pRSF and pCDF Vectors

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Description

Coexpression of multiple target genes in *E. coli* is advantageous for studying protein complexes. Coexpression often achieves optimal yield, solubility, and activity and may protect individual subunits form degradation (1–7). The pCDF-1b and pRSF-1b T7 promoter-based expression plasmids feature capabilities to coexpress and express fusion proteins with an N-terminal His•Tag® sequence can be removed after purification by cleavage with the appropriate protease. Each vector contains a T7lac promoter for high-level protein expression, followed by an optimal ribosome binding site, multiple cloning site (MCS) region, and T7 terminator. The MCS regions have restriction sites that facilitate the transfer of inserts from existing pET vector constructs. pRSF-1b carries a replicon derived from RSF1030 (8, 9) and a marker for kanamycin resistance and pCDF-1b carries a replicon derived from CloDF13 (10) and a marker for streptomycin/spectinomycin resistance. These vectors can be used together or with existing pET and Duet vector recombinants with compatible replicons and antibiotic resistance markers.

Components

Expression Systems

- 10 µg pRSF-1b or pCDF-1b DNA
- 0.2 ml BL21 Glycerol Stock
- 0.2 ml BL21(DE3) Glycerol Stock
- 0.2 ml BL21(DE3)pLysS Glycerol Stock

Expression Systems plus Competent Cells

All the components in the Expression Systems plus:

- 0.2 ml NovaBlue Competent Cells
- 0.2 ml BL21(DE3) Competent Cells
- 0.2 ml BL21(DE3)pLysS Competent Cells
- 2 ng Test Plasmid (ampicillin resistant)
- 2 × 2 ml SOC Medium

Storage

Store Competent Cells and Glycerol Stocks at –70°C. Store all other components at –20°C.

pRSF-1b and pCDF-1b

Each plasmid carries a T7*lac* promoter, ribosome binding site, multiple cloning site (MCS) and T7 terminator. The plasmids also have the *lacI* gene to ensure the expression of sufficient *lac* repressor to control basal expression. In each of these two vectors, the MCS has an *Nco* I restriction site at the ATG (Met) translation initiation site that can be used to produce unfused protein and several restriction sites common to most pET vectors (*Bam*H I, *Eco*R I, *Sac* I, *Sal* I, *Hin*d III, and *Not* I) for easy transfer of inserts. The MCS also encodes a six amino acid (aa) amino-terminal His•Tag® sequence for detection and purification and an optional 15 aa carboxy terminal S•Tag™ sequence for detection, purification and quantification. (See Technical Bulletins 392 and 393 for detailed maps of pCDF-1b and pRSF-1b, respectively). Ek/LIC cloning versions of the pCDF and pRSF vectors are also available (pCDF-2 Ek/LIC and pRSF-2 Ek/LIC, see Technical Bulletin 163).

The plasmids differ in antibiotic resistance markers, replicons, and copy numbers. The CloDF13 replicon was combined with *aadA* gene encoding streptomycin and spectinomycin resistance to create pCDF1-b and confers resistance a final concentration of 50 µg/ml of one or 50 µg/ml of each antibiotic (Cat. Nos. 5711 and 567570, respectively). A slightly modified version of the RSF1030 replicon was combined with a kanamycin resistance marker to create pRSF-1b and confers resistance to a final concentration of 30 µg/ml of kanamycin (Cat. No. 420311). Both replicons are compatible with each other and with the ColE1 replicon (pET) and P15A replicon (pACYC) (11).

Vector and Host Strain Compatibility

Vector compatibility

Table 1 summarizes the replicons and compatible replicons used in Novagen *E. coli* expression vectors and strains. Note that the vectors must also have different antibiotic resistance markers for compatibility. The difference in target gene dosage attributed to plasmid copy number between any of the plasmids could be used to influence relative target protein expression levels according to the relative plasmid copy number.

*Note:The combination of a "plain" T7 promoter pET plasmid [i.e., pET-3a-d, pET-20b(+), etc.] with a T7*lac *promoter plasmid is not recommended.*

Table 1 Plasmid replicons and compatibility

Plasmid(s)	Replicon (source)	Copy Number*	Compatible replicons
pET (all), pETDuet™-1	ColE1 (pBR322)	~40	P15A, Mini-F/RK2, CloDF13, RSF1030
pACYCDuet™-1, pLysS, pLysE, pLacI, pRARE	P15A (pACYC184)	10–12	ColE 1, Mini-F/RK2, CloDF13, RSF1030
pCDF-1b, pCDFDuet™-1	CloDF13	~20–40	ColE 1, P15A, RSF1030
pRSF-1b, pRSFDuet™-1	RSF1030	> 100	ColE 1, P15A, CloDF13
pETcoco™ (all)	Mini-F/RK2 (pBeloBAC11, RK2)	1 amplifiable to ~40	ColE 1, P15A

* Copy number was estimated based on gel analysis (12, 13).

