invitrogen™

Prokaryotic Expression Guide

NEW

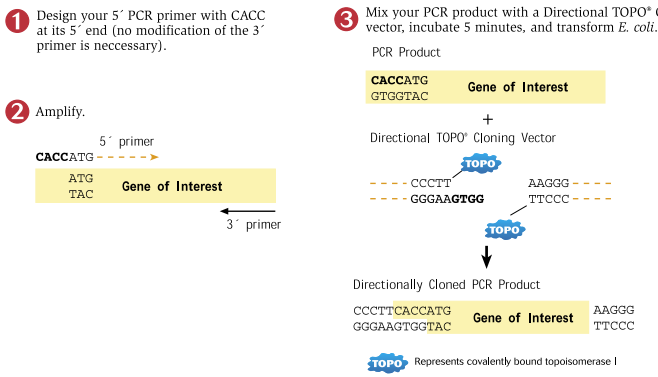
Superior cloning technologies

Directional TOPO® Cloning

The most effective technology available for cloning DNA

In the 5-minute Directional TOPO® Cloning reaction, the enzyme topoisomerase I is used in place of DNA ligase, greatly increasing the success and speed of the cloning reaction. Directional TOPO® Cloning allows the cloning of PCR products in a specific 5´ to 3´ orientation.

Directional TOPO® Cloning procedure

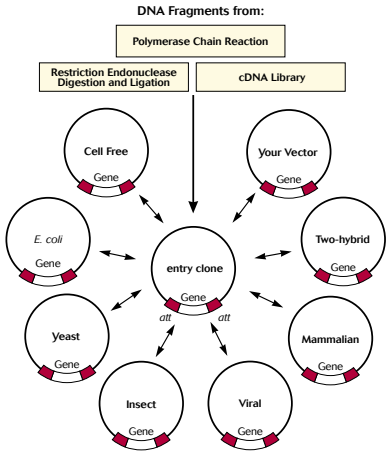


Gateway® Cloning Technology

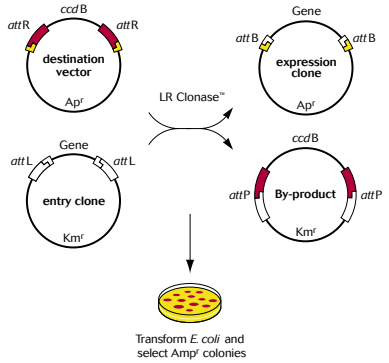
The universal cloning and expression platform

Get rapid, highly efficient cloning of DNA segments across multiple systems and vectors with Gateway® Technology. After generating an entry clone, you can quickly and easily move a gene into different expression vectors.

The flexibility of Gateway® Technology



The Gateway® LR recombination reaction



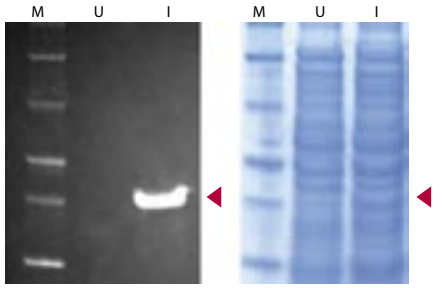
Advanced labeling and detection tool

Lumio™ Technology

Specific, sensitive, and rapid detection of protein expression

The versatile Lumio™ platform uses a small, six-amino acid novel recognition sequence and target-specific fluorescent labeling reagents, enabling you to quickly detect proteins in gels using a standard UV transilluminator or laser based scanner. It eliminates protein staining and time-consuming western blot procedures.

Lumio™ detection of CAT under UV light (left) and Coomassie® stained (right)



M: Fluorescent marker
U: pET161-DEST/CAT uninduced
I: pET161-DEST/CAT Induced

CAT was expressed from the Champion™ pET161-DEST vector. Samples were run on a 12% NuPAGE® Bis-Tris gel with MES running buffer with the Lumio™ Green Detection Reagent and optimized Lumio™ Gel Sample Buffer. The gel was visualized under ultraviolet light (left) and then stained with Coomassie® Blue (right).

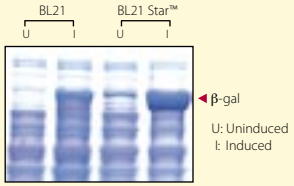
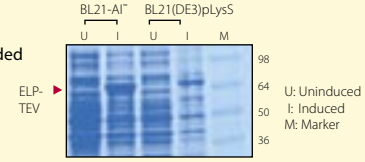
Diverse Champion™ pETvector options for E. *coli* expression

