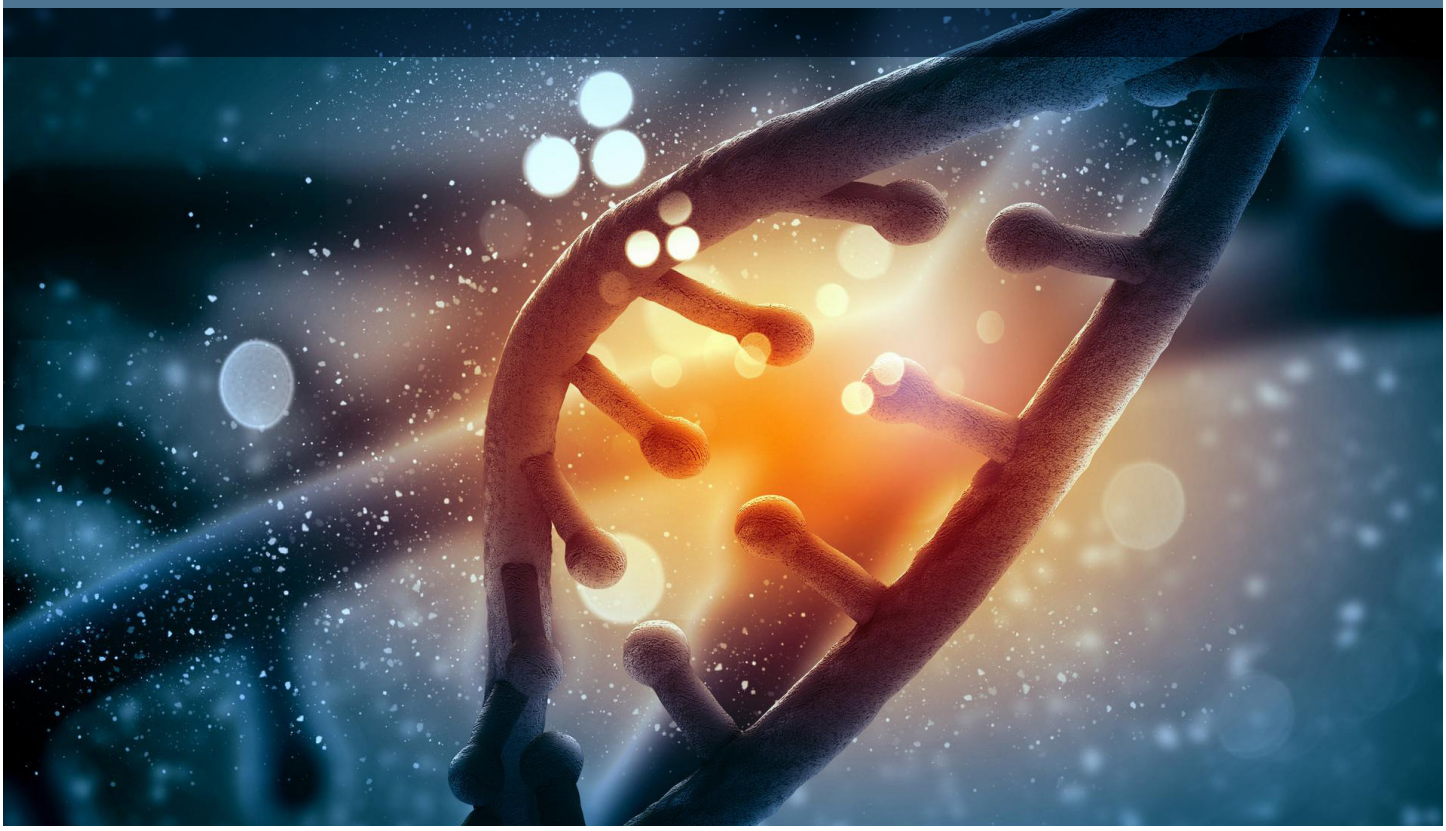
Agilent

Trusted Answers

SureVector Next-Generation Cloning Kits

Citation Reference Guide



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Your Vision. Your Vectors.

