Functional Tags For ORF Clones For Your Research

GeneCopoeia, Inc.

Presenter:

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Senior Application Scientist
GeneCopoeia, Inc.
Goals of this presentation

- How to use expression-ready ORF clones in research
- How to choose tagged clones
- How to order expression-ready ORF clones from GeneCopoeia
Outline

➢ Introduction to GeneCopoeia

➢ OmicsLink™ Expression-ready clones

➢ Applications for ORF clones

➢ How to order GeneCopoeia ORF clones
Outline

- Introduction to GeneCopoeia
- OmicsLink™ Expression-ready clones
- Applications for ORF clones
- How to order GeneCopoeia ORF clones
What do we do?

- Original manufacturer and seller of a variety of research reagents and tools.
- Biological research service provider to world-wide customers.
## Products for Functional Genomics

<table>
<thead>
<tr>
<th>Products &amp; Services</th>
<th>Genome editing</th>
<th>Transcription regulation</th>
<th>Translation regulation</th>
<th>Gene Profiling</th>
</tr>
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<tbody>
<tr>
<td>ORF clones</td>
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<td>Pathway- and disease focused gene analysis</td>
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</table>
Outline

- Introduction to GeneCopoeia
- OmicsLink™ Expression-ready clones
- Applications for ORF clones
- How to order GeneCopoeia ORF clones
Post-genomics era functional studies

DNA → mRNA → Protein

Human Genome Project

Post-Human Genome Project: ORFs and Expression Regulation

Reverse Transcription
Why Use ORF clones?

- Biochemical studies *in vitro*
  - Macromolecular interactions (protein-protein, protein-DNA, etc.)

- Biological function studies *in vivo*

- Orthologous studies

- Reverse/rescue mutant phenotype

- Drug target validation
GeneCopoeia Full-length ORF clones

- Only company with genome-wide untagged ORF clones
- Choice of numerous functional tags
- All ORFs sequence-verified & guaranteed
Sequence- vs. expression validation

- **Sequence validation**: Confirm that sequence is correct, untagged protein will have natural function

- **Expression validation, tagged or untagged**: Could be expressing protein that is non-functional or has acquired non-physiological function
OmicsLink™ Expression-ready clones

Advantages

- Ready for immediate in vivo and in vitro expression
- No cloning steps required
- Full-length ORFs without 5’ or 3’ UTRs
- >45,000 human & mouse ORFs
- Available for human, mouse, & zebrafish
- Available in lentiviral or non-viral vectors
OmicsLink™ Expression-ready clones

**Applications & advantages**

- High-throughput and large scale protein production and purification
- Ready for various functional assays, including protein immobilization, cellular localization, and other functional assays
- Suitable for reverse transfection arrays and nucleic acid programmable protein arrays (NAPPA)
- Resources and time savings
ORF Clone Collections

- Human ORFs
  - 21,000+ ORFs
  - Fully sequenced

- Mouse ORFs
  - ~15,000 genes
  - ~13,000 unique genes

- ORFs from other species
  - Zebrafish
  - etc
Promoters and Fusion Tags in Expression Systems

**Promoters:**
- CMV
- EFa1
- Lenti/CMV/EFa1
- T7
- Trac
- GAL1
- AcMNPV polyhedrin

**Fusion Tags:**
- Hisx6
- Flag/3xFlag
- GST
- MBP
- SUMO
- GFP, YFP, RFP
- 3xHA
- c-Myc
- Halo-Tag®
- AviTag™
- AviTag-IRES-Biotin Ligase
- Luciferase, secreted and non-secreted

**Mammalian Selection Markers:**
- Puromycin
- Neomycin
- Hygromycin
- Blasticidin
- Zeomycin
Clone Sets: Genome-wide or families

- CMV based mammalian expression ready clone sets
  - Untagged: No potential for tag effects
  - C-terminal Flag
  - C-terminal monomeric EGFP
- Lentiviral + CMV expression ready clone sets
  - Untagged: No potential for tag effects
  - C-terminal V5
Outline

- Introduction to GeneCopoeia
- OmicsLink™ Expression-ready clones
- Applications for ORF clones
- How to order GeneCopoeia ORF clones
Using untagged ORF clones

Why choose to NOT tag?

