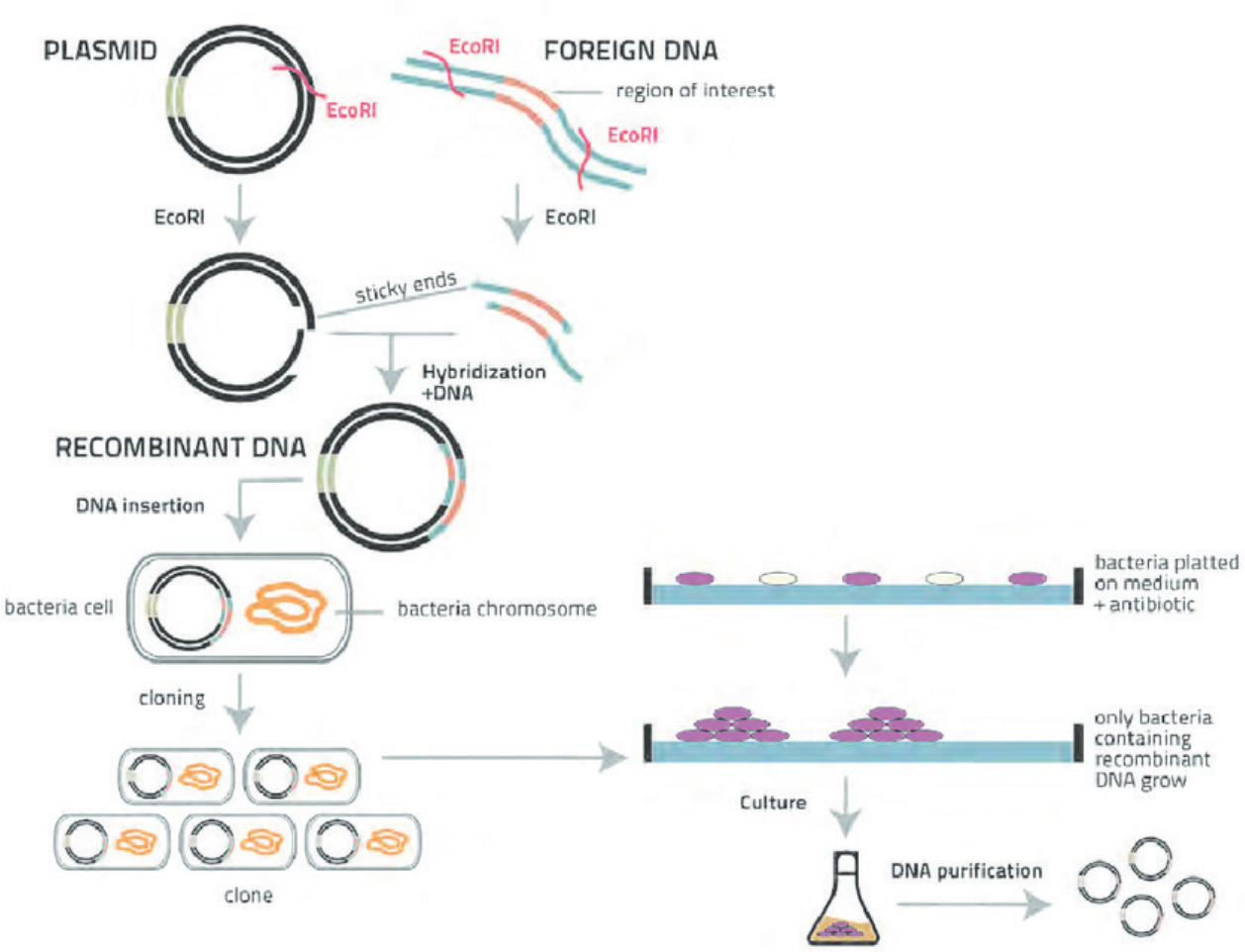

Figure 3 Cell Based Cloning Example
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Each of these steps can take a significant amount of time to optimize and require scientific expertise to assess, preventing the automation of much of this process. In cloning specifically, there are often limitations based on size of plasmid and target genes to be amplified (e.g., genes encoding for toxic proteins are difficult, if not impossible to clone using E. coli). Further, when using these plasmids in the context of drug manufacturing, the purification process is critical and needs to be well characterized to ensure that all cell-based byproducts are removed to ensure the biosafety of end product(s) so there is not an increased risk for patients. Where speed and efficiency is of the essence, each of these steps ultimately results in delayed patient care and economic burden.

As we look forward, novel strides in synthetic biology, third generation sequencing and various other fields demanding larger and larger DNA constructs will surely continue to grow. Synthetic biology specifically requires flexible and high-performance technologies for the synthesis of genome scale DNA. We are seeing progressive and innovative companies such as Ginkgo Bioworks focused on designing and building entirely customized organisms, applications of which have the potential to impact all industries where biology plays a part (e.g., agriculture, space exploration, global warming). Accurately engineering genetic material is a critical success factor to getting the right medicines to the right patients; it is also becoming just as important across industries.