Vector	Cat. no.	Position	Tag	Cleavage Protease	Antibiotic resistance	Benefit
pET100/D-TOPO®	K100-01	N-term	6xHis-Xpress™	EK	Amp	Cleavable detection and purification tag
pET101/D-TOPO®†	K101-01	C-term	V5-6xHis	–	Amp	Detection and purification tag
pET102/D-TOPO®	K102-01	N-term	Thioredoxin	EK	Amp	Cleavable thioredoxin tag enhances protein translation and solubility
		C-term	V5-6xHis	–		Detection and purification tag
pET151/D-TOPO®	K151-01	N-term	6xHis-V5	TEV	Amp	Cleavable thioredoxin tag enhances protein translation and solubility
pET160/GW/D-TOPO®	K160-01	N-term	6xHis-Lumio™	TEV	Amp	Direct fluorescent in-gel detection and cleavable purification tag
pET161/GW/D-TOPO®	K161-01	C-term	Lumio™-6xHis	–	Amp	Direct fluorescent in-gel detection and cleavable purification tag
pET200/D-TOPO®	K200-01	N-term	6xHis-Xpress™	EK	Kan	Cleavable detection and purification tag
pET SUMO	K300-01	N-term	6xHis-SUMO	–	Kan	Highest protein solubility with the SUMO tag; cleavable detection and purification tag; generation of native proteins and peptides
pET-DEST42	12276-010	C-term	V5-6xHis	–	Amp	Detection and purification tag
pET160-DEST	12583-035	N-term	6xHis-Lumio™	TEV	Amp	Direct fluorescent in-gel detection and cleavable purification tag
pET161-DEST	12583-043	C-term	Lumio™-6xHis	–	Amp	Direct fluorescent in-gel detection and purification tag
pET104-DEST	K104-01	N-term	BioEase™	EK	Amp	*in vivo* biotinylation tag for detection and purification with streptavidin

† Useful for native protein expression by inserting a stop codon before the C-terminal fusion sequence.

Improved expression host strains

Recombinant protein expression strains

Product	Cat. no.	Benefits
BL21 Star™(DE3)	C6010-03	Maximize protein production in BL21 *E. coli* by enhancing mRNA stability (see β-gal expression compared in BL21 and BL21 Star™ *E. coli* systems). 
BL21 Star™(DE3)pLysS	C6020-03	Maximize protein production in BL21 *E. coli*. Addition of pLysS lowers basal-level expression for expression of proteins that are slightly growth inhibitive to *E. coli*.
BL21-AI™	C6070-03	Tight regulation and strong expression of proteins from the T7 promoter. Recommended for expression of toxic genes. 

Efficient removal of fusion tags

AcTEV™ Protease: a stabilized TEV protease

Recognition Sequence

Glu Asn Leu Tyr Phe Gln Gly
^TEV/AcTEV cleavage site

Features	Quantity	Cat. no.
• Highly specific cleavage	1,000 units	12575-015
• Active from 4°C to 30°C and from pH 6.0 to pH 8.5	10,000 units	12575-023
• Increased enzyme stability for prolonged activity		
• 6xHis sequence facilitates removal following cleavage		

SUMO (ubiquitin-like protein) Protease

Features	Quantity	Cat. no.
• Highly specific cleavage activity*	100 units	12588-018
• Recognizes tertiary structure of SUMO		
• Active from 2°C to 37°C		
• 6xHis sequence facilitates removal following cleavage		
• Does not leave extra amino acids from protease recognition sequence following cleavage		

**The SUMO Protease can cleave any fusion to the SUMO protein fusion, except fusions that begin with proline.*

EKMax™ Enterokinase, recombinant

Recognition Sequence

Asp Asp Asp Asp Lys
^EK cleavage site

Features	Quantity	Cat. no.
• Highly specific cleavage activity	250 units	E180-01
• Does not leave extra amino acids from protease recognition sequence following cleavage	1,000 units	E180-02
• Uses EK-Away™ Resin for protease removal		

Convenient media and selection

imMedia™ pre-mixed, pre-sterilized media: complete media in 5 minutes (no autoclaving of water or glassware required)

Product	Quantity	Cat. no.
For the preparation of liquid medium*		
imMedia™ Ampicillin Liquid	20 pouches	Q600-20
imMedia™ Kanamycin Liquid	20 pouches	Q610-20
imMedia™ Zeocin™ Liquid	20 pouches	Q620-20
For the preparation of agar plates**		
imMedia™ Ampicillin Agar	20 pouches	Q601-20
imMedia™ Kanamycin Agar	20 pouches	Q611-20
imMedia™ Zeocin™ Agar	20 pouches	Q621-20
For the preparation of agar plates with IPTG and X-gal**		
imMedia™ Ampicillin Blue	20 pouches	Q602-20
imMedia™ Kanamycin Blue	20 pouches	Q612-20

Effective concentrations of imMedia™ components: ampicillin (100 µg/ml), kanamycin (50 µg/ml), Zeocin™ (25 µg/ml), IPTG (100 µg/ml), and X-gal (100 µg/ml).

*Each pouch contains sufficient reagents to prepare 200 ml of media.
**Each pouch contains sufficient reagents to prepare 8-10 100 mm agar plates.

Traditional media

Product	Quantity	Cat. no.
LB Agar (Lennox L Agar)	500 g	22700-025
LB Broth (1X) (liquid)	500 ml	10855-021
LB Broth Base (Lennox Broth Base)	500 g	12780-052
Luria Broth Base (Miller's LB Broth Base)	500 g	12795-027
SELECT Agar	500 g	30391-023
S.O.C. Medium (liquid)	10 X 10 ml	15544-034
Terrific Broth	500 g	22711-022

Media components

Product	Quantity	Cat. no.
X-gal	1 g	15520-018
Bluo-gal (produces darker blue than X-gal)	1 g	15519-028
IPTG	1 g	15529-019

Antibiotics

Product	Quantity	Cat. no.
Ampicillin	200 mg	11593-019
Carbenicillin, disodium salt	5 g	10177-012
Kanamycin sulfate	5 g	11815-024
Kanamycin sulfate (liquid) (100X)	100 ml	15160-054
Streptomycin sulfate	100 g	11860-038
Zeocin™	1 g	R250-01

For more additional products and detailed information, visit www.invitrogen.com or call a Technical Service representative at 800 955 6288, option 2.

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