- Tag could interfere with normal protein folding
- Tag could interfere with normal protein activity
- Tag could interfere with normal protein localization
- Tag could create toxic fusion protein
- Have native antibody available, tag not needed
Using untagged ORF clones

GeneCopoeia untagged expression vectors
Using untagged ORF clones

Express native protein for phenotype

Al-Zoubi, et al. (2013). Cell Cycle 12, 480

Used GeneCopoeia OmicsLink™ clone to demonstrate that TNFα overexpression eliminates breast cancer growth in vivo
Aplication Example: Validation of RNAi Silencing

- mRNA
  - 5' UTR
  - Full Length Protein coding ORF
  - 3' UTR

- dsRNA or shRNA against UTRs
  - Transfect cells
  - Co-Transfect cells

- Silence GENE function
  - No effect, function rescue/recovery?
Why use tags?

- No native antibody available
- Enable purification
- Enhance solubility during purification
- Interaction with macromolecules
- Localization/live cell visualization
<table>
<thead>
<tr>
<th>Fusion Tag</th>
<th>Purification</th>
<th>Increase Solubility</th>
<th>ab IP</th>
<th>Cellular Labeling</th>
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<tr>
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Applications for tagged ORF clones

Protein tagging for purification

N-terminal tag

C-terminal tag
How to choose tagged ORF clones

Considerations for choosing N- or C-terminus

- Choose end that is not buried in protein core
- Choose end that does not disrupt localization (signal peptide)
Applications for tagged ORF clones

Protein purification

His tag

Features

- 6xHis tag permits use of classic metal columns or beads for purification
- Can be fused at N- or C-terminus
Applications for tagged ORF clones

Protein purification

His tag

![Diagram of ORF clones with His tag](image-url)
Applications for tagged ORF clones

Protein purification

His tag

- Mammalian cells
- Bacteria
- Cell-free
- Insect cells
- Etc.

Isolate proteins
Applications for tagged ORF clones

Protein purification

His tag

Affinity purification - typically column or beads (agarose or magnetic)
Applications for tagged ORF clones

Protein purification

Flag tag

- Small tag less likely to interfere with protein function
- Multiple applications: Purification, western, IP, IF
- Can place at N- or C-terminus

Flag

Protein

DYKDDDDK

Protein

Flag

DYKDDDDK
Applications for tagged ORF clones

Protein purification

Flag tag

pReceiver-M11 Expression Clone

pReceiver-M13 Expression Clone
Applications for tagged ORF clones

Protein purification

Flag tag

- Mammalian cells
- Bacteria
- Cell-free
- Insect cells
- Etc.

Lysis cells
Applications for tagged ORF clones

Protein purification

Flag tag

Affinity purification—typically column or beads (agarose or magnetic)
Applications for tagged ORF clones

Live cell imaging

GFP tag

Features

- Classical fluorescent marker from jellyfish
- Multifunctional tag: Usable for live cell imaging, purification, western, IP, & more
- Can be fused at either N- or C-termini
Applications for tagged ORF clones

Live cell imaging

GFP tag
Applications for tagged ORF clones

Live cell imaging

GFP tag

- Mammalian cells
- Bacteria
- Cell-free
- Insect cells
- Etc.

View cells under microscope
Applications for tagged ORF clones

**Live cell imaging**

HaloTag® 7

- Genetically engineered derivative of a dehalogenase
- Multifunctional tag: Usable for live cell imaging, purification, western, IP, & more
- Efficiently forms a covalent bond with various synthetic HaloTag® ligands
- Can be fused at either N- or C-termini

![Halo Tag Diagram]

**Features**

[Diagram showing the fusion of Halo and Protein]
Applications for tagged ORF clones

Live cell imaging

HaloTag®
Applications for tagged ORF clones

Live cell imaging

HaloTag® 7
Applications for tagged ORF clones

**Pull-down**

Avi Tag™

- Specific and reversible binding of avidin or streptavidin to biotin
- Biotinylated by biotin ligase in vitro or in vivo
- Can be fused at either N- or C-termini
- Biotinylated by biotin ligase in vitro or in vivo
- Small tag less likely to interfere with protein function
Applications for tagged ORF clones

Pull-down

Avi Tag™

pReceiver-M05 Expression Clone

pReceiver-M17 Expression Clone
Applications for tagged ORF clones

Pull-down

Avi Tag™

- Mammalian cells
- Bacteria
- Cell-free
- Insect cells
- Etc.

Isolate proteins for western and/or mass spectrometry
Outline

- Introduction to GeneCopoeia
- OmicsLink™ Expression-ready clones
- Applications for ORF clones
- How to order GeneCopoeia ORF clones
How do I order a GeneCopoeia clone?
How do I order a GeneCopoeia clone?
How do I order a GeneCopoeia clone?

