USER GUIDE

invitrogen

GeneArt™ PerfectMatch TALs and GeneArt™ Precision TALs

TAL effector expression system for genome editing

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Contents

About this guide

Revision history

Revision	Date	Description
B.0	August 2015	Add LRRK2 TAL vector information
A.0	November 2014	New document

Product information

Product description

Introduction

Invitrogen™ GeneArt™ PerfectMatch TALs and GeneArt™ Precision TALs are optimized to deliver transcriptional effectors to cells in a sequence specific manner. These TALs are provided as Gateway™-adapted entry vectors. The sequence is transferred from the entry vector to a destination vector by LR recombination, resulting in high-level expression of the TAL effectors. The GeneArt™ PerfectMatch TALs are also provided as a CMV expression vector, for high-level expression in mammalian cells without an LR recombination step.

Kit contents and storage

Ordering information

GeneArt™ PerfectMatch TALs functional domain	Catalog no.
Truncated N-TAL FokI	816508DE
Truncated N-TAL FokI CMV	816509DE

GeneArt™ Precision TALs functional domain	Catalog no.
Native TAL FokI	816510DE
Truncated TAL FokI	816511DE
Native TAL vp16 activator	816512DE
Native TAL vp64 activator	816514DE
Native TAL MCS	816516DE
Truncated TAL MCS	816517DE
Native TAL KRAB repressor	816518DE

Validated GeneArt™ Precision TAL	Catalog no.
Truncated TAL FokI LRRK2 specific	816012DE

DNA binding domain	Catalog no.
18 Nucleotide Binding Domain (containing your specific TAL)	816010DE
24 Nucleotide Binding Domain (containing your specific TAL)	816011DE

Contents

5 µg of vector DNA, lyophilized. The TAL vector contains a functional domain and an 18 or 24 nucleotide DNA binding domain.

Shipping/storage

All GeneArt™ PerfectMatch TALs and GeneArt™ Precision TALs are shipped at room temperature. Do not store lyophilized DNA for a prolonged time.

Upon receipt, resuspend the vector and store at –20°C.

Resuspend the vector DNA

Add 50 µL of distilled water or 10 mM Tris-HCl (pH 8.0) to the tube containing the vector and incubate for one hour at room temperature.

Resuspend the vector DNA by gently pipetting up and down 5–10 times.

Store the resuspended vector DNA at –20°C.

Antibiotic resistance markers are indicated on each tube label. The standard delivery amount of DNA is 5 µg.

Contents description

TAL effectors

Transcription activator-like (TAL) effector proteins are naturally occurring transcriptional activators secreted by *Xanthamonas spp*. into their plant hosts. They are injected into plant host cells via a Type III secretion system and travel to the nucleus where they bind to, and activate specific promoter sequences that lead to changes that are permissive for bacterial infection (Boch and Bonas, 2010). TAL effector proteins consist of constant N and C terminal domains (containing translocation and nuclear localization/activation signals respectively) flanking a central repeat domain. Each repeat is 34–35 amino acids in length, with two centrally located residues that make up a repeat variable domain (RVD) that dictates the affinity of the repeat for different nucleotide targets. Combination and order of various repeat types define the genomic target site specificity of a particular TAL effector. The deciphering of this TAL effector 'code' led to the engineering of designer TAL effector proteins that function as a vehicle to target functionality of essentially any open region of the chromosomes of plants, bacteria, yeast, flies and mammalian cells (Boch *et al.*, 2009; Moscou and Bogdanove, 2009). Activities such as activators, repressors and nucleases have been demonstrated to be addressable via this powerful system (Li *et al.*, 2011; Scholze and Boch, 2011; Mussolino and Cathomen, 2012). These tools have applications from efficient genomic editing and gene knock out for manipulating the chromosome to modulation of specific promoter activities to allow simple and complex metabolic manipulation in various species of cells.

GeneArt™ PerfectMatch TALs and GeneArt™ Precision TALs

The two versions of TALs available are: GeneArt™ PerfectMatch TALs and GeneArt™ Precision TALs. With GeneArt™PerfectMatch TALs and GeneArt™ Precision TALs the researcher can determine the exact DNA loci they would like to have their functionality delivered to and have specific TAL genes built to perform the function. The researcher will receive a Gateway™-adapted entry vector containing the coding sequence for a TAL nuclease or activator designed to bind a specific 18 or 24 base DNA sequence of choice. The GeneArt™ PerfectMatch TALs are also available in a CMV expression vector.

GeneArt™ PerfectMatch TALs

GeneArt™ PerfectMatch TALs can be designed to target any locus in the genome since there are no restrictions for the 5' base. Previously, target sites for customized TAL effectors required a 5′ T in the target sequences for maximal binding activities. The 5′ T constraint limited the flexibility of TAL effector target sites in the genome and prevented some specific sites in the genome from being targeted. Structure studies suggested the N-terminal domain (NTD) of the TAL effectors, not the central repeat domain, is responsible for the interaction with the 5′ T of the target. We developed our second generation TALs, GeneArt™ PerfectMatch TALs, by mutating the N-terminal domain to reduce its specificity for 5′ T. GeneArt™ PerfectMatch TALs can target DNA sequences with any 5′ base (T, G, C or A) with performance comparable to that of GeneArt™ Precision TALs.

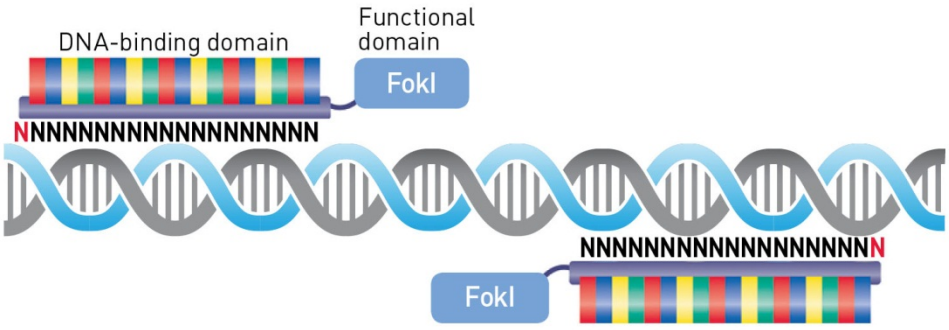
N-TAL FokI and N-TAL FokI CMV

GeneArt™ PerfectMatch TALs contain a truncated TAL engineered with FokI nuclease. The FokI TAL nuclease pair binds to duplex DNA at the target sites designated by the DNA binding domains to cleave the DNA.

There are two versions of GeneArt™ PerfectMatch TALs:

- **N-TAL FokI**: a Gateway™-adapted entry vector which allows easy transfer through a LR recombination reaction to destination vectors designed to facilitate high-level expression of the TAL effectors in your cells of choice.

- **N-TAL FokI CMV**: a CMV expression vector which contains a CMV promoter to drive high-level expression of the TAL in mammalian systems. It can be directly used without extra subcloning.



GeneArt™ Precision TALs

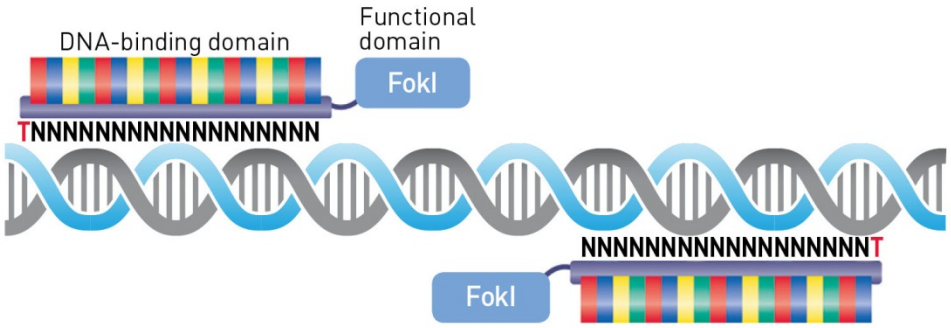
Unlike GeneArt™ PerfectMatch TALs, Precision TALs have a conserved T binding motif at the N-terminus of the TAL effector protein and so require a 5' T for maximal binding activity. GeneArt™ Precision TALs are available as the following Gateway™-adapted entry vectors: TAL FokI, TAL FokI LRRK2 (validated), TAL VP16, TAL VP64, TAL KRAB, and TAL MCS.

