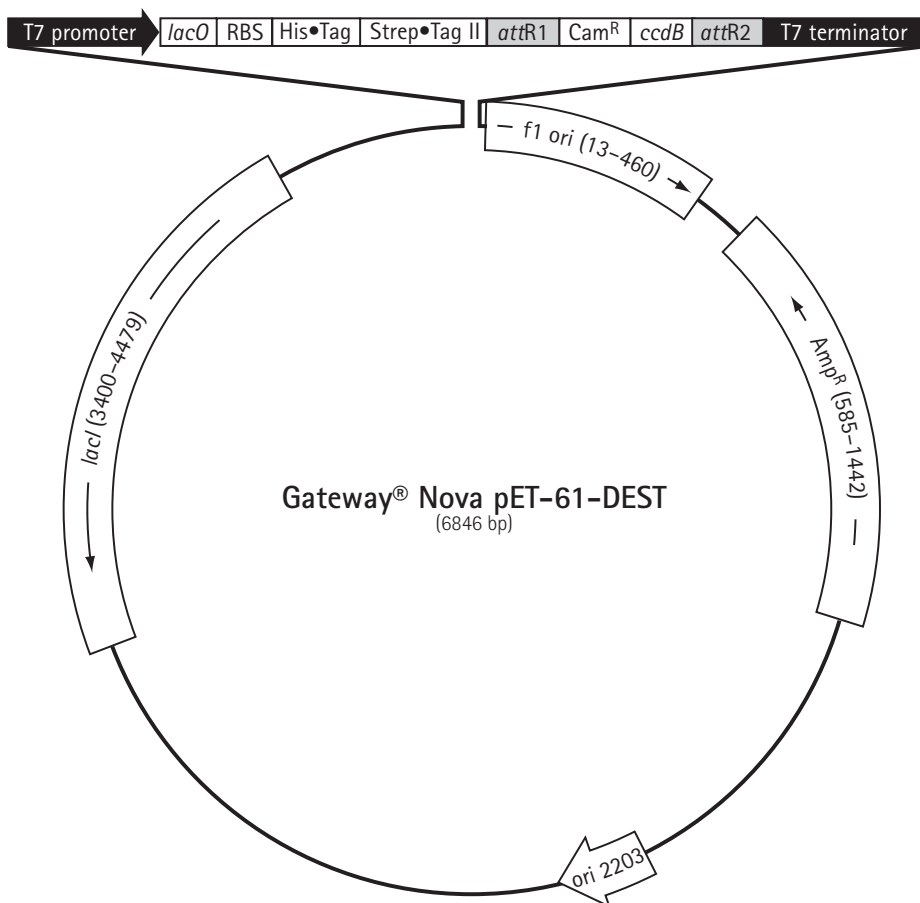


The Gateway® Nova pET-61-DEST™ vector is designed to create expression clones (pEXPR) that are fused to consecutive N-terminal His•Tag® and Strep•Tag® II coding sequences. For appropriately designed inserts lacking a stop codon, a stop codon is provided by the vector after the *attB2* sequence. The consecutive purification tags enable consecutive “gentle” purification procedures.

Feature	Location	Cat No.
Gateway® Nova pET-61-DEST™ DNA		71852-3
f1 origin	13-460	
Amp ^R	585-1442	
pBR322 ori	2203	
lacI coding sequence	3400-4479	
T7 promoter	4870-4886	
T7 transcription start	4887	
His•Tag® coding sequence	4963-4980	
Strep•Tag® II coding sequence	4987-5010	
<i>attR1</i>	5020-5144	
Cam ^R	5253-5909	
<i>ccdB</i>	6254-6556	
<i>attR2</i>	6600-6724	
T7 terminator	6774-6821	



USA and Canada

Tel (800) 526-7319
novatech@novagen.com

France
Freephone
0800 126 461

Germany
Freecall
0800 100 3496

Ireland
Toll Free
1800 409 445

United Kingdom
Freephone
0800 622 935

All other
European Countries
+44 115 943 0840

All Other Countries

Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

techservice@merckbio.eu

www.novagen.com

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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 pET-61-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 5027 and 6710 of the original Gateway Nova pET-61-DEST vector.