Learn more about expression-ready ORF cDNA clones collection

Search full-length ORF cDNA clones

1. Simple search by gene symbol, aliases, description, nucleotide accession, Entrez gene ID, catalog or product ID
   Select species:
   • Human
   • Mouse
   • Zebrafish
   Enter Keyword(s): [Search]

2. Search by a specific field
   Select species:
   • Human
   • Mouse
   • Zebrafish
   Select field:
   • Description
   • Symbols/Aliases
   • Nucleotide Accession
   • UniGene ID
   • Entrez Gene ID
   • Catalog/product ID
   Enter Keyword(s): [Search]

3. Browse genes by signal pathway
   • Over 60 signaling pathways
   • Easy to search and quick to order

4. Search by sequence homology (BLAST)
   Select species:
   • Human
   • Mouse
   • Zebrafish
   Select sequence type:
   • Protein
   • Nucleotide
   Enter query sequence:

Submit
How do I order a GeneCopoeia clone?

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   - Enter query sequence:

Budget Friendly ORF Clones
- $199 and up
- Mammalian, lentiviral and Gateway clones at next day shipping!
**How do I order a GeneCopoeia clone?**

<table>
<thead>
<tr>
<th>ORF cDNA clones</th>
<th>ORF donor clones</th>
<th>Promoter clones</th>
<th>qPCR primers</th>
<th>shRNA clones</th>
<th>mRNA products</th>
<th>mRNA target clones</th>
<th>Lentivirus</th>
<th>Proteins</th>
</tr>
</thead>
</table>

Learn more about expression-ready ORF cDNA clones collection

**Search full-length ORF cDNA clones**

1. Simple search by gene symbol, aliases, description, nucleotide accession, Entrez gene ID, catalog or product ID
   - Select species:
     - Human
     - Mouse
     - Zebrafish
     - SARS
     - Shigella flexneri
   - Enter Keyword(s):
     - SOX2
   - Search

2. Search by a specific gene symbol
   - Select species:
     - Human
     - Mouse
   - Select field:
     - Description
     - Nucleotide Accession
     - Entrez Gene ID
   - Enter Keyword(s):
     - SOX1
   - Search

3. Browse genes by signal pathway
   - Over 60 signaling pathways
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4. Search by sequence homology (BLAST)
   - Select species:
     - Human
     - Mouse
     - Zebrafish
   - Select sequence type:
     - Protein
     - Nucleotide
   - Enter query sequence:
   - Submit

Budget Friendly ORF Clones $199 and up
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How do I order a GeneCopoeia clone?

1. Simple search by gene symbol, aliases, description, nucleotide accession, Entrez gene ID, catalog or product ID
   - Select species:
     - Human
     - Mouse
     - Zebrafish
   - Enter Keywords:

2. Search by a specific:
   - Select species:
     - Human+Mouse
   - Select field:
     - Description
     - Nucleotide Accession
     - Entrez Gene ID
   - Enter Keywords:

3. Browse genes by signal pathway
   - Over 60 signaling pathways
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4. Search by sequence homology (BLAST)
   - Select species:
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     - Nucleotide
   - Enter query sequence:

Budget Friendly ORF Clones
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How do I order a GeneCopoeia clone?

Search Result: ORF Clones

<table>
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<tr>
<th>Accession</th>
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</table>

Search related products for "SOX2":

- Promoter
- qPCR
- shRNA
- miRNA
- miRNA target
- Lentivirus
- Protein

About GeneCopoeia
- GeneCopoeia Overview
- Contact GeneCopoeia
- Order Support
- Worldwide Distributions

Products
- Clone collections
- Lentiviral system
- miRNA solutions
- qPCR Arrays

Custom Services
- Custom TALEN and TALE-TF
- Custom promoter
- Custom 3' UTR target
- Custom lentivirus

Resources
- ORF cDNA Vectors
- Publications
- Manuals, Protocols, and MSDS
- Brochures and Product Profiles
How do I order a GeneCopoeia clone?

Add clones to shopping cart to view shipping formats, prices and proceed with order

<table>
<thead>
<tr>
<th>Mammalian (36)</th>
<th>Lentiviral (96)</th>
<th>Bacterial (15)</th>
<th>Yeast (3)</th>
<th>Insect (1)</th>
<th>Wheat germ (8)</th>
<th>Gateway (4)</th>
<th>Budget-friendly (6)</th>
<th>Controls and Kits (188)</th>
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</thead>
</table>

**Mammalian (36 types)**

<table>
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<tr>
<th>Buy</th>
<th>Catalog#</th>
<th>Promoter</th>
<th>Host Cell</th>
<th>Selection Marker</th>
<th>Tag</th>
<th>Protease Site</th>
<th>Vector</th>
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Add clones to shopping cart to view shipping formats, prices and proceed with order

- Add to Shopping Cart

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How do I order a GeneCopoeia clone?