Host strain compatibility

For protein production, the pRSF and pCDF recombinants are transferred to an *E. coli* expression host (DE3) containing a chromosomal copy of the gene for T7 RNA polymerase. The choice of an expression host strain is based on strain characteristics and expression vector compatibility. Review the Competent Cells Technical Bulletin (TB009) for complete descriptions of the host strain characteristics. Use the following tables to determine compatibility.

Note: pRSF and pCDF plasmids are not compatible with pETcoco™ vectors.

Table 2 Vector and host strain compatibility

Compatible Vector Combinations				Number of coexpressed target proteins	Compatible expression host strains
Vector 1	**Vector 2**	**Vector 3**	**Vector 4**		
pRSF-1b (Kn^R)	pCDF-1b or pCDFDuet™-1 (Sm^RSp^R)	pACYCDuet™-1 (Cm^R)	pET or pETDuet™-1 (Ap^R)	5–7	Group A
pCDF-1b (Sm^RSp^R)	pRSF-1b or pRSFDuet™-1 (Kn^R)	pACYCDuet-1 (Cm^R)	pET or pETDuet-1 (Ap^R)	5–7	Group A
pRSF-1b (Kn^R)	pCDF-1b or pCDFDuet-1 (Sm^RSp^R)	pET or pETDuet-1 (Ap^R)		3–5	Group B
pCDF-1b (Sm^RSp^R)	pRSF-1b or pRSFDuet-1 (Kn^R)	pET or pETDuet-1 (Ap^R)		3–5	Group B
pRSF-1b (Kn^R)	pET or pETDuet-1 (Ap^R)			2–3	Group B
pRSF-1b (Kn^R)	pCDF-1b or pCDFDuet-1 (Sm^RSp^R)			2–3	Group B
pRSF-1b (Kn^R)	pACYCDuet-1 (Cm^R)			2–3	Group A
pCDF-1b (Sm^RSp^R)	pET or pETDuet-1 (Ap^R)			2–3	Group C
pCDF-1b (Sm^RSp^R)	pRSF-1b or pRSFDuet-1 (Kn^R)			2–3	Group B
pCDF-1b (Sm^RSp^R)	pACYCDuet-1 (Cm^R)			2–3	Group A

Ap: ampicillin/carbenicillin, Kn: kanamycin, Cm: chloramphenicol, Sm: streptomycin, Sp: spectinomycin

Table 3 Strain groups

Group A	Group B	Group C
BL21(DE3)	BL21(DE3)	BL21(DE3)
B834(DE3)	BL21(DE3)pLysS	BL21(DE3)pLysS
BLR(DE3)	B834(DE3)	B834(DE3)
HMS174(DE3)	B834(DE3)pLysS	B834(DE3)pLysS
NovaBlue(DE3)	BLR(DE3)	BLR(DE3)
Tuner™(DE3)	BLR(DE3)pLysS	BLR(DE3)pLysS
	HMS174(DE3)	HMS174(DE3)
	HMS174(DE3)pLysS	HMS174(DE3)pLysS
	NovaBlue(DE3)	NovaBlue(DE3)
	NovaBlue(DE3)pLysS	NovaBlue(DE3)pLysS
	Rosetta™(DE3)	Origami™(DE3)*
	Rosetta(DE3)pLysS	Origami(DE3)pLysS*
	Rosetta 2(DE3)	Origami B(DE3)
	RosettaBlue™(DE3)pLysS	Origami B(DE3)pLysS
	Tuner(DE3)	Rosetta(DE3)
	Tuner(DE3)pLysS	Rosetta(DE3)pLysS
		Rosetta 2(DE3)
		Rosetta-gami™(DE3)*
		Rosetta-gami(DE3)pLysS*
		Rosetta-gami B(DE3)
		Rosetta-gami B(DE3)pLysS
		Tuner(DE3)
		Tuner(DE3)pLysS

* Selection for pCDF vectors require the use of spectinomycin since these strains carry the *rpsL* mutation, conferring streptomycin resistance.

Procedures

Cloning

Cloning procedures, including vector and insert preparation and ligation reactions, are in the pET System Manual (Technical Bulletin 055). Unique restriction sites can be incorporated into an insert by PCR amplification with primers that contain the desired restriction sites (14, 15). Novagen recommends the use of the robust, high fidelity KOD HiFi, Hot Start, or XL DNA polymerases to greatly decrease the probability of generating PCR-based mutations. A high efficiency *recA*$^-$, *endA*$^-$ host strain such as NovaBlue (Cat. No. 70181-3) should be used for cloning.

