

The Gateway® Nova pCOLA-2-DEST™ vector carries the COLA replicon from ColA and the kanamycin resistance gene. This DEST vector is NOT compatible with pENTR vectors derived from pDONR221 or other pENTR vectors carrying Kan^R on their backbone. It is compatible with pENTR vectors created using pDONR vectors that do not carry Kan^R on their backbone, such as pDONR223, the pDONR vector used in the human ORFeome project (Rual 2004). pEXPR recombinants created using Gateway Nova pCOLA-2-DEST can be used for coexpression with Gateway Nova pET-DEST vectors carrying the ColE1 replicon and Amp^R gene.

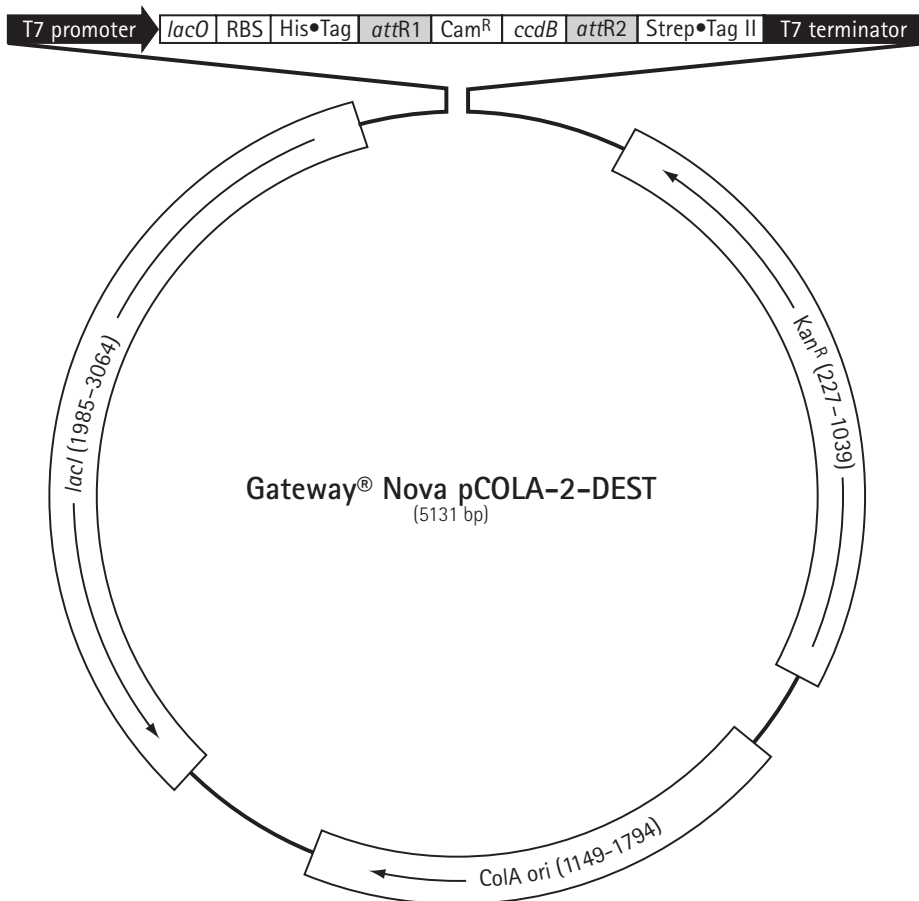
The Gateway Nova pCOLA-2-DEST is designed to create expression clones (pEXPR) that are fused to an N-terminal His•Tag® coding sequence. If the pENTR clone lacks a stop codon and is appropriately designed for a C-terminal fusion, the target gene in the resulting pEXPR clone will also be fused to a C-terminal Strep•Tag® II coding sequence (Skerra 2000). The presence of "gentle elution" tags at both the N- and C-termini is ideal for dual purification strategies designed to isolate full-length fusion proteins (Fiedler 2002).

Fiedler, M. et al. 2002. *Protein Eng.* 15, 931.

Rual, J. F. et. al. 2004. *Genome Res.* 14, 2128.

Skerra, A. and Schmidt, T.G.M. 2000. *Meth. Enzymol.* 326, 271.

Feature	Location	Cat No.
Gateway® Nova pCOLA-2-DEST™ DNA		71855-3
Kan ^R	227-1039	
ColA ori	1149-1794	
<i>lacI</i> coding sequence	1985-3064	
T7 promoter	3188-3204	
T7 transcription start	3205	
His•Tag® coding sequence	3281-3298	
<i>attR1</i>	3302-3426	
Cam ^R	3535-4191	
<i>ccdB</i>	4536-4838	
<i>attR2</i>	4882-5006	
Strep•Tag® II coding sequence	5014-5037	
T7 terminator	5078-5125	