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How do I order a GeneCopoeia clone?

OmicsLink™ Expression Clone (CMV Promoter)

Vector Features

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How do I order a GeneCopoeia clone?

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- Add to Shopping Cart

| Mammalian (36 types) |

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How do I order a GeneCopoeia clone?

**Select delivery format**

Standard delivery format for all other clones: Varies

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[Add to Shopping Cart]

* Mammalian (36 types)

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<th>Buy</th>
<th>Catalog#</th>
<th>Promoter</th>
<th>Host Cell</th>
<th>Selection Marker</th>
<th>Tag</th>
<th>Protease Site</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EX-T2547-M01</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>N-His</td>
<td>N/A</td>
<td>pReceiver-M01</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M77</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>C-His</td>
<td>N/A</td>
<td>pReceiver-M77</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M51</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>C-His+IRES-eGFP</td>
<td>N/A</td>
<td>pReceiver-M51</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M02</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>N/A</td>
<td>N/A</td>
<td><strong>pReceiver-M02</strong></td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M68</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Puromycin</td>
<td>N/A</td>
<td>N/A</td>
<td>pReceiver-M68</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M67</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Hygromycin</td>
<td>N/A</td>
<td>N/A</td>
<td>pReceiver-M67</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M29</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>N-eGFP</td>
<td>N/A</td>
<td>pReceiver-M29</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M98</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>C-eGFP(monomeric)</td>
<td>N/A</td>
<td>pReceiver-M98</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M03</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>C-eGFP</td>
<td>N/A</td>
<td>pReceiver-M03</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M15</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>N-eYFP</td>
<td>N/A</td>
<td>pReceiver-M15</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M16</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>C-eYFP</td>
<td>N/A</td>
<td>pReceiver-M16</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M32</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>N-eCFP</td>
<td>N/A</td>
<td>pReceiver-M32</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M33</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>C-eCFP</td>
<td>N/A</td>
<td>pReceiver-M33</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M04</td>
<td>CMV</td>
<td>Mammalian</td>
<td>Neomycin</td>
<td>N-GST</td>
<td>EK</td>
<td>pReceiver-M04</td>
</tr>
</tbody>
</table>

*Note: pReceiver-M02 is highlighted as an example.*
How do I order a GeneCopoeia clone?

![Select delivery format table](image)

<table>
<thead>
<tr>
<th>Buy</th>
<th>Catalog#</th>
<th>Delivery format</th>
<th>Delivery time</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Selected)</td>
<td>EX-T2547-M02-B</td>
<td>Bacterial stock</td>
<td>Next day shipment</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M02-10</td>
<td>10µg purified plasmid</td>
<td>3-5 days (add $50)</td>
</tr>
<tr>
<td></td>
<td>EX-T2547-M02-50</td>
<td>50µg purified plasmid</td>
<td>3-5 days (add $100)</td>
</tr>
</tbody>
</table>

Add to Shopping Cart
How do I order a GeneCopoeia clone?

* Discount applies to: (1) control clones when purchased together with non-control clones in the same corresponding vector (type); (2) ORF clones when purchased together with the same ORF product ID in a different vector type.
How do I order a GeneCopoeia clone?
# How do I order a GeneCopoeia clone?

## Ready-to-express ORF clone pricing

<table>
<thead>
<tr>
<th>Clone type</th>
<th>List price</th>
<th>30% off promotional price (through February 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral &amp; non-viral</td>
<td>Start at $805.00</td>
<td>Start at $564.00</td>
</tr>
<tr>
<td>Budget-friendly</td>
<td>Start at $250.00</td>
<td>Start at $199.00</td>
</tr>
<tr>
<td>Secondary clones</td>
<td>Start at $175.00</td>
<td>Start at $175.00</td>
</tr>
</tbody>
</table>
Budget-friendly expression clones

What are budget-friendly expression clones?