The Agilent SureVector Cloning system is a state-of-the-art modular vector solution that harnesses the power of synthetic biology to provide quick, user-friendly customization of cloning and expression vectors. The SureVector Cloning system offers a unique set of standard modules such as selectable markers, origins of replication, expansion fragments, and promoters. These modules can easily be assembled into an expansive range of custom vectors, all supported by the validated SureVector assembly system.

Fast, flexible, and reliable

Rapid custom vector generation: Go from design to vector in less than a day, compared to four weeks for other custom vector services.

Reliable and precise assembly: SureVector modules are extensively validated to ensure they can be easily interchanged without loss of functionality.

More flexible than traditional systems: As experimental needs evolve, quickly assemble new vectors in the lab using the same established workflow rather than ordering new ones.

Control your experiments: Take control of your experiments by optimizing your own DNA assembly, rather than troubleshoot your service provider's.

How does SureVector work?

A single SureVector Cloning kit contains a set of DNA fragments comprising the functional components of most cloning and expression vectors. The cloning method in this kit enables multiple DNA modules to be assembled into a recombinant plasmid that contains the target gene of interest (GOI) and any additional features that need to be expressed. A single reaction using the proprietary SureVector enzymes allows assembly of up to seven fragments within a circularized plasmid. The kit includes all the buffers, enzymes, and nucleotides required to generate plasmids in less than 20 minutes of hands-on time.

The SureVector Core kit provides functionality in *E.coli*, yeast, and mammalian cells. Additionally, the SureVector product line offers a wide variety of fragments to expand functionality in *E.coli,* yeast, and mammalian cells.

Technical Overview

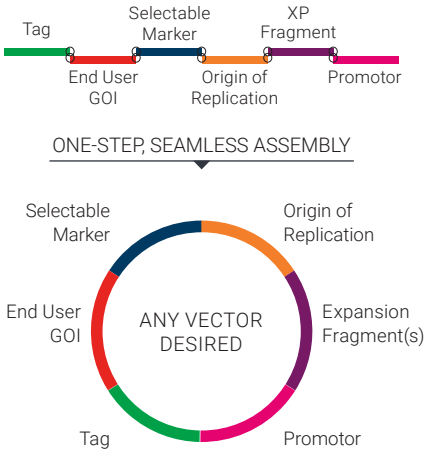

Figure 1. Components of the SureVector core kit are shown above. Combinational shuffling of individual components leads to a total of 216 possible vectors from the core kit alone! The number of possible SureVector constructs increases to over 700,000 with the addition of the fragments available in the expansion kit.

SureVector Core Kit

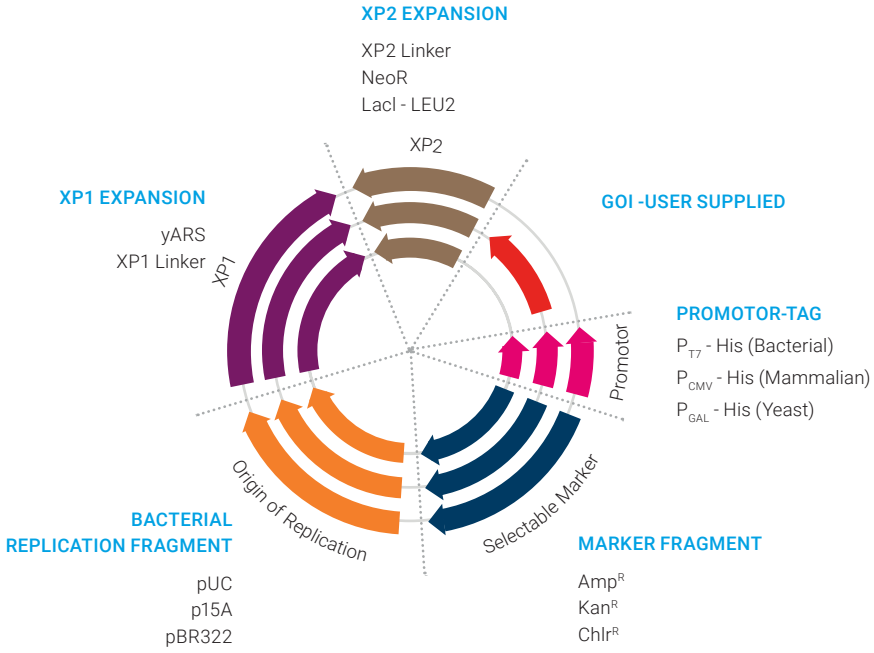

Figure 2. Choose the fragments you want, combine the reagents in a tube, and run the assembly protocol. Additional steps both up and downstream in your workflow remain unchanged compared to existing cloning technologies.