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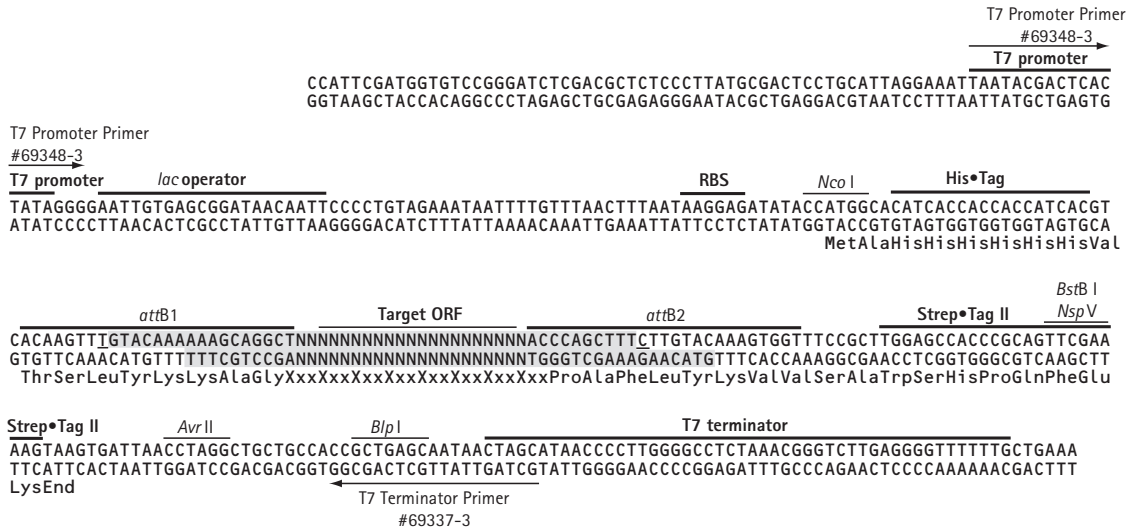
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Sequence of pEXPR derivative



The DNA sequence shown above represents a pEXPR clone derived from a Clonase® LR reaction between a pENTR clone and Gateway® Nova pCOLA-2-DEST™. The Cam^R and *ccdB* genes between the original *attR1* and *attR2* regions of this DEST vector are replaced by the target gene from the pENTR clone during the recombination reaction between the *attR* and *attL* sequences. The open reading frame, fusion tags, and *attB* sites of the resulting pEXPR recombinant are detailed. The shaded regions correspond to those DNA sequences transferred from the entry clone into the DEST vector and the non-shaded regions are derived from the DEST vector. The underlined nucleotides flanking the shaded region correspond to bases 3309 and 4992 of the original Gateway Nova pCOLA-2-DEST vector.

Restriction sites in Gateway® Nova pCOLA-2-DEST™

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
Acell	1	1144	BsrDI	3	2303 2669 3768	NspV	1	5031
AcellII	4	1884 2215 2943 3764	BsrGI	3	3310 4593 4995	PfiMI	3	348 3135 3981
AclI	2	3050 3836	BssHII	2	2299 4243	PinAI	1	52
AgeI	1	52	Bst1107I	1	4284	Ppu10I	2	492 758
AlwNI	1	4528	BstAPI	1	3034	Psp1406I	2	3050 3836
ApaBI	1	3035	BstBI	1	5031	PspOMI	1	2503
Apal	1	2507	BstEII	1	2528	PstI	1	4875
ApaLI	2	2730 4649	BstXI	4	2664 2787 2916 4737	PvuI	1	1741
Asel	5	218 407 1968 2027 3188	BstZ17I	1	4284	PvuII	3	2021 2114 3649
AvrII	1	5050	Bsu36I	1	3	RcaI	2	211 1088
BamHI	2	3499 4202	BtrI	3	1394 3299 4654	Sall	1	4877
Bbel	1	2075	BtsI	5	29 662 749 1983 2351	Scal	1	4166
Bbr7I	2	2220 2559	CjeI	10	1416 1603 2381 2577 2582	SexAI	1	1649
BbsI	2	2225 2564			2846 2922 4512 4671 5045	SfoI	1	2073
BbvCI	1	4474	CjePI	11	529 781 1538 1603 2431	SmaI	2	734 4620
Bcgl	1	2390			2577 2846 3259 3299 4671	SphI	1	1140
BciVI	4	214 1090 1167 2258	ClaI	1	915	SrfI	1	4620
BclI	1	2696	DraI	2	3669 4008	SspI	3	683 1057 4061
BfrBI	2	494 760	DrdI	1	123	TaqII	2	356 1917
BioHII	1	4875	EagI	1	3429	Tth111I	1	123
BlpI	1	5068	EarI	4	792 1048 1818 3093	VspI	5	218 407 1968 2027 3188
BmgBI	3	1394 3299 4654	Ecil	2	1216 2925	XbaI	1	1790
Bmrl	3	1912 2552 2789	Eco57I	1	1850	XcmI	3	2325 2343 2859
Bpml	3	2389 2878 3871	EcoNI	3	695 1593 3178	XmaI	2	732 4618
Bpu10I	3	588 3522 4474	EcoRI	1	3749	XmnI	1	219
BpuEI	5	1 1510 1738 1902 4348	EcoRV	1	1566			
BsaBI	1	4633	EheI	1	2073	Enzymes that do not cut:		
Bsal	1	131	FalI	1	157	AarI	AatII	Acc65I
BsaXI	2	141 2042	HpaI	1	2208	AfeI	AfilI	AhdI
BseYI	3	1425 2176 2311	KasI	1	2071	AleI	Alol	Ascl
BsgI	2	2665 2865	MluI	2	2710 4196	BglI	BglII	BpII
BsmBI	3	588 2095 3973	MscI	3	4016 4645 4741	BsiWI	BspLU11I	BssSI
BsmI	4	649 726 3742 4149	NarI	1	2072	DraIII	DrallI	Ecl136II
Bsp24I	4	1635 2544 2878 4638	NcoI	1	3274	EcoICRI	FseI	FspAI
BspEI	1	3745	NheI	1	1140	FspI	Fspl	HindIII
BspHI	2	211 1088	NotI	1	3429	MfeI	MunI	NaeI
BspMI	1	4864	NruI	1	951	Pacl	Pcil	Pmel
BsrBI	3	209 1094 3218	Nsil	2	496 762	PsiI	Psrl	RleAI
						RsrII	Sacl	SaclI
						SanDI	SapI	SbfI
						SgrAI	SnaBI	SpeI
						StuI	SunI	Swal
							XhoI	Zral
								Sse232I
								Sse8387I
								Sse8647I