4. Addressing the Challenge Technologies

We should expect that there will continue to be incremental improvements to PCR and cell-based cloning, however, major improvements in time and cost efficiency as well as issues in scalability will require a major re-think. Imagine, if you will, a new approach that accurately, consistently, and quickly amplifies up to chromosome size DNA and drastically reduces the development timeline; not by improving on these existing technologies, but through providing a fundamentally different approach.

In 2019, OriCiro was founded to address these challenges and aims to revolutionize our approach to genetic engineering. By leveraging the "tools" used within E. coli during the DNA replication process, they have successfully developed a rapid DNA amplification platform that overcomes challenges associated with PCR and cloning techniques to enable rapid advancements in synthetic biology and gene therapies. Having isolated and reconstituted the critical DNA replication components used in E. coli, they have effectively created a cell-free cloning technology that results in circular DNA amplification that is freed of limitations such as sequences with high-GC content and cytotoxic sequences. As with cell-based cloning, amplification takes place at a constant temperature eliminating the need for thermal cyclers.

	PCR	*E. coli* cloning	OriCiro® Technology
DNA size	<30kbp	<50kbp	<50kbp *1
Operation	Need to design thermocyclic process	Cumbersome process Involving techniques that require several days to perform	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) *2
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

*1 OriCiro® Research Service enable to amplify up to 200kbp size
*2 Will be enhanced to match that of E.coli cloning by introducing the error-repair mechanism.

Figure 4 How DNA amplification technologies compare and highlights the benefits offered by OriCiro's platform.

An amplification technology that does not require thermal cycling, nor the setup of a lab to support cells and the corresponding filtration steps, lending itself to high-throughput automation opportunities to drastically speed up the development process.

In contrast to the standard E. coli cloning process, OriCiro's cell-free approach, has the potential to revolutionize this space by drastically reducing costs and increasing throughput, while also improving the safety profile of the end-product using a simpler, more efficient process. Fig 6

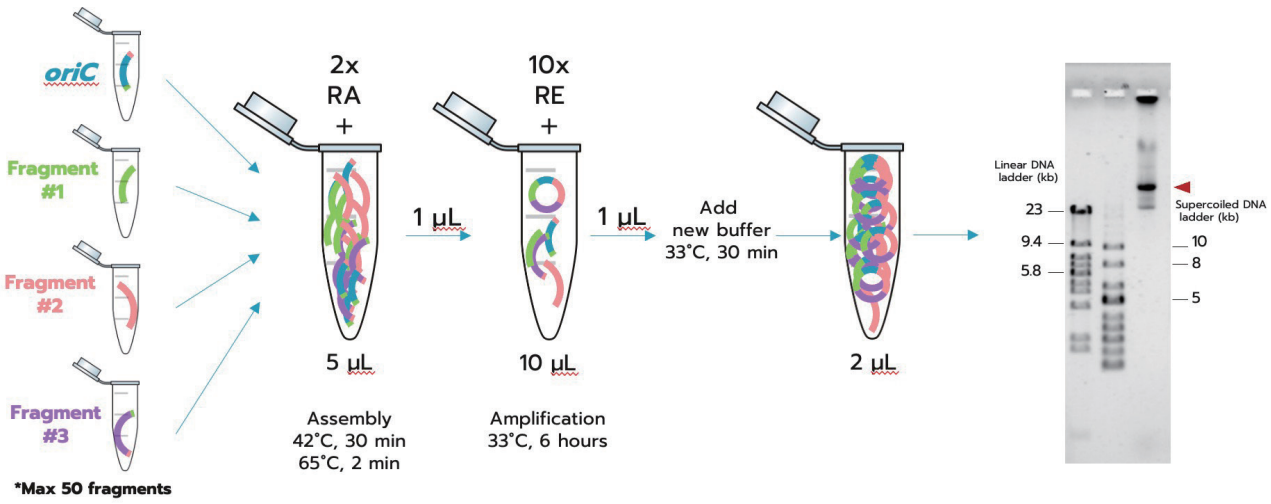

Figure 5 OriCiro's simplifies approach

Assumption – Process enhancement

	E. coli fermentation
Cell Banking	Application of Cartagena Protocol for E. coli
	MCB and WCB construction
	Quality Inspection & Test Production
Up stream	Multiple step of pre-culture, fermentation, cell harvest and alkaline lysis.
Down stream	Ultrafiltration (Buffer exchange)
	Anion exchange chromatography (Endotoxin removal)
	Size exclusion chromatography (RNA removal)
	Hydrophobic interaction chromatography (Host cell protein removal)
	Ultrafiltration & filling
QA/QC	pDNA quality inspection

	OriCiro
Plasmid Banking	**Plasmid construction by cell-free**
	Quality Inspection & Test Production
Up stream	**Single step** of isothermal incubation
Down stream	Size exclusion chromatography (RNA removal)
	Plasmid selection chromatography (Open circular DNA removal)
	Ultrafiltration & filling
QA/QC	pDNA quality inspection

Figure 6 Comparison of methods

To further illustrate the potential for this new capability, we've illustrated the difference in process between these two approaches in Fig 7.