TAL FokI and Validated TAL FokI

GeneArt™ Precision TALs engineered with the FokI nuclease can be used for targeting specific genes for silencing. GeneArt™ Precision TAL LRRK2 vectors have been designed and validated for silencing the LRRK2 gene by FokI nuclease.

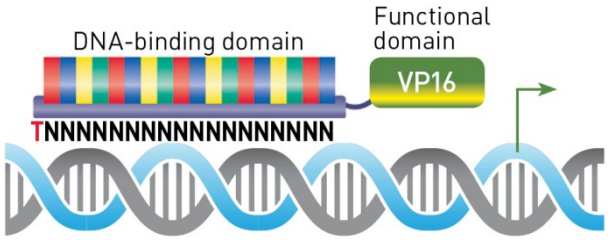
Note: see References for validation details.

FokI is a type IIS restriction endonuclease from *Flavobacterium okeanokoites*, consisting of an N-terminal DNA-binding domain and a non-specific DNA cleavage domain at the C-terminal. A FokI nuclease pair binds to duplex DNA at the target sites designated by the DNA binding domains to cleave the DNA.



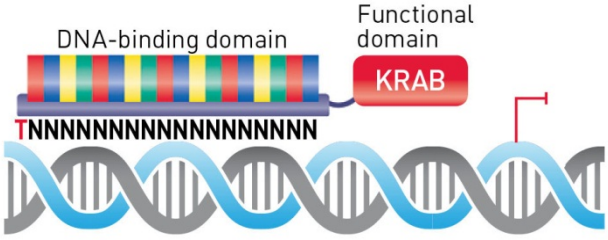
TAL VP16 and TAL VP64

GeneArt™ Precision TALs engineered with the VP16 or VP64 activators can be used to increase the expression level of endogenous or recombinant genes. VP16 is a trans-acting protein originating from the herpes simplex virus that forms a complex with host transcription factors to induce immediate early gene transcription. VP64 is a tetrameric form of the VP16 minimal activation domain.



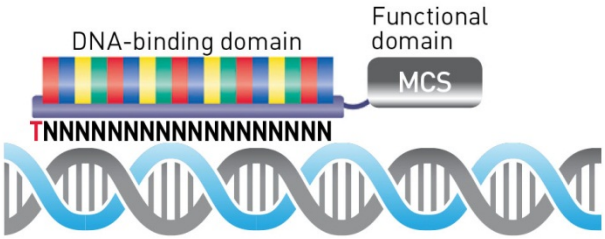
TAL KRAB

GeneArt™ Precision TALs engineered with the KRAB repressor can be used to down-regulate the expression level of endogenous or recombinant genes.



TAL MCS

GeneArt™ Precision TALs that include a multiple cloning site (MCS) allow the user to clone any desired effector domain, and target the protein to any locus within the genome.



Gateway™ Technology

The Gateway™ Technology is a cloning method based on the bacteriophage lambda site-specific recombination system which facilitates the integration of lambda into the *E. coli* chromosome and the switch between the lytic and lysogenic pathways (Ptashne, 1992). In Gateway™ Technology, the components of the lambda recombination system are modified to improve the specificity and efficiency of the system (Bushman *et al.*, 1985).

LR recombination reaction

An LR recombination reaction is performed between the entry clone and the destination vector of choice to generate an expression clone. The LR recombination reaction is mediated by LR Clonase™ II Enzyme Mix, a mixture of the bacteriophage λ Integrase (Int) and Excisionase (Xis) proteins, and the *E. coli* Integration Host Factor (IHF) protein.

Experimental outline

Experimental outline steps

The table below outlines the steps required to express your GeneArt™ PerfectMatch TALs and GeneArt™ Precision TALs in cells.

Step	Action	Page
1	Determine the sequence of the binding site for your TAL effector protein.	8
2	Synthesize TAL sequence and clone into a Gateway™-adapted entry vector of choice to generate an entry clone. Or, clone TAL sequence into the CMV expression vector	—
3	Perform an LR recombination reaction by mixing the entry clone and the appropriate destination vector with Gateway™ LR Clonase™ II Enzyme Mix. **Note:** This step is not required with the CMV vector.	12
4	Transform the recombination reaction into competent *E. coli* cells and select for expression clones.	14
5	Analyze transformants for the presence of insert by restriction enzyme digestion or colony PCR.	15
6	Prepare purified plasmid DNA and transfect the cell line of choice.	16

Create a TAL sequence

GeneArt™ PerfectMatch TALs binding site rules

The following are guidelines and rules for generating the PerfectMatch TAL sequence:

- The GeneArt™ PerfectMatch TALs offering allows the construction of TAL effector functional proteins directed to either 18 or 24 base DNA target sites.
- GeneArt™ PerfectMatch TALs are provided in two types of vectors:

  1) Gateway™-adapted entry vector:

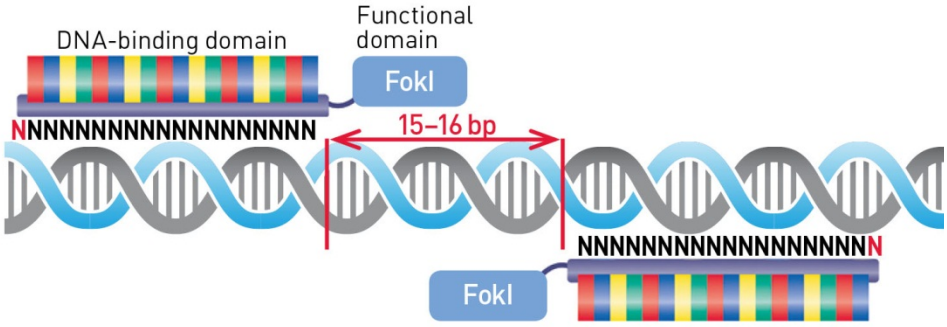
     Gateway™-adapted entry vectors allow easy transfer of target specific TAL domain through a LR recombination reaction into destination vectors designed to facilitate high-level expression of the TAL effectors in your cell line of choice. A Gateway™-adapted destination vector is needed for expression plasmid generation. Choose a destination vector from our Gateway™-adapted vector portfolio.

  2) CMV expression vector (mammalian expression vector):

     The mammalian expression vector contains CMV promoter which drives high-level expression of the TAL in mammalian systems. PerfectMatch TALs provided in this vector can be directly used for expression in mammalian systems without the need for any intermediate sub-cloning steps.

     Optional: PerfectMatch TAL cassette can be transferred directly into your expression vector of choice with the restriction enzymes Not I and Hind III.

- **Each target site sequence is preceded by a 5′ N**. PerfectMatch TAL protein allows binding to a DNA sequence preceded by any DNA base. The letter N represents any base of A, G, C or T. The 5′ N does not count as one of the 18 or 24 bases to be selected for targeting your specific site.
- Design nuclease pairs with a spacing of 13–18 bp between the target sites on opposite strands of the DNA. However, we recommend a spacing of 15–16 bp between the target sites in order to achieve maximal nuclease activity. The target sites can be either 18 or 24 bp in length. Use the following image as a reference for the orientation of the binding domains.