The compendium

In this compendium, we have selected five peer-reviewed publications that feature a variety of applications utilizing the SureVector Cloning system. These papers highlight the versatility and functionality of the SureVector Cloning kits and illustrate how they helped researchers advance their studies.

Seamless assembly of DNA parts into functional devices and higher-order multidevice systems

PLoS ONE, 2019

Authors

Jeffrey Carl Braman, Peter J. Sheffield.

Abstract

A new method is introduced allowing seamless assembly of independent, functionally tested, blunt-end double-strand nucleic acid parts (DNA fragments not supplied in vectors such as plasmids) into more complex biological devices (e.g. protein expression vectors) and higher-order multidevice systems (e.g. biochemical pathways). Individual parts include bacterial selection markers and origins of replication, promoters useful in a variety of species, transcription terminators, shuttle sequences and a variety of ***N*** and ***C*** terminal solubility/affinity protein tags. Parts are not subjected to pre-assembly manipulation with nucleic acid-modifying enzymes. Instead, they are simply mixed in appropriate predefined combinations and concentrations and then seamlessly linked into devices employing a specialized thermostable enzyme blend. Combinatorial assembly of parts is an inherent time-saving feature of the new method, in contrast to hierarchical binary assembly (***one part at a time***) methods. This feature substantially simplifies and speeds optimization of device and system development. The versatility and functionality of the new method were shown by combinatorial assembly of parts into vector devices, one of which optimally expressed protein from a model gene. Also, a four-enzyme biosynthetic pathway system was re-created by combinatorial construction from parts and devices. Concepts discussed in this paper provide synthetic biologists, chemists and bio-engineers with improved and expanded capability to create novel biological molecules and systems.

Reference

Braman, JC.; Sheffield, PJ. Seamless assembly of DNA parts into functional devices and higher order multi-device systems. PLoS ONE 2019, 14 (6): e0199653. https://doi.org/10.1371/journal.pone.0199653

A rapid combinatorial approach to assembling synthetic prokaryotic and eukaryotic protein expression vectors

Synthetic Biology, 2018

Authors

Rebecca Mullinax, Sarah E. Johns, Denise Rhodes, Vivian Zhang, Nancy McKinney, Katherine A. Felts, Carsten P. Carstens, Peter Sheffield.

Abstract

Vector construction and gene cloning are ubiquitous techniques essential to all fields of biological and medical research. They are the first steps in many endeavors leading to expressing proteins to understand gene function and regulation. However, they can often be rate-limiting, particularly in multigene studies, due to the time and effort required to assemble gene constructs and to identify the optimal constructs for protein expression.

The SureVector system was developed to address this by enabling the rapid and reliable assembly of multiple DNA modules into a recombinant plasmid containing a gene-of-interest (GOI). It harnesses the power of synthetic biology to combine DNA modules from standard parts into a customized vector that expresses proteins in bacterial, mammalian, or yeast cells. The key advantages of the innovative SureVector system include rapid custom vector generation, enhanced flexibility to assemble new vectors quickly as experimental requirements change, and the reliable and precise assembly of fully interchangeable standard DNA modules that retain their functionality. The SureVector system is the only next-generation plasmid assembly technology to guarantee the assembly of multiple functional DNA modules.

Reference

Mullinax, R.; Johns S.E.; Rhodes, D.; et al. A Rapid Combinatorial Approach to Assembling Synthetic Prokaryotic and Eukaryotic Protein Expression Vectors. In Synthetic Biology: Methods and Protocols; Methods in Molecular Biology series, 1772; Humana Press, 2018; pp 457–468.
https://doi.org/10.1007/978-1-4939-7795-6_27

Construction of CRISPR libraries for functional screening

Synthetic Biology, 2018

Authors

Carsten P. Carstens, Katherine A. Felts, Sarah E. Johns.