Analysis of recombinants

Plasmid DNA from candidate recombinants should be verified for the presence of the correct insert and reading frame. Verification should occur prior to cotransformation into an expression host in order to isolate and analyze a single plasmid clone. Several methods available for analysis of transformants include colony PCR, restriction analysis, sequencing, and *in vitro* transcription/translation. These methods are described in the pET System Manual (Technical Bulletin 055).

pCDF-1b and pRSF-1b plasmid DNA can be isolated for transformation into expression hosts, restriction mapping, *in vitro* transcription/translation, and sequence analysis. If provided, use a low-copy number protocol from scientific literature for isolation of pCDF1b DNA and a high-copy number protocol from scientific literature for isolation of pRSF-1b DNA. For *in vitro* transcription/translation analysis, it is important that the template be RNase-free. Plasmid DNA isolated with SpinPrep™ Plasmid Kits or methods from scienfic literature may require an additional phenol:chloroform:isoamyl alcohol extraction to eliminate RNases as described in the pET system Manual.

Primers

The ACYCDuetUP1 Primer (Cat. No. 71178-3) is an appropriate upstream primer for *in vitro* transcription/translation analysis or for colony screening of pRSF-1b and pCDF-1b vectors. The T7 Promoter Primer (Cat. No. 69348-3) is an appropriate upstream primer for colony screening only. The recommended downstream primer for screening the pCDF-1b and pRSF-1b by either *in vitro* transcription/translation or colony screening is the T7 Terminator Primer (Cat. No. 69337-3).

Transformation into expression host strains

Follow the protocols given in TB009 for the transformation of pCDF-1b and pRSF-1b vectors into competent cells. For cotransformations into expression strains using supercoiled plasmid, add 1 µl containing 1–10 ng of each plasmid. Perform the hour outgrowth prior to plating 10–70 µl of the transformation mixture. Note that antibiotics appropriate for both vectors must be included in the plates and medium when cotransforming two vectors. Use the tables on pages 4–5 to determine appropriate expression hosts.

Induction

After the plasmids are established in a λDE3 lysogen, expression of the target DNA can be induced by using medium generated with Overnight Express™ Autoinduction System components (16) or by adding IPTG. Medium produced with Overnight Express components directs high-density cell growth in the absence of induction followed by autoinduction at high cell density (See Technical Bulletin 383 for more information). If using IPTG for induction, a final concentration of 1 mM IPTG is recommended for full induction (T7*lac* promoter) at an OD_{600} of 0.6. Induce for 3 h. Follow the induction protocols in the pET System Manual (TB055).

Induction analysis, protein detection, purification, and quantification

For recommendation and protocols regarding induction analysis and optimization, sample preparation, purification, detection, and quantification, review the pET System Manual (TB055) and the following Technical Bulletins as appropriate.

Coexpression experiments can result in different expression levels of target proteins (17, 18). These differences may be due to unique translation rates or the unequal copy numbers of multiple expression plasmids (13).