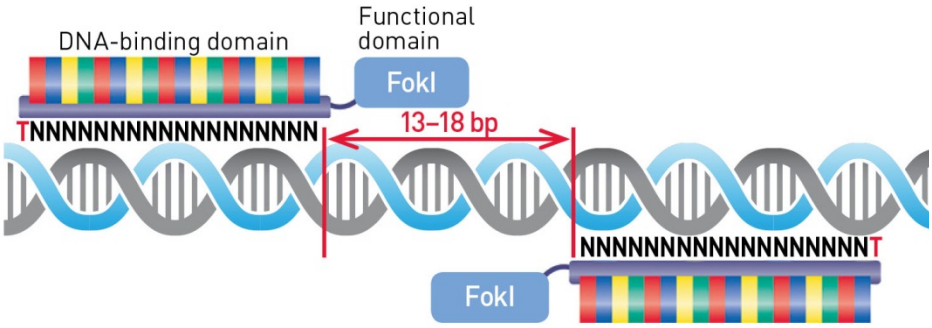
- The contribution of individual binding motifs within the DNA binding domain to TAL effector binding efficiency is thought to differ, since strong and weak binding motifs exist. The A and T binding motifs are thought to fall within the "weak binder" category, while the C and G binding motifs are thought to be "strong binders". **Stretches of more than 5 weak binders should be avoided at the extreme 5′ end of the binding domain (not counting the 5′ N), or if they are not flanked by Cs**. It is recommended to select a TAL effector with a DNA binding domain composed of mixed binding motifs for best results.

- In the context of the living cell, DNA accessibility also determines TAL effector efficiency. Chromatin structure, DNA methylation, and/or proteins bound to the DNA may interfere with TAL binding.

GeneArt™ Precision TALs binding site rules

The following are guidelines and rules for generating the Precision TAL sequence:

- The GeneArt™ Precision TALs offering allows the construction of TAL effector functional proteins directed to either 18 or 24 base DNA target sites.
- **Each target site must be preceded by a 5′ T** because the N-terminus of the TAL effector protein contains a conserved T binding motif. The 5′ T does not count as one of the 18 or 24 bases to be selected for targeting your specific site.
- Nuclease pairs need to be designed with a spacing of 13–18 bp between the target sites on opposite strands of the DNA. Both target sites must be preceded by a 5′ T. The target sites can be either 18 or 24 bp in length. The following image should be used as a reference for the orientation of the binding domains.



- The contribution of individual binding motifs within the DNA binding domain to TAL effector binding efficiency is thought to differ, since strong and weak binding motifs exist. The A and T binding motifs are thought to fall within the "weak binder" category, while the C and G binding motifs are thought to be "strong binders". **Stretches of more than 5 weak binders should be avoided at the extreme 5′ end of the binding domain (not counting the 5′ T), or if they are not flanked by Cs**. It is recommended to select a TAL effector with a DNA binding domain composed of mixed binding motifs for best results.
- In the context of the living cell, DNA accessibility also determines TAL effector efficiency. It is possible that chromatin, DNA methylation, and/or proteins bound to the DNA may interfere with TAL binding.
- Although promoter structure varies, and specific rules regarding design are currently lacking, it is recommended that TAL transcription factors used for transcriptional activation of natural promoters be positioned upstream of the TATA box, or in some cases downstream of the transcriptional start site. Selecting a target site directly over the TATA box, or other known transcription factor binding site is not recommended. Be sure that the natural ATG is present, and that no premature ATG which may interfere with the natural translational start is transcribed.

Create an expression clone

Introduction

To create an expression clone, perform the LR recombination reaction to transfer the gene of interest from the Gateway™-adapted entry vector into your destination vector of choice. We recommend that you review this section and the next section entitled **Perform the LR recombination reaction** (pages 12–13) before proceeding.

Note: This step is not required when using the GeneArt™ PerfectMatch TAL-N-TAL FokI CMV.

Resuspend the vectors

Each destination vector is supplied as 6 µg of lyophilized plasmid. To use, resuspend the destination plasmid in 40 µL of TE Buffer, pH 8.0 to a final concentration of 150 ng/µL.

Note: Destination vectors are supplied as supercoiled plasmids. The linearization of the destination vector is **NOT** required to obtain optimal results for any downstream application.

Propagate the vectors

Entry clone

Propagate and maintain your entry clone using a *rec*A, *end*A *E. coli* strains like TOP10, TOP10F', DH5α, JM109, or equivalent for transformation. Select transformants on LB plates containing 50–100 µg/mL kanamycin. Prepare a glycerol stock of each plasmid for long-term storage.

Destination vector

If you wish to propagate and maintain your destination vectors prior to recombination, we recommend using One Shot™*ccd*B Survival T1^R Chemically Competent *E. coli* (Cat. no. C7510-03) for transformation. The One Shot™*ccd*B Survival T1^R *E. coli* strain is resistant to the toxic effects of the *ccdB* gene and can support the propagation of plasmids containing the *ccd*B gene. To maintain the integrity of the vector, select for transformants in media containing 50–100 µg/mL ampicillin and 15–30 µg/mL chloramphenicol.

IMPORTANT! Do **NOT** use general *E. coli* cloning strains including TOP10 or DH5α for propagation and maintenance of destination vectors as these strains are sensitive to the toxic effects of the *ccdB* gene.

Create an expression clone, continued

Gateway™ recombination reactions

The LR reaction facilitates recombination of an *att*L substrate (entry clone) with an *att*R substrate (destination vector) to create an *att*B-containing expression clone (see diagram below). This reaction is catalyzed by LR Clonase™ II Enzyme Mix.

Recombination region of the expression clone

In the following example, the recombination region of the expression clone resulting from the LR reaction between a TAL entry clone and the Gateway™ pcDNA™-DEST40 destination vector sequence is shown.

Features of the recombination region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the destination vector by recombination.
- Non-shaded regions are derived from the destination vector.
- The underlined nucleotides flanking the shaded region correspond to bases 918 and 2601, respectively, of the Gateway™ pcDNA™-DEST40 destination vector sequence.

Perform the LR recombination reaction

Introduction

Perform an LR recombination reaction between the entry clone and the appropriate destination vector. We recommend that you include a positive control (see below) and a negative control (no LR Clonase™ II Enzyme Mix) in your experiment.

Note: This step is not required when using the GeneArt™ PerfectMatch TAL-N-TAL FokI CMV.

Positive control

The pENTR™-gus plasmid is used as a positive control for LR recombination and expression. Using the pENTR™-gus entry clone in an LR recombination reaction with a destination vector will allow you to generate an expression clone containing the gene encoding β-glucuronidase (*gus*). The pENTR™-gus positive control is supplied with the LR Clonase™ II Enzyme Mix.

LR Clonase™ II Enzyme Mix

The LR Clonase™ II Enzyme Mix is available separately. The LR Clonase™ II Enzyme Mix combines the proprietary enzyme formulation and 5X LR Clonase™ Reaction Buffer (previously supplied as separate components in LR Clonase™ Enzyme Mix) into a single-tube format. Use the protocol provided on page12 to perform the LR recombination reaction using LR Clonase™ II Enzyme Mix.

Note: You may perform the LR recombination reaction using LR Clonase™ Enzyme Mix, if desired. To use LR Clonase™ Enzyme Mix, follow the protocol provided with the product. **Do not** use the protocol for LR Clonase™ II Enzyme Mix provided in this manual as reaction conditions differ.

Materials needed

You should have the following materials on hand before beginning:

- Purified plasmid DNA of your entry clone (50–150 ng/µL in TE, pH 8.0)
- Destination vector (150 ng/µL in TE, pH 8.0)
- LR Clonase™ II Enzyme Mix (Cat. no. 11791-020, 11791-100)
- TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- Proteinase K solution (supplied with the LR Clonase™ II Enzyme Mix)
- pENTR™-gus positive control (supplied with the LR Clonase™ II Enzyme Mix)

Perform the LR recombination reaction, continued

Set up the LR recombination reaction

Follow this procedure to perform the LR reaction between your entry clone and the destination vector. If you want to include a negative control, set up a separate reaction but omit the LR Clonase™ II Enzyme Mix.

