Certificate of Analysis

iDimerize™ Reverse Dimerization Vector Set 1

Contents

Catalog No.
635062 (Not sold separately)

Lot Number
Specified on product label.

Product Information

The iDimerize Reverse Dimerization Vector Set 1 is provided in the iDimerize Reverse Dimerization System (Cat. No. 635066), which lets you control the subcellular localization, catalytic activity, and secretion of a protein of interest in live cells via a membrane-permeant compound. The iDimerize Reverse Dimerization Vector Set 1 contains three mammalian expression vectors encoding fusion tags (i.e., self-assembly domains in combination with different localization sequences) that can be easily added to your protein of interest. The aggregation state of the resulting chimeric protein can be controlled by the addition of a small, membrane-permeant compound to the cell medium. In the absence of the compound, the tagged protein expressed by the system self-assembles into complexes; when the compound is added to the cell medium, the complexes disaggregate, allowing the now soluble tagged proteins to regain their normal function. The vector set also includes two linear selection markers for hygromycin and puromycin resistance. **NOTE:** prHom-1, prHom-Nuc1, and prHom-Sec1 are identical to vectors pC_4-F_M2E, pC_4EN-F_M3, and pC_4S_1-F_M4-FCS-hGH, respectively, previously supplied in the RPD Regulated Secretion/Aggregation Kit from ARGENT Pharmaceuticals, Inc.

Package Contents

- 20 µl prHom-1 Vector (500 ng/µl)
- 20 µl prHom-Nuc1 Vector (500 ng/µl)
- 20 µl prHom-Sec1 Vector (500 ng/µl)
- 40 µl Linear Hygromycin Marker (50 ng/µl)
- 40 µl Linear Puromycin Marker (50 ng/µl)

Storage Conditions

- Store at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Shipping Conditions

- Dry ice (–70°C)

Product User Manuals

User manuals for Clontech products are available for download at **www.clontech.com/manuals**
The following user manual applies to this product:

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(PA124440)

- iDimerize Reverse Dimerization System User Manual (PT5180-1)