Detection/Assay Tools for Fusion Tags

His•Tag® detection	Cat. No.	Size	Technical Bulletin No./Applications
His•Tag Monoclonal Antibody	70796-4 70796-3	3 µg 100 µg	TB283 immunofluorescence, immunoprecipitation, Western blotting
His•Tag AP Western Reagents	70972-3	25 blots	TB283 colorimetric detection
His•Tag AP LumiBlot™ Reagents	70973-3	25 blots	TB283 chemiluminescent detection
His•Tag HRP LumiBlot Reagents	70974-3	25 blots	TB283 chemiluminescent detection
S•Tag™ detection	**Cat.No.**	**Size**	**Technical Bulletin No./Applications**
S-protein AP Conjugate	69598-3	50 µl	TB097 Western blotting
S-protein HRP Conjugate	69047-3	50 µl	TB136 Western blotting
Biotinylated S-protein	69218-3	250 µl	Western blotting
S-protein FITC Conjugate	69060-3	200 µl	TB143 Immunofluorescence
S•Tag AP Western Blot Kit	69213-3	25 blots	TB082 colorimetric detection
S•Tag AP LumiBlot Kit	69099-3	25 blots	TB164 chemiluminescent detection
S•Tag HRP LumiBlot Kit	69058-3	25 blots	TB145 chemiluminescent detection
Quantitative Assay	**Cat. No.**	**Size**	**Technical Bulletin No./Sensitivity**
FRETWorks™ S•Tag Assay Kit	70724-3 70724-4	100 assays 1000 assays	TB251 fluorescent assay, Limit < 1 fmol
S•Tag Rapid Assay Kit	69212-3	100 assays	TB082 Limit 20 fmol
Western Blot Protein Markers	**Cat. No**	**Size**	**Technical Bulletin No./Size Standards**
Perfect Protein™ Western Markers	69959-3	25 lanes	TB102; 15, 25, 35, 50, 75, 100 and 150 kDa
Trail Mix™ Western Markers	70982-3	25 lanes	TB310; 15, 25, 35, 50, 75, 100 and 150 kDa, and 15, 16, 100 kDa prestained markers
Extraction Reagents	**Cat. No.**	**Size**	**Technical Bulletin No./Capacity and Features**
BugBuster® Protein Extraction Reagent	70584-3 70584-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste. Tris-buffered.
BugBuster HT Protein Extraction Reagent	70922-3 70922-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste. Tris-buffered and pre-mixed with Benzonase® Nuclease.
BugBuster 10X Protein Extraction Reagent	70921-3 70921-4 70921-5	10 ml 50 ml 100 ml	TB245 Dilute to 1X with choice of buffer and use 5 ml/g wet cell paste.
BugBuster (primary amine-free) Extraction Reagent	70923-3 70923-4	100 ml 500 ml	TB245 Use 5 ml/g wet cell paste. PIPPS-buffered.
PopCulture® Reagent	71092-3 71092-4 71092-5	15 ml 75 ml 250 ml	TB323 Use 0.1 volume per ml of culture.
rLysozyme™ Solution	71110-3 71110-4 71110-5	300 KU 1200 KU 6000 KU	TB334 and TB323 Use 40 U per 1 ml of culture volume with PopCulture Reagent and 1 KU per 1 ml BugBuster Reagent.
Benzonase Nuclease, Purity > 90%	70746-3 70746-4	10,000 U 2,500 U	TB245, 323, 261 Use 25 U per ml original culture volume with PopCulture and BugBuster Reagent
Lysonase™ Bioprocessing Reagent	71230-3 71230-4 71230-5	0.2 ml 1 ml 5 × 1 ml	TB361. Optimized blend of rLysozyme Solution and Benzonase Nuclease. Use 3 µl per ml lysis buffer
His•Tag® purification	**Cat. No.**	**Size**	**Technical Bulletin No./Capacity and Features**
Ni-NTA His•Bind® Resin	70666-3 70666-4 70666-5	10 ml 25 ml 100 ml	TB273 Capacity is 5–10 mg/ml settled resin
Ni-NTA Superflow™	70691-3 70691-4 70691-5	10 ml 25 ml 100 ml	TB273 Capacity is 5–10 mg/ml settled resin, high flow rates and pressures
Ni-NTA Buffer Kit	70899-3		TB273 All buffers for native purification using Ni-NTA His•Bind and Ni-NTA Superflow resins.

His•Tag® purification	Cat. No.	Size	Technical Bulletin No./Capacity and Features
His•Bind® Resin	69670-3 69670-4 69670-5	10ml 50 ml 100ml	TB054 Capacity is 8 mg/ml settled resin
His•Bind Buffer Kit	69755-3		TB054 All buffers for native purification using His•Bind Resin
His•Bind Columns	70971-3 70971-4	pkg/5 pkg/25	TB054 pre-packed, pre-charged; Capacity is 10 mg per column
His•Mag™ Agarose Beads	71002-3 71002-4	2 ml 10 ml	TB054 magnetic agarose beads, pre-charged, Capacity is 5 mg per ml settled beads
His•Bind Purification Kit	70239-3		TB054 10 ml His•Bind Resin, Buffers and Chromatography Columns
BugBuster® Ni-NTA His•Bind Purification Kit	70751-3		TB273 10 ml Ni-NTA His•Bind Resin, BugBuster, Benzonase and Chromatography Columns
BugBuster His•Bind Purification Kit	70793-3		TB054 10 ml His•Bind Resin and Buffer, BugBuster, Benzonase and Chromatography Columns
PopCulture® His•Mag Purification Kit	71114-3		TB054 Process 40 × 3 ml cultures purifying up to 375 µg per 3 ml culture
RoboPop™ His•Mag Purification Kit	71103-3		TB327 Purify up to 12 mg per 96 wells
RoboPop Ni-NTA His•Bind Kit	71188-3		TB346 Purify up to 96 mg per 96 wells
S•Tag™ purification	**Cat. No.**	**Size**	**Technical Bulletin No./Capacity and Features**
S-protein Agarose	69704-3 69704-4	2 ml 5 × 2 ml	TB087, TB160 Purify up to 1 mg per 2 ml settled resin
S•Tag Thrombin Purification Kit	69232-3		TB087 Purify and cleave up to 1 mg target protein per kit (2 ml settled resin)
S•Tag rEK Purification Kit	69065-3		TB160 Purify and cleave up to 1 mg target protein per kit (2 ml settled resin)

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