1. Add the following components to 1.5 mL microcentrifuge tubes at room temperature and mix. Set up an additional set of reactions for your negative control. You will not add LR Clonase™ II Enzyme Mix to these reactions

Component	Sample	Positive control
Entry clone (50–150 ng/reaction)	1–7 µL	—
Destination vector (150 ng/µL)	1 µL	1 µL
pENTR™-gus (50 ng/µL)	—	2 µL
TE Buffer, pH 8.0	to 8 µL	5 µL

2. Remove the LR Clonase™ II Enzyme Mix from –20°C and thaw on ice (~ 2 minutes).
3. Vortex the LR Clonase™ II Enzyme Mix briefly twice (2 seconds each time).
4. Add 2 µL of LR Clonase™ II Enzyme Mix to each sample or positive control reaction listed above. Mix well by pipetting up and down. Do not add LR Clonase™ II Enzyme Mix to negative control reactions.

   Reminder: Return LR Clonase™ II Enzyme Mix to –20°C immediately after use.
5. Incubate reactions at 25°C for 1 hour.

   Note: For most applications, 1 hour will yield a sufficient number of colonies for analysis. Depending on your needs, the length of the recombination reaction can be extended up to 18 hours. For large plasmids (≥10 kb), longer incubation times will yield more colonies.
6. Add 1 µL of Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Proceed to **Transform competent *E. coli* cells**, next page.

   Note: You may store the LR reaction at –20°C for up to 1 week before transformation, if desired.

Transform competent *E. coli* cells

Introduction

Once you have performed the LR recombination reaction, transform chemically competent *E. coli* with the resulting expression clone.

Materials needed

You should have the following materials on hand before beginning:

- LR recombination reaction (from Step 7, previous page)
- Chemically competent *E. coli* cells
- S.O.C. Medium (warm to room temperature)
- pUC19 control (use as a control for transformation if desired)
- LB plates containing 100 µg/mL ampicillin (two for each transformation; warm at 37°C for 30 minutes)
- 42°C water bath
- 37°C shaking and non-shaking incubator

Transformation procedure

1. For each transformation, aliquot 50 µL of chemically competent *E. coli* cells into a sterile microcentrifuge tube.
2. Add 1 µL of the LR recombination reaction (from **Set up the LR recombination reaction**, Step 7, previous page) into the tube containing 50 µL of competent cells and mix gently. **Do not mix by pipetting up and down.**
3. Incubate on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Immediately transfer the tubes to ice.
6. Add 450 µL of room temperature S.O.C. Medium.
7. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
8. Spread 20 µL and 100 µL from each transformation on a prewarmed selective plate and incubate overnight at 37°C. We generally plate 2 different volumes to ensure that at least 1 plate has well-spaced colonies.
9. An efficient LR recombination reaction should produce >5000 colonies if the entire LR reaction is transformed and plated.

Analyze transformants

Analyze positive clones

1. Pick 5 colonies and culture them overnight in LB or SOB medium containing 100 µg/mL ampicillin.
2. Isolate plasmid DNA using your method of choice. We recommend using the PureLink™HiPure Plasmid MiniPrep Kit (Cat. no. K2100-02) or the PureLink™HQ Mini Plasmid Purification Kit (Cat. no. K2100-01). See Additional products (p30).
3. Analyze the plasmids by restriction analysis to confirm the presence of the insert.

Analyze transformants by PCR

You can also analyze positive transformants using PCR. For PCR primers, use a primer that hybridizes within the vector (*e.g.* T7 Promoter Primer; Catalog no. N560-02) and one that hybridizes within your insert. You will have to determine the amplification conditions. If you are using this technique for the first time, you may want to perform restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template. The protocol below is provided for your convenience. Other protocols are suitable.

Materials needed:

PCR SuperMix High Fidelity (Cat. no. 10790-020)

Appropriate forward and reverse PCR primers (20 µM each)

Note: To avoid PCR errors due to highly repetitive sequences, we recommend designing primers that hybridize to the N-terminal domain of the TAL sequence.

Procedure:

1. For each sample, aliquot 48 µL of PCR SuperMix High Fidelity into a 0.5 mL microcentrifuge tube. Add 1 µL each of the forward and reverse PCR primer.
2. Pick 5 colonies and resuspend them individually in 48 µL of the PCR SuperMix (remember to make a patch plate to preserve the colonies for further analysis).
3. Incubate reaction for 10 minutes at 94°C to lyse cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at +4°C.
6. Visualize by agarose gel electrophoresis.

Confirm the expression clone

The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccdB* gene will be ampicillin- and chloramphenicol-resistant. To check your putative expression clone, test for growth on LB plates containing 30 µg/mL chloramphenicol. A true expression clone will not grow in the presence of chloramphenicol.

Transfection

Introduction

Once you have generated your expression clone, you are ready to transfect the plasmid into the mammalian cell line of choice. You may perform transient transfection experiments or use Geneticin™ selection to generate stable cell lines. The neomycin resistance gene in pcDNA dest 40 Gateway™ vector allows for the selection of stable cell lines using Geneticin™ antibiotic. We recommend that you include a positive control (see below) and a negative control (mock transfection) in your experiment to evaluate your results.

Plasmid preparation

Plasmid DNA for transfection in eukaryotic cells must be very clean and free from contamination with phenol and sodium chloride. Contaminants will kill the cells, and salt may interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.™ MidiPrep Kit (Cat. no. K1910-01), PureLink™ HQ Mini Plasmid Purification Kit (Cat. no. K2100-01), or CsCl gradient centrifugation.

Positive control

If you used the pENTR™-gus control vector in an LR recombination reaction with a destination vector, you can use the resultant expression clone as a positive control for mammalian cell transfection and expression. A successful transfection will result in β-glucuronidase expression that can be detected by western blot or functional assay.

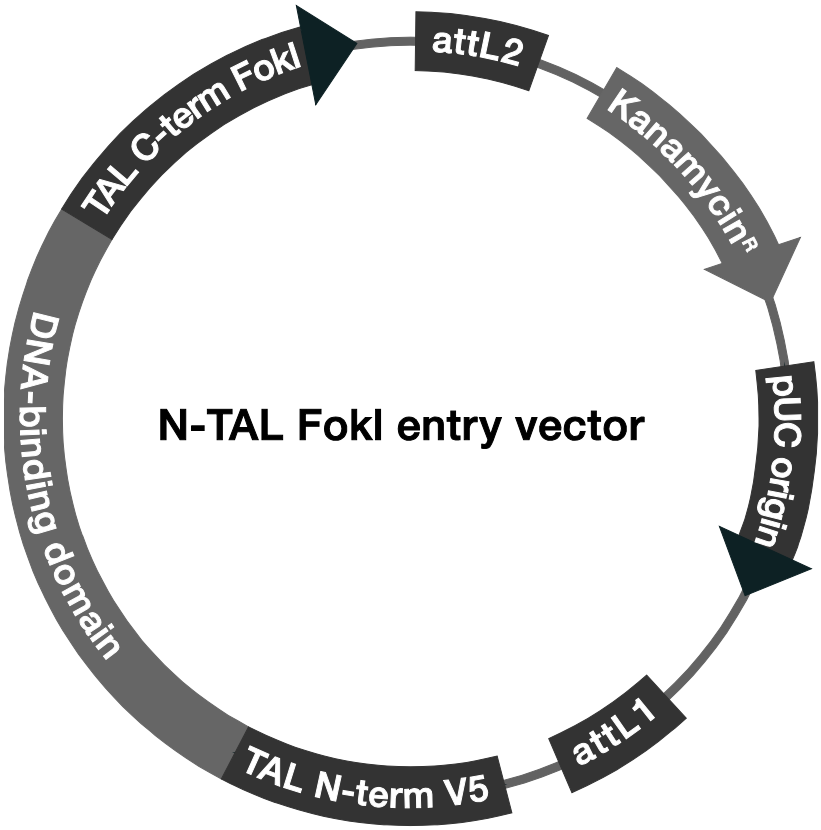
Methods of transfection

We recommend using Lipofectamine™ 3000 Reagent (Catalog no. L3000015) or the transfection method recommended by the supplier of the cell type being used. For more information, refer to **www.thermofisher.com** transfection or contact Technical Support (see page 31).