prHom-1, prHom-Nuc1 and prHom-Sec1 Vector Information

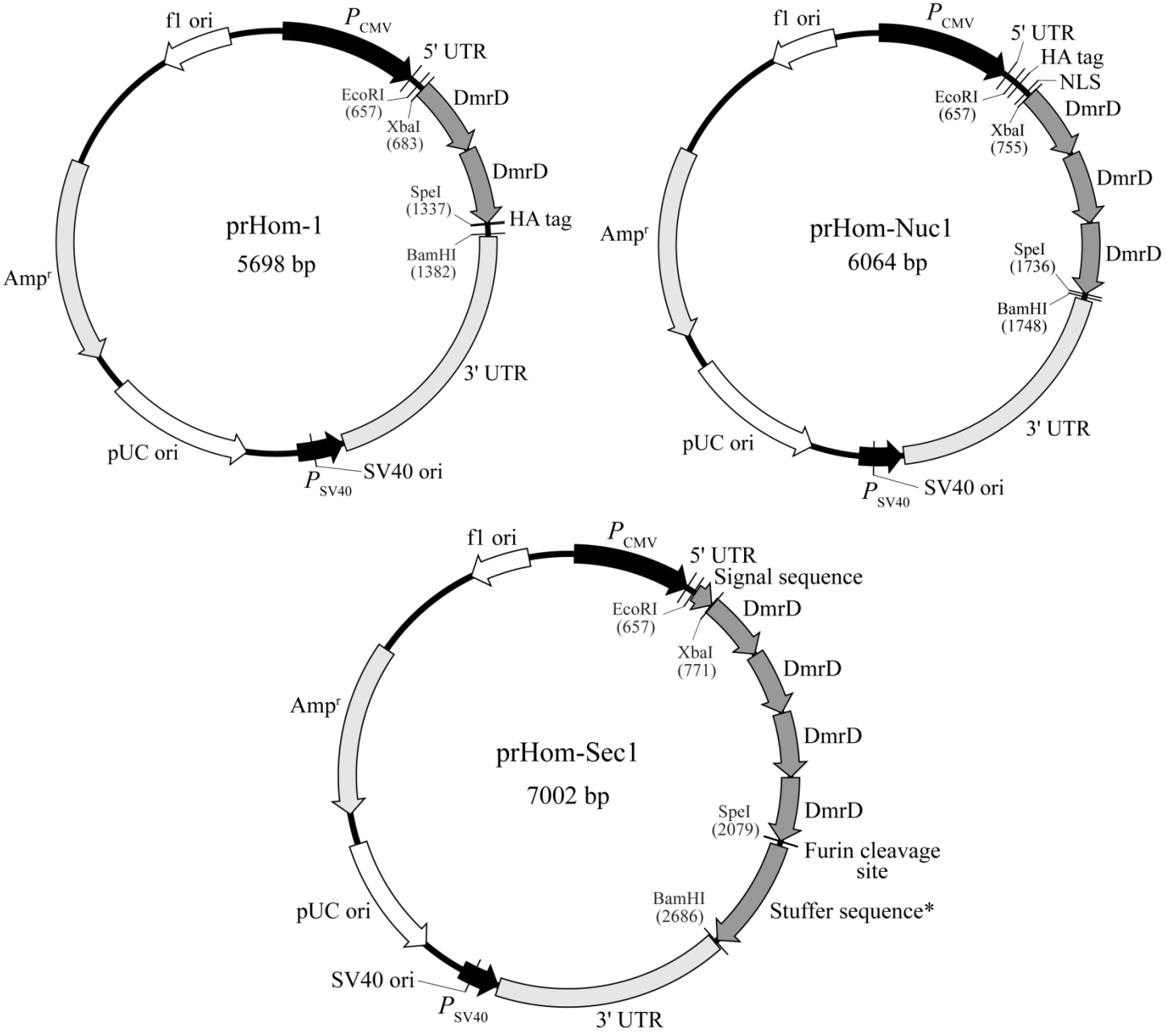

Figure 1. prHom-1, prHom-Nuc1 and prHom-Sec1 Vector Maps. **Cloning Strategy: For prHom-1**, use the EcoRI and XbaI sites to clone your gene of interest upstream of the DmrD domains, and the SpeI and BamHI sites to clone your gene of interest downstream of the DmrD domains. **For prHom-Nuc1 and prHom-Sec1**, use the XbaI site to clone your gene of interest upstream of the DmrD domains, and the SpeI and BamHI sites to clone your gene of interest downstream of the DmrD domains. See the iDimerize Reverse Dimerization System User Manual (PT5180-1) for more information on how to clone your gene of interest. ***NOTE:** The "stuffer sequence" in prHom-Sec1encodes human growth hormone; replace this sequence with your gene of interest.

Description

The vectors in this vector set allow a protein of interest to be tagged with a multivalent, self-assembly domain, DmrD, and expressed in mammalian cells. The tagged protein automatically forms multimeric complexes that can be rapidly and reversibly dissolved by the addition of the membrane permeant compound D/D Solubilizer, (1). This reversible process can be used to control processes such as protein secretion, or to reversibly block the catalytic site to prevent protein activation.

The iDimerize Reverse Dimerization Vector Set 1 contains three mammalian expression vectors that encode different numbers of DmrD domains and different localization tags:

- **prHom-1** constitutively expresses a protein of interest fused to two copies of the self-assembly domain DmrD.
- **prHom-Nuc1** constitutively expresses a protein of interest fused to three copies of DmrD and a nuclear localization sequence (NLS). As a result of the NLS tag, a chimeric protein of interest expressed by this vector will localize to the nucleus.
- **prHom-Sec1** constitutively expresses a protein of interest fused to a secretory signal sequence and four copies of DmrD. As a result of these fusion tags, a chimeric protein of interest expressed by this vector will localize to the endoplasmic reticulum (ER) and self-assemble into complexes that prevent it from being transported through the secretory pathway. The addition of D/D Solubilizer to the medium will dissolve the complexes, allowing the protein to be secreted by the cell. This approach allows cells to rapidly (15 min) secrete a pool of protein in response to D/D Solubilizer (2). A furin cleavage site, located between the protein of interest and the DmrD domains, allows the DmrD-tag to be removed from the secreted protein. **NOTE:** The "stuffer sequence" in prHom-Sec1 encodes human growth hormone; replace this sequence with your gene of interest.
- The Dmr tags encoded by prHom-1 and prHom-Nuc1 also include a hemagglutinin (HA) tag. This tag is useful for determining subcellular protein localization, facilitating protein purification, identifying associated proteins, and characterizing new proteins by immunoprecipitation (HA-Tag Polyclonal Antibody, Cat. No. 631207).