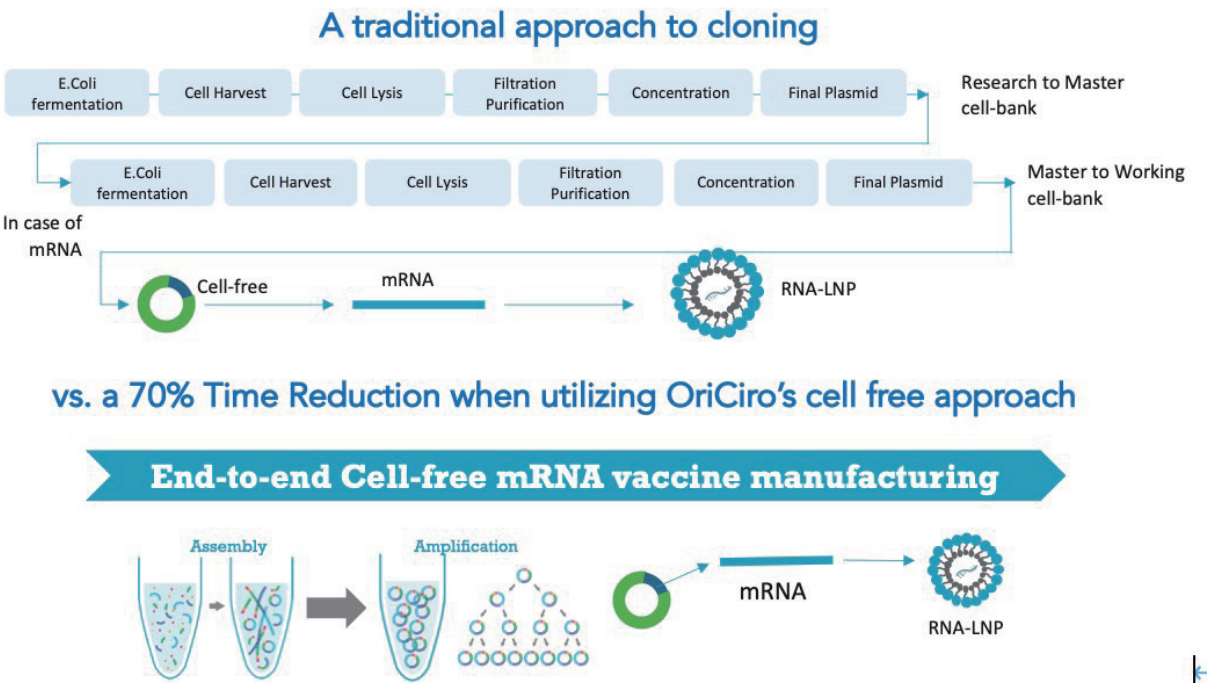

Figure 7 Process optimization

5. Conclusion

DNA is the central building block that enables the production of next-generation gene and cell therapies. Where speed to market is critical, optimizing the approach to designing, constructing, and producing high quality plasmids with minimal error rates and high levels of purity remain critical success factors.

Focusing on cell-free DNA technology, OriCiro is eliminating the time-consuming process of growing and purifying DNA from cellular vehicles (e.g., E. Coli) while still maintaining the fidelity of amplification for which this approach is often used. By having isolated the components necessary for chromosomal replication in E. coli, they have enabled large sequence length amplification (>50kbp) in hours instead of days and have eliminated the challenges that are posed by cytotoxic products that ultimately cause cloning approaches to fail.

OriCiro has also eliminated the challenges arising from amplifying high GC-rich regions that pose challenges in PCR cycling while still enabling amplification that does not require the purification processes of traditional methods. Like PCR, however, the cell-free approach remains a viable option for high-throughput 96-well experimentation.

Whether the need is for early design and construction of complex DNA templates or the manufacturing of nucleic acid based therapeutics, the systems OriCiro Genomics offers can greatly increase throughput and potential as well as streamline and consolidate many steps of the manufacturing process. Thinking differently might accelerate development, reduce costs and equipment needed for experimentation, and increase the safety profile of the next generation of cell and gene therapies; if you are interested in finding out more about OriCiro's capabilities, please contact:

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20/15 Visioneers

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OriCiro's Cell-Free cloning Services

Reagent kit

Cell-Free Cloning System

- Assemble to Amplify maximum 50kb DNA with in a day
- 10,000 times more accurate amplification vs PCR (Taq polymerase)
- Any DNA sequence including toxic,GC-rich,AT-rich and repeat seaquence can be amplified

Research Service

- Amplification of long DNA (up to 200kb) and sequences difficult to amplify by PCR
- Efficient cloning of any DNA sequence including cytotoxic and repeat GC-rich
- Making a library of Plasmid DNA for transfection
- Phage/Virus genome production
- DNA repair templates for gene editing in T cells therapy

Quality Management

- ISO 9001:2015 certified.

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