Map of N-TAL FokI Entry Vector

N-TAL FokI Entry Vector map

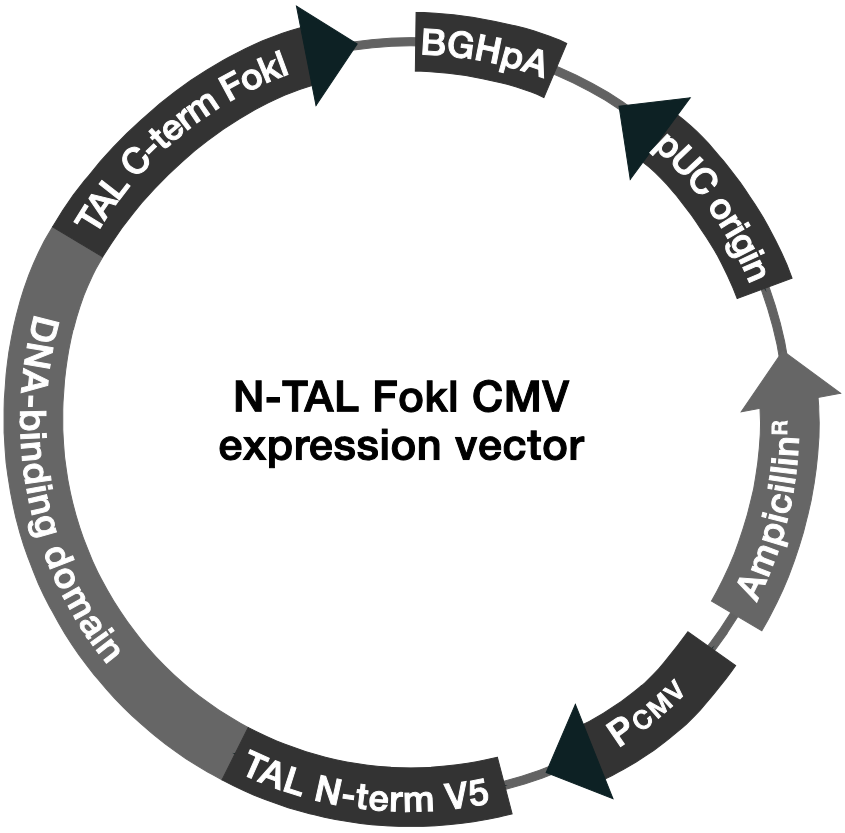
The map below shows the elements of the N-TAL FokI Entry Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Map of N-TAL FokI CMV Expression Vector

N-TAL FokI CMV Expression Vector map

The map below shows the elements of the N-TAL FokI CMV Expression Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Features of GeneArt™ PerfectMatch TAL vectors

Common N-TAL FokI/ FokI CMV vector features

The following elements are found in the GeneArt™ PerfectMatch TALs: N-TAL FokI, and N-TAL FokI CMV.

Feature	Description
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of the recombinant fusion protein by the Anti-V5 antibodies (Southern *et al.*, 1991).
pUC origin	Allows high-copy number replication and growth in *E. coli.*
DNA binding domain	Allows targeting of the TAL effector to specific DNA sequences. DNA repeat variable domain
TAL N-term	N-terminus domain of the TAL containing translocation and nuclear localization signal tag. It contains 3 amino acids mutated from T-TALs.
NLS	Truncated versions of the vector contain the SV40 nuclear localization signal (NLS).
TAL C-term	C-terminus domain of the TAL containing activation domain.
FokI	FokI nuclease domain of the TAL

Specific N-TAL FokI/ FokI CMV vector features

The following features are found in the specific GeneArt™ PerfectMatch TAL vector noted.

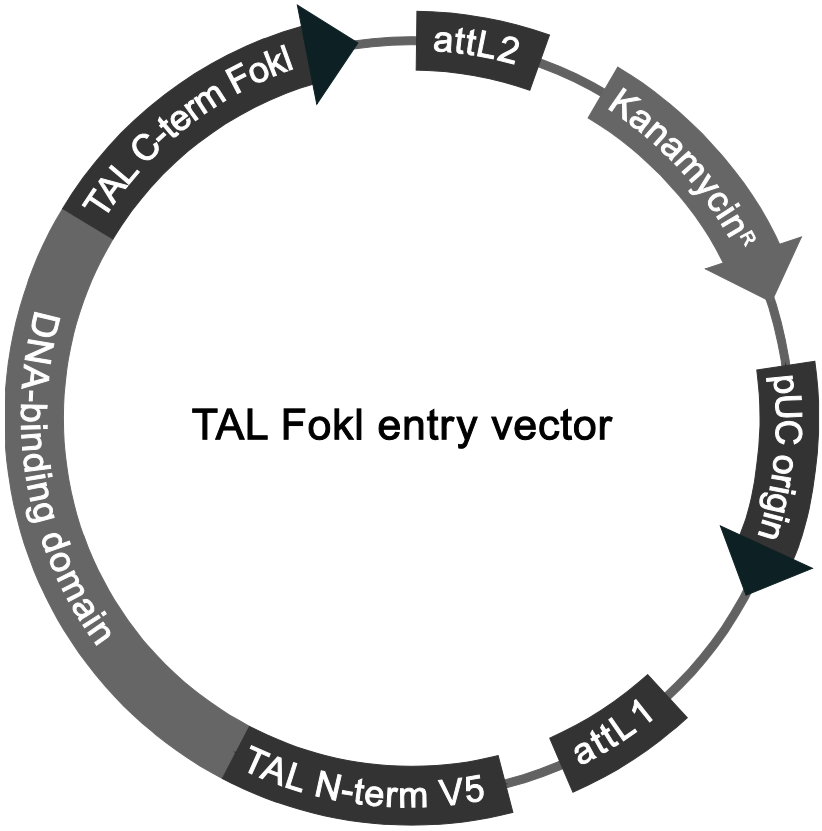
Vector	Feature	Description
N-TAL FokI	*rrn*B T1 and T2 transcription terminators	Protects the cloned gene from expression by vector-encoded promoters, thereby reducing possible toxicity (Orosz et al., 1991).
N-TAL FokI	M13 Forward (–20) priming site	Allows sequencing in the sense orientation.
N-TAL FokI	*att*L1 and *att*L2 sites	Allows recombinational cloning of the gene of interest from an entry clone (Landy, 1989).
N-TAL FokI	Kanamycin resistance gene	Allows selection of the plasmid in *E. coli*.
N-TAL FokI CMV	P_{CMV}, Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows efficient, high-level expression of TAL-FokI protein.
N-TAL FokI CMV	BGHpA, Bovine growth hormone (BGH) polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA.
N-TAL FokI CMV	Ampicillin resistance gene (C)	Allows selection of the plasmid in *E. coli*.
N-TAL FokI CMV	T7 promoter/priming site	Allows *in vitro* transcription in the sense orientation and sequencing through the insert.

C= complementary strand

Map of TAL FokI Entry Vector

TAL FokI Entry Vector map

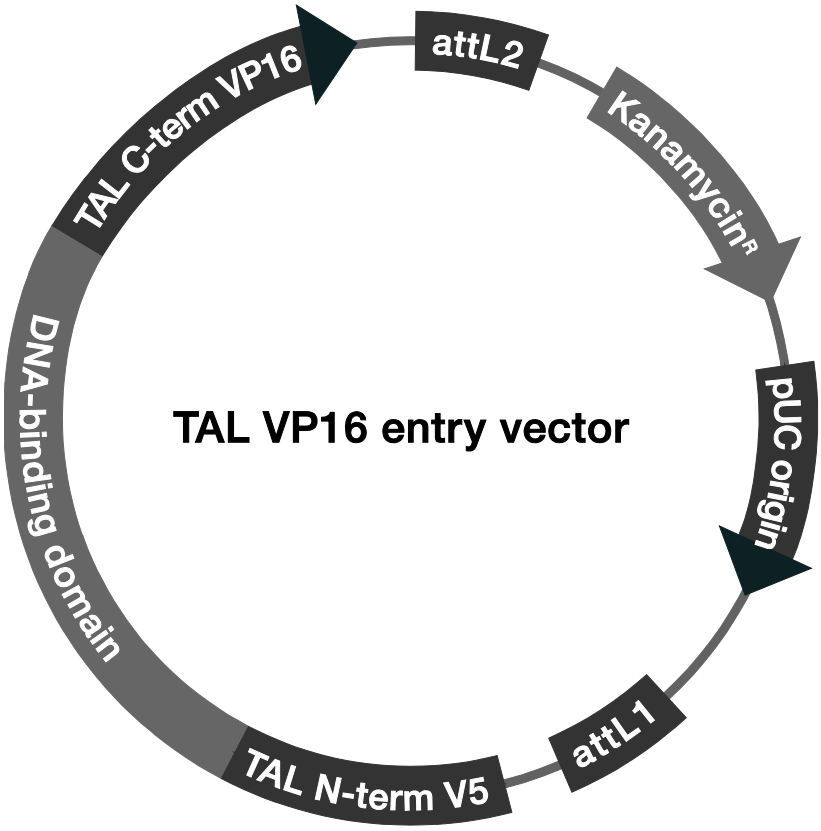
The map below shows the elements of the TAL FokI Entry Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Map of TAL vp16 Entry Vector