Restriction sites in Gateway® Nova pET-61-DEST™

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
AceII	9	399 955 2309 2611 2752	BspEI	3	3072 5463 6841	NotI	1	5147			
AclI	6	3299 3630 4358 5482	BspHI	4	1460 1492 1541 4727	NspV	1	5004			
AfeI	2	456 882 1255 2938 4465	BspLU111	1	2261	PciI	1	2261			
AhdI	1	5554	BspMI	1	6582	PfiMI	2	4550 5699			
Alol	1	3011 4724	BsrBI	4	99 1498 2332 4900	Ppil	3	282 1288 1555			
AlwNI	2	656	BsrDI	5	717 891 3718 4084 5486	Psil	1	371			
ApaBI	1	282	BssHII	3	5028 6311 6713	Psp1406I	6	456 882 1255 2938 4465			
Apal	1	1852 6246	BssIII	2	3714 5961			5554			
ApaLI	5	4450	BssSI	2	1320 2088	PspOMI	1	3918			
Asel	5	3922	Bst1107I	2	2494 6002	PstI	1	6593			
AvrII	1	1323 1947 2447 4145 6367	BstAPI	1	4449	PvuI	1	1026			
BamHI	2	828 3383 3442 4870 6734	BstBI	1	5004	PvuII	4	2674 3436 3529 5367			
BbeI	4	6746	BstEII	1	3943	RcaI	4	1460 1492 1541 4727			
Bbr7I	3	5217 5920	BstXI	4	4079 4202 4331 6455	Sall	1	6595			
BbsI	3	3490 4672 4786 4807	BstZ17I	2	2494 6002	SapI	1	2378			
BbvCI	1	3148 3635 3974	BtrI	1	6372	Scal	2	1136 5884			
BcgI	4	3143 3640 3979	BtsI	4	1056 1076 3398 3766	SfoI	4	3488 4670 4784 4805			
BciVI	3	6192	CjeI	16		SgrAI	1	4806			
BclI	1	1195 2690 3805 5028	CjePI	13		SmaI	1	6338			
BglI	1	1494 2063 3673	ClaI	1	4850	SphI	1	4658			
BglII	1	4111	DraI	5	522 541 1233 5387 5726	SrfI	1	6338			
BioHIII	1	776	DraIII	1	246	SspI	2	451 5779			
BlpI	1	6593	DrdI	3	290 2159 2574	TaqII	5	341 1004 1189 2365 3332			
BmgBI	1	6764	EagI	1	5147	Tth111I	1	2519			
Bmrl	6	6372	EarI	3	1451 2378 4508	VspI	5	828 3383 3442 4870 6734			
		696 2527 3327 3967 4204	Ecil	4	799 2059 2205 4340	XbaI	1	4917			
		4601	Eco47III	2	3011 4724	XcmI	3	3740 3758 4274			
		726 2740 3804 4293 5008	Eco57I	2	1323 1719	XmaI	1	6336			
		5589	EcoNI	1	4593	XmnI	2	1255 2707			
Bpu10I	3	3156 5240 6192	EcoRI	1	5467						
BpuEI	7	1261 1637 1878 2176 3317	EheI	4	3488 4670 4784 4805	Enzymes that do not cut:	AarI	AatII	Acc65I		
		6066 6828	FspI	1	878	AceII	AfiII	AgeI	AleI	Ascl	AsiSI
BsaBI	4	3068 4846 4856 6351	HpaI	1	3623	BaeI	BfrBI	BglII	BpII	BseRI	BsiWI
Bsal	1	717	KasI	4	3486 4668 4782 4803	Bsu36I	Ecl136II	EcoCRI	EcoRV	Fall	FseI
BsaXI	2	282 3457	MluI	2	4125 5914	FspAI	HindIII	KpnI	MfeI	MunI	NdeI
BseYI	3	1957 3591 3726	MscI	3	5734 6363 6459	NheI	NruI	NsiI	Pacl	PinAI	PmeI
BsgI	4	3107 4080 4280 5034	NaeI	2	140 4817	PmlI	Ppu10I	PshAI	Psrl	RleAI	RsrII
BsmBI	3	2620 3510 5691	NarI	4	3487 4669 4783 4804	SacI	SacII	SanDI	Sbfl	Scil	SexAI
BsmI	2	5460 5867	NcoI	1	4956	SfiI	SgfI	SnaBI	SpeI	Sse232I	Sse8387I
Bsp24I	6	1567 1745 3959 4293 4812	NgoAIV	2	138 4815	Sse8647I	StuI	SunI	Swal	XhoI	ZraI
		6356	NgoMIV	2	138 4815						