All three vectors drive expression of the protein of interest from the human cytomegalovirus enhancer/promoter element (P_{CMV}). To enhance expression, the vectors also contain the 5′ untranslated region (UTR) from the herpes simplex virus thymidine kinase gene, as well as a portion of the rabbit beta-globin gene 3′ UTR that includes the final intron and a polyA signal. In addition, each vector contains a pUC origin of replication and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*, and an f1 origin for single-stranded DNA production.

Location of Features

prHom-1 Vector

- P_{CMV} (human cytomegalovirus promoter): 19–613
- 5′ UTR (HSV TK 5′ untranslated region): 615–655
- DmrD (self-assembly domain): 682–1008 & 1012–1335
- HA Tag (hemagglutinin epitope tag): 1342–1368
- 3′ UTR (rabbit beta-globin 3′ untranslated region, includes polyA signal): 1387–2548
- P_{SV40} (SV40 promoter): 2557–2752 (complementary)
- SV40 origin of replication: 2635–2712 (complementary)
- pUC origin of replication: 2974–3593 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 3748–4620 (complementary)
- f1 origin of replication: 5189–5495 (complementary)

prHom-Nuc1 Vector

- P_{CMV} (human cytomegalovirus promoter): 19–613
- 5′ UTR (HSV TK 5′ untranslated region): 615–655
- HA Tag (hemagglutinin epitope tag): 685–711
- NLS (nuclear localization signal): 733–753
- DmrD (self-assembly domain): 754–1080; 1087–1407; & 1411–1734
- 3′ UTR (rabbit beta-globin 3′ untranslated region, includes polyA signal): 1753–2914
- P_{SV40} (SV40 promoter): 2923–3118 (complementary)
- SV40 origin of replication: 3001–3078 (complementary)
- pUC origin of replication: 3340–3959 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 4114–4986 (complementary)
- f1 origin of replication: 5555–5861 (complementary)

prHom-Sec1 Vector

- P_{CMV} (human cytomegalovirus promoter): 19–613
- 5′ UTR (HSV TK 5′ untranslated region): 615–655
- Secretory signal sequence (from human growth hormone): 692–769
- DmrD (self-assembly domain): 767–1096; 1103–1423; 1427–1750; & 1754–2077
- Furin cleavage site: 2081–2104
- Stuffer sequence: 2105–2677
- 3′ UTR (rabbit beta-globin 3′ untranslated region, includes polyA signal): 2691–3852
- P_{SV40} (SV40 promoter): 3861–4056 (complementary)
- SV40 origin of replication: 3939–4016 (complementary)
- pUC origin of replication: 4278–4897 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 5052–5924 (complementary)
- f1 origin of replication: 6493–6799 (complementary)

Additional Information

See the iDimerize Reverse Dimerization System User Manual (PT5180-1) for information on how to clone your gene of interest into your vector of choice. Cotransfection of the vectors with Linear Hygromycin or Puromycin Markers allows antibiotic selection of stable transfectants (see user manual for details).

In the absence of D/D Solubilizer, the protein tagged with DmrD self-assembles into complexes. The addition of D/D Solubilizer to the cell medium causes these complexes to dissolve.

Propagation in *E. coli*

- Recommended host strain: Stellar™ Competent Cells. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

References

1. Rollins, C. T. *et al.* (2000) *Proc Natl Acad Sci USA* **97**(13):7096–7101.
2. Rivera, V. M. *et al.* (2000) *Science* **287**(5454):826–830.

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzymes	Fragment Sizes
prHom-1	SpeI	5.7 kb
	EcoRI & BamHI	0.7 & 5.0 kb
prHom-Nuc1	SpeI	6.1 kb
	EcoRI & BamHI	1.1 & 5.0 kb
prHom-Sec1	SpeI	7.0 kb
	EcoRI & BamHI	2.0 & 5.0 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

Linear Selection Marker Identity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Marker	Enzymes	Fragments
Linear Hygromycin Marker	HindIII & XbaI	0.5, 0.6 & 1.1 kb
Linear Puromycin Marker	HindIII & XbaI	0.45, 0.6, & 0.75 kb

Functional Testing of Linear Markers

- HEK 293 cells were transfected with 200 ng of either the Linear Hygromycin Marker or the Linear Puromycin Marker. After 5 hr at 37°C, the transfection solution was removed and the cells were given fresh medium. 48 hr later, the cells were plated in two 10 cm plates. 48 hr after plating, medium containing either hygromycin or puromycin was added to the plates. After 2–3 weeks, >20 clones were identified.

Notice to Purchaser

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CATALOG NO.
635062

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This document has been reviewed and approved by the Clontech Quality Assurance Department.

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