TAL vp16 Entry Vector map

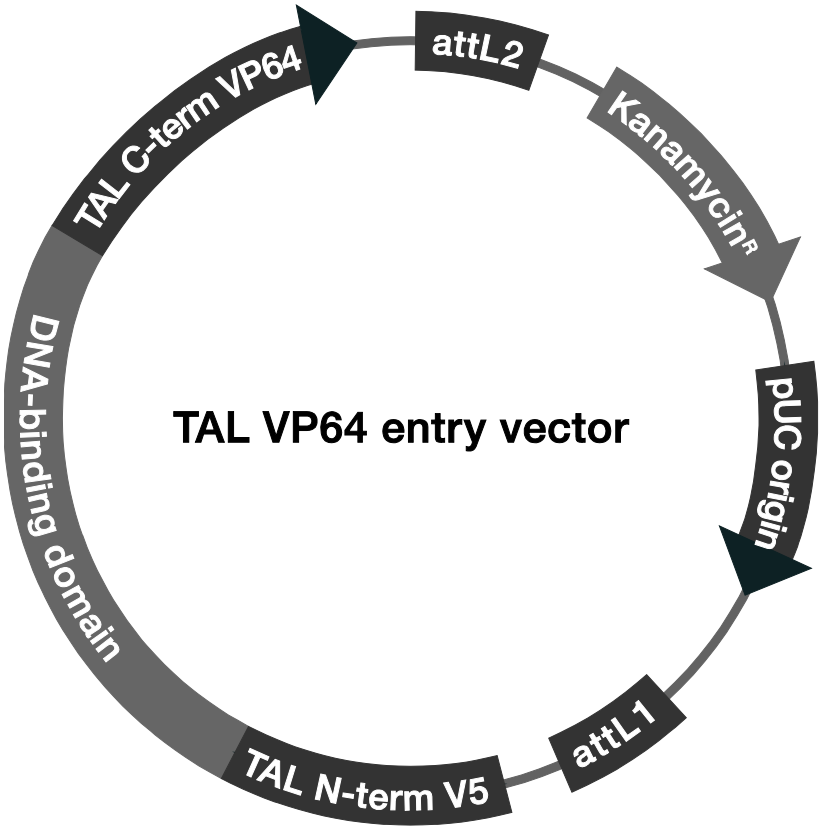
The map below shows the elements of the TAL vp16 Entry Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Map of TAL vp64 Entry Vector

TAL vp64 Entry Vector map

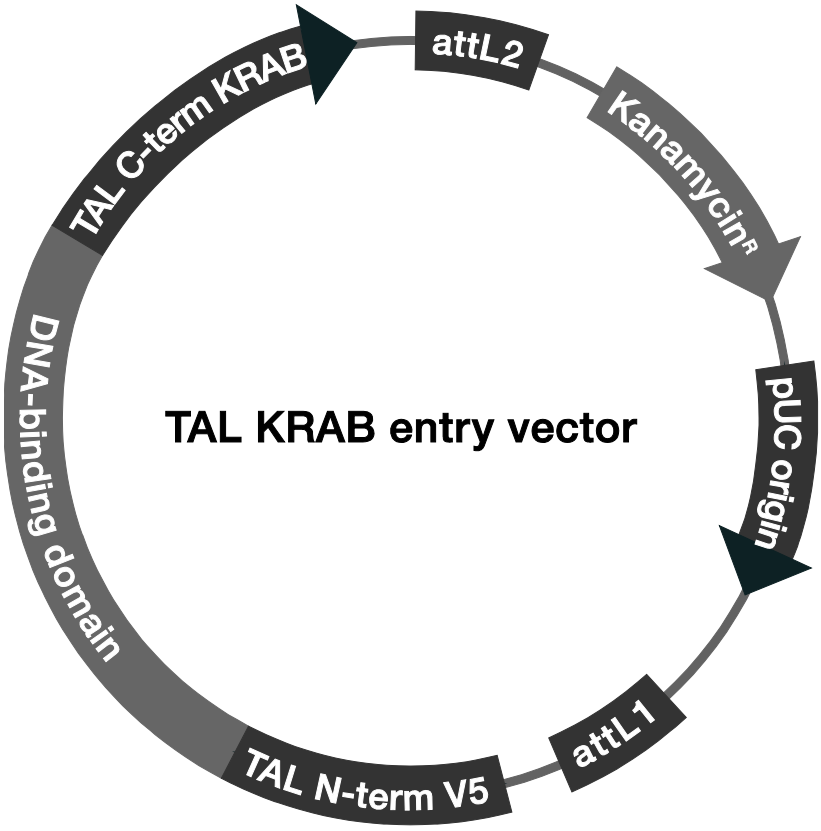
The map below shows the elements of the TAL vp64 Entry Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Map of TAL KRAB Entry Vector

TAL KRAB Entry Vector map

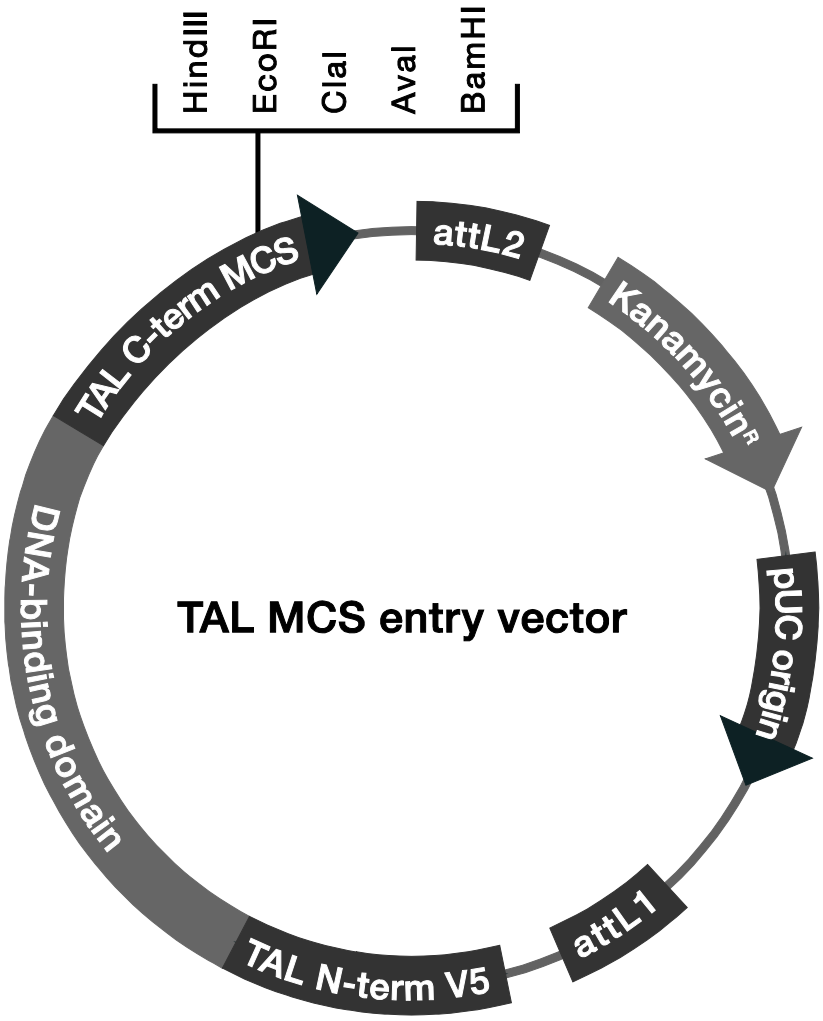
The map below shows the elements of the TAL KRAB Entry Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Map of TAL MCS Entry Vector

TAL MCS Entry Vector map

The map below shows the elements of the TAL MCS Entry Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Multiple cloning site of TAL MCS Entry Vector

Native TAL MCS

The multiple cloning site for the Native TAL MCS entry clone is shown below. The sequence of the TAL C-terminus is in bold. The MCS is underlined. Restriction sites are labeled to indicate the cleavage site.