Abstract

Identification of gene function has been aided by the ability to generate targeted gene knockouts or transcriptional repression using the CRISPR/CAS9 system. Using pooled libraries of guide RNA expression vectors that direct CAS9 to a specific genomic site allows identification of genes that are either enriched or depleted in response to a selection scheme, thus linking the affected gene to the chosen phenotype. The quality of the data generated by the screening is dependent on the quality of the guide RNA delivery library with regard to error rates and especially the evenness of distribution of the guides. Here, we describe a method for constructing complex plasmid libraries based on pooled designed oligomers with high representation and tight distributions. The procedure allows the construction of plasmid libraries of > 60,000 members with a $95^{th}/5^{th}$ percentile ratio of less than 3.5.

Reference

Carstens, C.P.; Felts, K.A.; Johns, S.E. Construction of CRISPR Libraries for Functional Screening. In Synthetic Biology: Methods and Protocols; Methods in Molecular Biology Series, 1772; Humana Press, 2018; pp 139–150.
https://doi.org/10.1007/978-1-4939-7795-6_7

Biotechnology and Bioengineering

Unknown areas of activity of human ribonuclease dicer: a putative deoxyribonuclease activity

Molecules, 2020

Authors

Marta Wojnicka, Agnieszka Szczepanska, Anna Kurzynska-Kokorniak.

Abstract

The Dicer ribonuclease plays a crucial role in the biogenesis of small regulatory RNAs (srRNAs) by processing long double-stranded RNAs and single-stranded hairpin RNA precursors into small interfering RNAs (siRNAs) and microRNAs (miRNAs), respectively. Dicer-generated srRNAs can control gene expression by targeting complementary transcripts and repressing their translation or inducing their cleavage. Human Dicer (hDicer) is a multidomain enzyme comprising a putative helicase domain, a DUF283 domain, platform, a PAZ domain, a connector helix, two RNase III domains (RNase IIIa and RNase IIIb) and a dsRNA-binding domain. Specific, ~20-base pair siRNA or miRNA duplexes with 2 nucleotides (nt) 3'-overhangs are generated by Dicer when an RNA substrate is anchored within the platform-PAZ-connector helix (PPC) region. However, increasing number of reports indicate that in the absence of the PAZ domain, binding of RNA substrates can occur by other Dicer domains. Interestingly, truncated variants of Dicer, lacking the PPC region, have been found to display a DNase activity. Inspired by these findings, we investigated how the lack of the PAZ domain, or the entire PPC region, would influence the cleavage activity of hDicer. Using immunopurified 3xFlag-hDicer produced in human cells and its two variants: one lacking the PAZ domain, and the other lacking the entire PPC region, we show that the PAZ domain deletion variants of hDicer are not able to process a premiRNA substrate, a dsRNA with 2 nt 3'-overhangs, and a blunt-ended dsRNA. However, the PAZ deletion variants exhibit both RNase and DNase activity on short single-stranded RNA and DNAs, respectively. Collectively, our results indicate that when the PAZ domain is absent, other hDicer domains may contribute to substrate binding and in this case, non canonical products can be generated.

Reference

Wojnicka, M.; Szczepanska, A.; Kurzynska-Kokorniak A. Unknown areas of activity of human ribonuclease dicer: a putative deoxyribonuclease activity. Molecules 2020, 25(6), 1414.
https://doi.org/10.3390/molecules25061414

The extraordinary resistance to UV radiations of a manganese superoxide dismutase of *Deinococcus radiodurans* offers promising potentialities in skin care applications

Journal of Biotechnology, 2019

Authors

Gianna Palmieri, Stefania Arciello, Marida Bimonte, Antonietta Carola, Annalisa Tito, Marta Gogliettino, Ennio Cocca, Carmela Fusco, Marco Balestrieri, Maria Gabriella Colucci, Fabio Apone.