- >20,000 human ORF clones
- Some ready for next-day shipping
- C-Flag tagged (non-viral only) or untagged
- CMV promoter
- Available in non-viral & lentiviral vectors
Clone Sets: Genome-wide or families

- CMV based mammalian expression ready clone sets
  - Untagged: No potential for tag effects
  - C-terminal Flag
  - C-terminal monomeric EGFP

- Lentiviral + CMV expression ready clone sets
  - Untagged: No potential for tag effects
  - C-terminal V5
## Clone Sets: Genome-wide Coverage

<table>
<thead>
<tr>
<th>Product</th>
<th># of genes</th>
<th># of clones</th>
<th>Vector type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human whole genome mammalian expression clone sets (no tag)</td>
<td>12,841</td>
<td>16,096</td>
<td>Non-viral</td>
</tr>
<tr>
<td>Human mammalian expression clone sets (no tag)</td>
<td>Varies</td>
<td>Varies</td>
<td>Non-viral</td>
</tr>
<tr>
<td>Human whole genome mammalian expression clone sets (with C-terminal Flag tag)</td>
<td>12,579</td>
<td>13,766</td>
<td>Non-viral</td>
</tr>
<tr>
<td>Human mammalian expression clone sets (with C-terminal Flag tag)</td>
<td>Varies</td>
<td>Varies</td>
<td>Non-viral</td>
</tr>
<tr>
<td>Human whole genome lentiviral expression clone sets (with no tag)</td>
<td>12,651</td>
<td>15,799</td>
<td>Lentiviral</td>
</tr>
<tr>
<td>Human lentiviral expression clone sets (with no tag)</td>
<td>Varies</td>
<td>Varies</td>
<td>Lentiviral</td>
</tr>
<tr>
<td>Human whole genome Gateway® PLUS™ shuttle clone sets (no promoter)</td>
<td>14,087</td>
<td>18,660</td>
<td>Gateway®</td>
</tr>
<tr>
<td>Human Gateway® PLUS™ shuttle clone sets (no promoter)</td>
<td>Varies</td>
<td>Varies</td>
<td>Gateway®</td>
</tr>
</tbody>
</table>
## Clone Sets: Gene families

<table>
<thead>
<tr>
<th>Species</th>
<th>Set type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>CDs</td>
</tr>
<tr>
<td>Human</td>
<td>Cytokines &amp; cytokine receptors</td>
</tr>
<tr>
<td>Human</td>
<td>Drug target genes</td>
</tr>
<tr>
<td>Human</td>
<td>GPCRs</td>
</tr>
<tr>
<td>Human</td>
<td>Histones</td>
</tr>
<tr>
<td>Human</td>
<td>Histone modification enzymes</td>
</tr>
<tr>
<td>Human</td>
<td>Ion channels</td>
</tr>
<tr>
<td>Human</td>
<td>Kinases</td>
</tr>
<tr>
<td>Human</td>
<td>Membrane-bound proteins</td>
</tr>
<tr>
<td>Human</td>
<td>Nuclear hormone receptors</td>
</tr>
<tr>
<td>Human</td>
<td>Phosphatases</td>
</tr>
<tr>
<td>Human</td>
<td>Proteases</td>
</tr>
<tr>
<td>Human</td>
<td>Secretory proteins</td>
</tr>
<tr>
<td>Human</td>
<td>Transcription factors</td>
</tr>
<tr>
<td>Various</td>
<td>Custom-selected</td>
</tr>
</tbody>
</table>

### Features

- **Non-viral**: Expression-ready, untagged or C-terminal FLAG-tagged
- **Lentiviral**: Expression-ready with native protein expression
- **Gateway® PLUS shuttle vectors**: No promoter
- **Custom-made sets available**
GeneCopoeia ORF clones

Other GeneCopoeia ORF clone products
Safe Harbor Site integration with ORF clones

- Over 18,000 sequence-verified human ORFs available
- Inserted between AAVS1 sites for ready safe harbor integration using TALEN
ORFEXPRESS™ Gateway® PLUS clones

Features

- Carry both recombination and multiple cloning sites
- 20,000 human, 15,000 mouse clones
- Simple & rapid transfer of protein-coding ORFs into any Gateway destination expression vector
- Available with and without stop codons
- Contain ribosomal binding sites (Shine Dalgarno, Kozak) for optimal translational context
GeneCopoeia Products & Services

- ORF clones
- Stable cell lines
- shRNA
- qPCR
- Proteins
- Lentivirus
GeneCopoeia OmicsLink™ clones are full-length ORF clones for human, mouse, & other systems, ready to express without any tedious cloning or verification steps.

GeneCopoeia OmicsLink™ ready-to-express clones come either untagged or with a large variety of functional tags for virtually any application.

GeneCopoeia is the only provider of genome-wide untagged expression-ready ORF clones. All expression-ready clones are sequence-verified & guaranteed.

Protein fusion tags can greatly facilitate your goals of detecting, purifying, and localizing your protein of interest.

Ordering OmicsLink™ ready-to-express clones from GeneCopoeia is as easy & convenient as the click of a mouse.
Thank you!

If you have any additional questions, please call 1-866-360-9531
Or visit us on the web:
www.genecopoeia.com

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Rockville, Maryland USA 20850