2849 **GAT CCT TTT GCC GGA ACA GCC GAT GAT TTC CCT GCC TTT AAT GAG GAA GAA**
Asp Pro Phe Ala Gly Thr Ala Asp Asp Phe Pro Ala Phe Asn Glu Glu Glu

*Pme*I *Hind*III

2900 **CTG GCC TGG CTG ATG GAA CTG CTG CCT CAG** GGT TCC CGT TTA AAC AAG CTT
Leu Ala Trp Leu Met Glu Leu Leu Pro Gln Gly Ser Arg Leu Asn Lys Leu

*Sal*I *Kpn*I *Eco*RI *Cla*I *Sca*I *Xho*I *Bam*HI *Sac*I *Bgl*II

2951 GTC GAC GGT ACC GAA TTC ATC GAT AGT ACT CTC GAG GGA TCC GAG CTC AAG
Val Asp Gly Thr Glu Phe Leu Ile Asp Ser Thr Leu Glu Gly Ser Glu Leu

3002 ATC TAG CTA AGT AGA CCC AGC TTT CTT GTA CAA AGT TGG CAT TAT AAG
Lys

Truncated TAL MCS

The multiple cloning site for the Truncated TAL MCS entry clone is shown below. The sequence of the TAL C-terminus is in bold. The MCS is underlined. Restriction sites are labeled to indicate the cleavage site.

1901 **TTT TTT CAG TGT CAC TCT CAC CCT GCC CAG GCC TTT GAT GAT GCC ATG ACA**
Phe Phe Gln Cys His Ser His Pro Ala Gln Ala Phe Asp Asp Ala Met Thr

1952 **CAG TTT GGC ATG AGC AGA CAC GGA CTG CTG CAG CTG TTT AGA AGA GTG GGA**
Gln Phe Gly Met Ser Arg His Gly Leu Leu Gln Leu Phe Arg Arg Val Gly

2003 **GTG ACA GAA CTG GAG GCC AGA TCC GGA ACC CTG CCT CCT GCC TCT CAG AGA**
Val Thr Glu Leu Glu Ala Arg Ser Gly Thr Leu Pro Pro Ala Ser Gln Arg

*Pme*I *Hind*III *Sal*I *Kpn*I

2054 **TGG GAT AGG ATT CTG CAG** GGT TCC CGT TTA AAC AAG CTT GTC GAC GGT ACC
Trp Asp Arg Ile Leu Gln Gly Ser Arg Leu Asn Lys Leu Val Asp Gly Thr

*Eco*RI *Cla*I *Sca*I *Xho*I *Bam*HI *Sac*I *Bgl*II

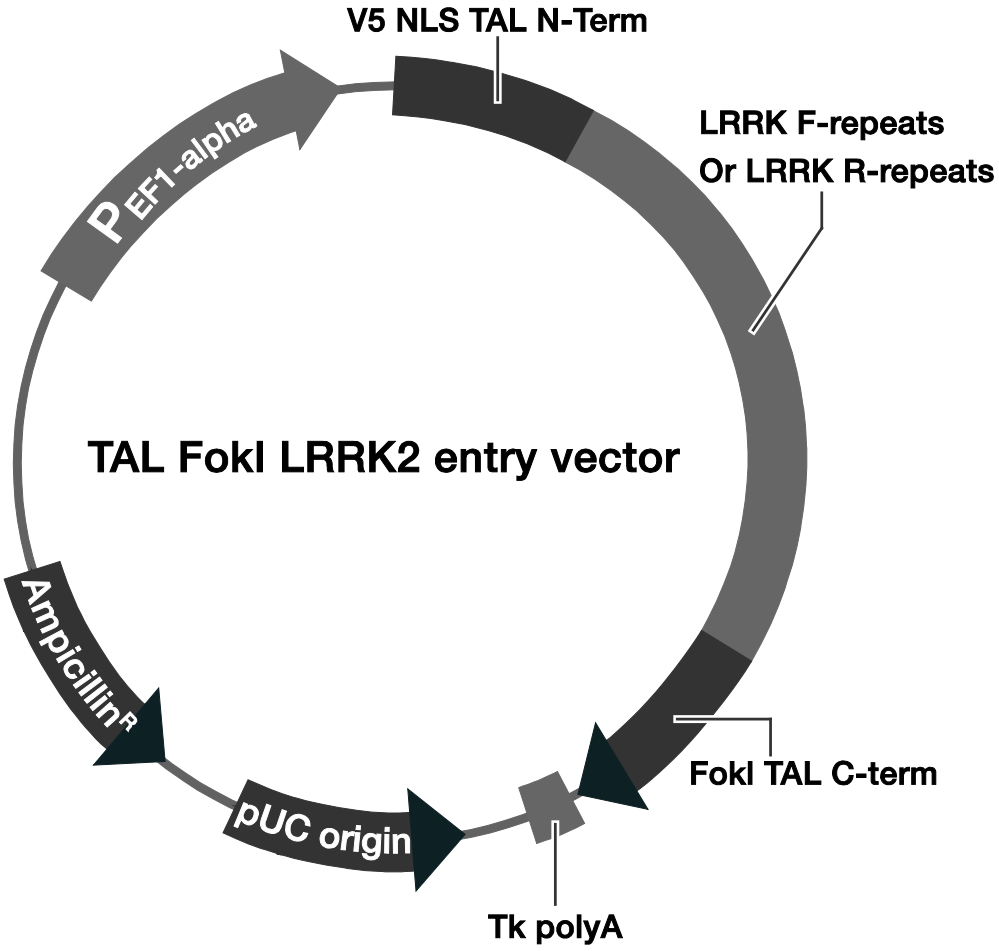
2105 GAA TTC ATC GAT AGT ACT CTC GAG GGA TCC GAG CTC AAG ATC TTA GCT AAG
Glu Phe Leu Ile Asp Ser Thr Leu Glu Gly Ser Glu Leu Lys

2156 TAG ACC CAG CTT TCT TGT ACA AAG TTG GCA TTA TAA GAA

Map of TAL FokI LRRK2 Entry Vector

TAL FokI LRRK2 Entry Vector map

The map below shows the elements of the TAL FokI LRRK2 Entry Vector. The region of the entry clone corresponding to the TAL is variable, depending upon the length of the sequence you ordered. **The complete sequence for your clone in .gb format is available on the disk provided with your clone.**



Features of GeneArt™ Precision TALs entry vectors

Common TAL entry vector features

The following elements are found in the GeneArt™ Precision TALs entry vectors: TAL FokI, TAL vp16/vp64, TAL MCS. All features have been functionally tested. These features do not apply to the TAL FolkI LRRK2 entry vector.