Abstract

An overproduction of free radicals or reactive oxygen species, often due to environmental factors, can alter the DNA structure and irreversibly modify proteins and lipids in the living cells. The superoxide anion (O2−) is one of the strongest oxidant molecules produced under oxidative stress conditions but it can be neutralized by the action of the enzymes SuperOxide Dismutases(SODs). In all the human tissues, SODs are essential for the prevention of serious diseases and the protection against oxidative stress damages. In the dermo-cosmetic sector, SODs have found promising applications, but their use is limited due to the loss of activity following the addition of the enzyme in the skin care formulas and the exposure of the skin to UV radiations and heat. Extremophile organisms, which proliferate in extreme physical and/or geochemical conditions, represent a potential source of stable SOD enzymes, able to function even in harsh conditions of high temperature, acid pH and long UV exposures. In the present study we investigated on a Mn-SOD deriving from the extremophilic bacterium *Deinococcus radiodurans* and, after its expression in *E.coli*, the Mn-SOD was characterized in terms of chemical and physical properties. Its extraordinary features in terms of UV resistance prompted us to investigate further about its potential applications in the dermo-cosmetic sector. It was expressed in Solanum lycopersicum (tomato) cell cultures with the main goal of developing a new ingredient, capable of keeping its ROS neutralizing activity once exposed to UV radiations and even when added to skin care formulas.

Reference

Palmieri, G.; Arciello, S.; Bimonte, M.; Carola, A.; et al. The extraordinary resistance to UV radiations of a manganese superoxide dismutase of *Deinococcus radiodurans* offers promising potentialities in skin care applications. Journal of Biotechnology 2019, 302, 101–111.
https://doi.org/10.1016/j.jbiotec.2019.07.002

SureVector Ordering Information

	E. coli	Mammalian	Yeast
Promoters	T7 (G7515A-B, G7518B-E)	CMV (G7516A-B)	GAL1 (G7517A-B)
	Tac (G7515A-B, G7518B-C)	SV40 (G7516A-B)	CUP1 (G7517A-B)
	Rhamnose (G7515A-B, G7518C)	EF-1α (G7516A-B)	ADH1 (G7517A-B)
Tags	CBP (G7515A-B, G7518E)	3xFLAG (G7516A-B)	3xFLAG (G7517A-B)
	DsbA (N-term only) (G7515A)	GFP (G7516A-B)	GFP (G7517A-B)
	GST (N-term only) (G7515A, G7518D)	3xHA (G7516A-B)	3xHA (G7517A-B)
	HA (C-term only) (G7515B)	6xHis (G7516A-B)	6xHis (G7517A-B)
	6xHis (G7515A-B, G7518B-C)	c-Myc (G7516A-B)	c-Myc (G7517A-B)
	MBP (N-term only) (G7515A, G7518D)	SBP (G7516A-B)	SBP (G7517A-B)
	c-Myc (C-term only) (G7515B)		
	SBP (G7515A-B, G7518D-E)		
	Thioredoxin (C-term only (G7515B, G7518E)		
Bacterial Selection	AmpR (G7514A, G7518A-E)	AmpR (G7514A, G7518A-E)	AmpR (G7514A, G7518A-E)
	CamR (G7514A, G7518A)	CamR (G7514A, G7518A)	CamR (G7514A, G7518A)
	KanR (G7514A, G7518A)	KanR (G7514A, G7518A)	KanR (G7514A, G7518A)
Bacterial Origins of Replication	pUC (G7514A, G7518A-E)	pUC (G7514A, G7518A-E)	pUC (G7514A, G7518A-E)
	p15A (G7514A)	p15A (G7514A)	p15A (G7514A)
	pBR322 (G7514A)	pBR322 (G7514A)	pBR322 (G7514A)
XP1 Fragments	XP1 (G7514A, G7518A-E)	XP1 (G7514A, G7518A-E)	yARS (G7514A)
			XP1 (G7514A, G7518A-E)
XP2 Fragments	LacI (G7514A, G7518A-E)	Blasticidin (G7516A-B)	URA3 (G7517A-B)
	XP2 (G7514A)	Hygromycin (G7516A-B)	HIS3 (G7517A-B)
		Puromycin (G7516A-B)	Hygromycin (G7517A-B)
		NeoR (G7514A)	LEU2 (G7514A)
		XP2 (G7514A)	XP2 (G7514A)
Promoter-Tag Fusions	T7-HIS6 (G7514A, G7518A-B, G7518D)	CMV-HIS6 (G7514A)	GAL1-HIS6 (G7514A)

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