Feature	Description
*rrn*B T1 and T2 transcription terminators	Protects the cloned gene from expression by vector-encoded promoters, thereby reducing possible toxicity (Orosz et al., 1991).
M13 Forward (–20) priming site	Allows sequencing in the sense orientation.
M13 Reverse (C) priming site	Allows sequencing in the antisense orientation.
*att*L1 and *att*L2 sites	Allows recombinational cloning of the gene of interest from an entry clone (Landy, 1989).
Kanamycin resistance gene	Allows selection of the plasmid in *E. coli*.
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of the recombinant fusion protein by the Anti-V5 antibodies (Southern *et al.*, 1991).
pUC origin	Allows high-copy number replication and growth in *E. coli.*
DNA binding domain	Allows targeting of the TAL effector to specific DNA sequences. DNA repeat variable domain.
TAL N-term	N-terminus domain of the TAL containing translocation and nuclear localization signal tag.
NLS	Truncated versions of the vector contain the SV40 nuclear localization signal (NLS), while native vectors contain the two endogenous NLS of the TAL.
TAL C-term	C-terminus domain of the TAL containing activation domain.

C= complementary strand

Specific TAL entry vector features

The following features are found in the specific GeneArt™ Precision TALs entry vector noted.

Vector	Feature	Description
TAL FokI	FokI	FokI nuclease domain of the TAL
TAL vp16/vp64	vp16 or vp64 activator	Effector domain of the TAL
TAL KRAB	KRAB repressor	Effector domain of the TAL
TAL MCS	MCS	Multiple cloning site for insertion of custom effector domains into the TAL
TAL MCS	Gly-Ser linker	Flexible peptide linker to prevent steric hindrance between domains

Specific TAL FokI LRRK2 entry vector features

The following features are found in the TAL FokI LRRK2 entry vector.

Feature	Description
EF1alpha promoter	Allows efficient, high-level expression of TAL-FokI protein.
V5 epitope tag	Allows detection of the recombinant fusion protein by the Anti-V5 antibodies (Southern et al., 1991).
SV40 nuclear localization signal (NLS)	For nuclear localization
Hax3 N-terminus	N-terminus domain of the TAL
DNA binding domain	Allows targeting of the TAL effector to LRRK2 DNA sequences.
Hax3 C-terminus	C-terminus domain of the TAL containing activation domain
FokI	FokI nuclease domain of the TAL
TK polyA	Herpes Simplex Virus Thymidine Kinase (TK) polyadenylation signal allows efficient transcription termination and polyadenylation of mRNA (Cole and Stacy, 1985).
Ampicillin resistance gene	Allows selection of the plasmid in *E. coli*.
attB1 and attB2	Sites for gateway adaption
pUC origin	Allows high-copy number replication and growth in *E. coli*.

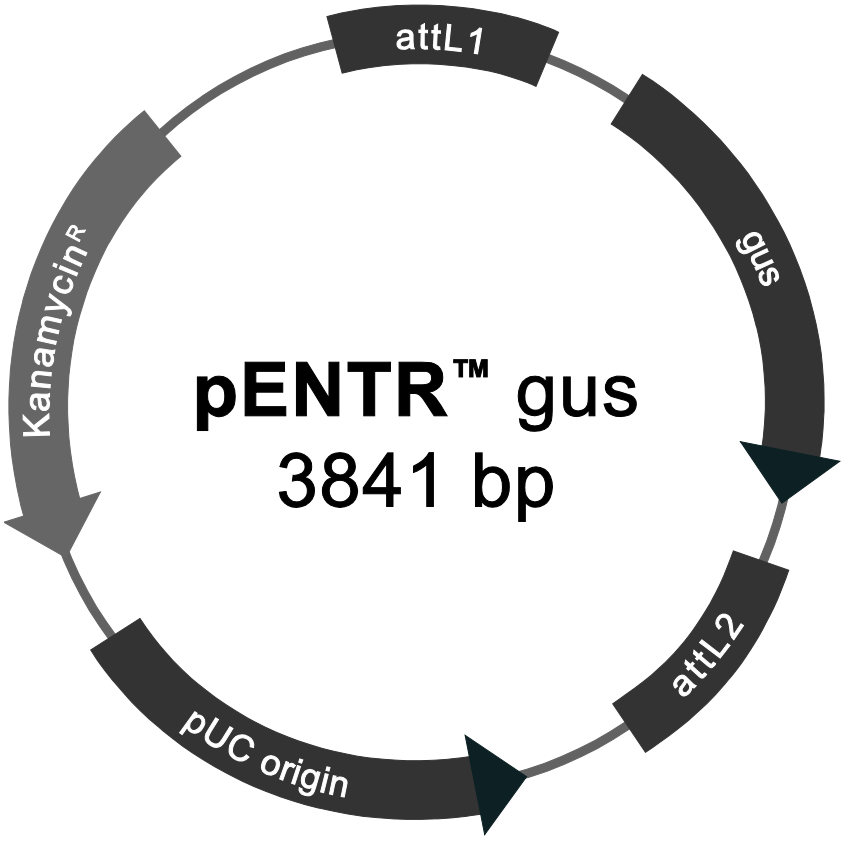
Map of pENTR™-gus Entry Vector

Description

pENTR™-gus is a 3841 bp entry vector containing the *Arabidopsis thaliana* gene for β-glucuronidase (*gus*) (Kertbundit *et al.*, 1991), and is included as a positive control with Gateway™LR Clonase™ II Enzyme Mix (Cat. nos. 11791-020 and 11791-100). The *gus* gene was amplified using PCR primers containing *att*B recombination sites. The amplified PCR product was then used in a BP recombination reaction with pDONR™ 201 to generate the entry clone. For more information about the BP recombination reaction, refer to the Gateway™Technology with Clonase™II manual.

Map of control vector

The figure below summarizes the features of the pENTR™-gus vector. **The complete sequence for pENTR™-gus is available from our web site (www.thermofisher.com/lifescience)** or by contacting Technical Support **(see page31).**



3841 nucleotides
*aff*L1: bases 228-2039
attL2: bases 2014-2140
pUC origin: bases 2200–2873 (C)
Kanamycin resistance gene: bases 2990–3804 (C)
C= complementary strand

Accessory products

Introduction

The products listed in this section may be used with GeneArt™ PerfectMatch TALs and GeneArt™ Precision TALs. For more information, refer to our website **www.thermofisher.com** or contact Technical Support (see page 31).

Additional products

Many of the reagents suitable for use with the vectors are available separately. Ordering information for these reagents is provided below. For more information, refer to our website **www.thermofisher.com**.

Item	Quantity	Catalog no.
Gateway™ LR Clonase™ II Enzyme Mix	20 reactions 100 reactions	11791-020 11791-100
Library Efficiency™ DH5™α Competent Cells	5 x 0.2 mL	18263-012
One Shot™ TOP10 Chemically Competent *E. coli*	20 reactions	C4040-03
One Shot™ TOP10 Electrocompetent *E. coli*	20 reactions	C4040-52
One Shot™ MAX Efficiency™DH10B™-T1 Phage-Resistant *E. coli*	20 reactions	12331-013
Ampicillin	200 mg	11593-027
Lipofectamine™ 3000 Transfection Reagent	1.5 mL	L3000015
Kanamycin Sulfate	5 g 25 g	11815-024 11815-032
Kanamycin Sulfate (100X), liquid	100 mL	15160-054
Geneticin™ Selective Antibiotic	1 g 5 g 20 mL (50 mg/mL) 100 mL (50 mg/mL)	11811-023 11811-031 10131-035 10131-027
PureLink™ HiPure Plasmid MiniPrep Kit	25 preps	K2100-02
PureLink™ HiPure Plasmid MidiPrep Kit	25 preps	K2100-04

Gateway™ destination vectors

A large selection of Gateway™ destination vectors are available to facilitate the expression of your gene of interest in virtually any protein expression system. For more information about the vectors available, refer to our website **www.thermofisher.com** or contact Technical Support (page 31).

Documentation and support

Customer and technical support

Visit **www.thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  – Product FAQs
  – Software, patches, and updates
- Order and web support
- Product documentation, including:
  – User guides, manuals, and protocols
  – Certificates of Analysis
  – Safety Data Sheets (SDSs; also known as MSDSs)
  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Quality Assurance Document

The Quality Assurance Document (QAD) is a certificate of analysis that provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on the disk provided with your clone.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on the Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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