

BioCat Special

- MGC premier cDNA Clones **15% OFF***
- MGC premier ORF Libraries **Special Price***

*Expires 31 December 2017

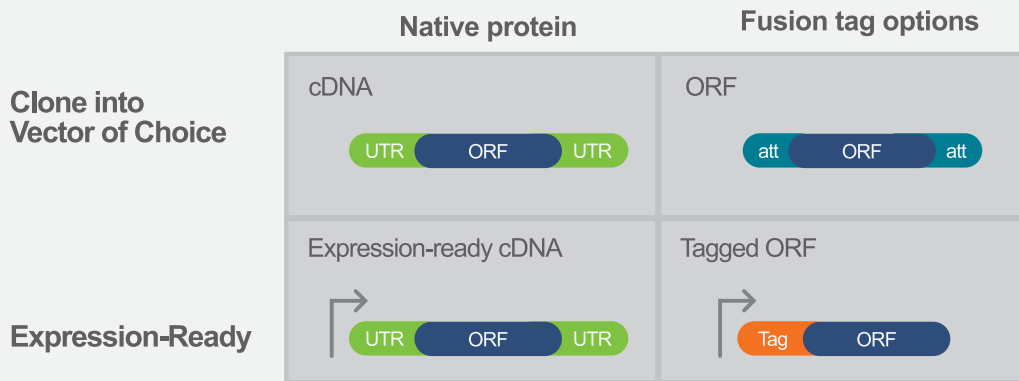
GENE

Expression
Silencing
Editing

- MGC premier[®] cDNA and ORFs
- shERWOOD-UltramiR shRNA Collections
- transEDIT[™] CRISPR/Cas9 Reagents

MGC premier[®] cDNA and ORFs

Choose from Full-Length, Sequence Verified cDNA, Entry ORFs and Tagged ORFs



Available Options:

- Mutagenesis
- Vector Development
- Lentiviral Cloning and Packaging

	Full-length cDNA clone	pCS6 Expression-Ready cDNA	pTCP/TCN Expression-Ready cDNA	Entry ORF	Tagged ORF	Lentiviral ORF	Custom Cloning
Fully Sequenced	X	X	X	X	X	X	X
Quick turnaround	X	X		X		X	
Easy to transfer	X			X			
Expression-ready		X	X		X	X	X
Fluorescent marker						X	X
Lentiviral Packaging					X	X	X
Species	H, M, R, B, X, Z	H, M, R	H, M, R	H, M, Z	H, M, R	H	H, M, R

H=Human, M=Mouse, R= Rat, B=Bovine, X=Xenopus, Z=Zebrafish

Gene Expression

Largest Collection of Pre-made, Full-Length cDNA and ORFs

transOMIC technologies provides you with the highest sequence quality and confidence when purchasing pre-made, full-length cDNA and ORF clones. All inserts have been rigorously sequenced and are backed by a 100% guarantee to be an exact match to the published sequence. (MGC Project Team, 2009; Yang *et al.*, 2011).

MGC premier cDNA

cDNA clones include the open reading frame and untranslated regions (UTR)

- Sequence guaranteed*
- Ideal for native protein expression*
- Human, mouse, rat, bovine, Xenopus and zebrafish genomes represented*

Clonal Isolation and End-Sequence Option

Want to confirm the identity of your cDNA before shipping? We can streak isolate a colony and end sequence it before delivery. Select the end sequenced format when choosing your cDNA. This risk-free option ensures you start with a verified clonal culture.

MGC premier ORFs

Open Reading Frame (ORF) clones contain only the protein coding sequence allowing for the addition of fusion tags. MGC premier entry ORF collections represent human, mouse and zebrafish genes.

- Gateway adapted entry vectors: easy to transfer*
- Stop codon option: N- or C-terminal fusion tags*
- End sequenced: clone confirmation prior to shipping*

Available as individual clones, sets or entire genome

References:

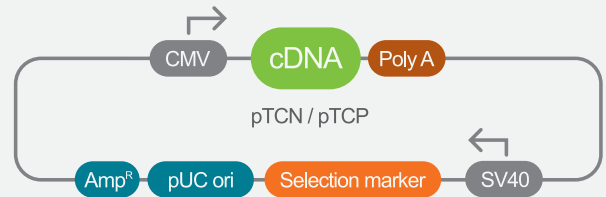
MGC Project Team, 2009. The completion of the Mammalian Gene Collection (MGC). *Genome Res.* 19: 2324-2333 and Supplemental Research Data.
Yang *et al.*, 2011. A public genome-scale lentiviral expression library of human ORFs. *Nat Methods.* 2011 Jun 26;8(8):659-61.

Guaranteed Sequence and Expression

MGC premier Expression-Ready cDNA

Best option for native protein expression

- Fully sequenced - better confidence in your results
- Strong promoter - robust expression
- Choice of selectable marker - transient or stable expression



pTCN/pTCP vector schematic

cDNA insert completely
resequenced after cloning
(pTCN and pTCP)

	Delivery Time	Mammalian Selectable Marker
pCS6	2 weeks	None
pTCN	4-6 weeks	Neomycin
pTCP	4-6 weeks	Puromycin

Gene Family and Pathway Focused Sets - cDNA and ORFs

Arrayed sets representing popular gene families and pathways are available for cDNA, entry ORFs and lentiviral ORFs. The table lists the available focused sets. Contact us for gene and clone information.

Apoptosis	Membrane transport
Breast Cancer 1000	Metastasis
Cell adhesion	Nuclear hormone receptor
Cell cycle	Phosphatases
Cytokine receptors	Protease
Cytokines	Transcription factor
Cytoskeleton	Ubiquitin ligases
Deubiquitinases (DUB)	Insulin signaling
Epigenetics-related gene set	Lymphocyte activation
Extracellular matrix	JAK/STAT pathway
GPCRs	Response to DNA damage
Helicases	B cell activation
Ion Channels	T cell activation
Kinases	p53 pathway

Don't see your gene family
or pathway of interest?
Contact info@biocat.com
for custom set options.

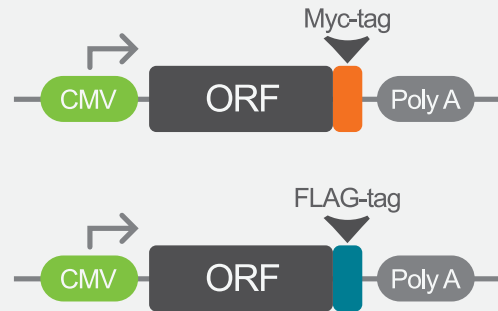
Gene Expression

Choice of Fusion Tags - Guaranteed Expression

MGC premier Tagged ORFs

Express over 40,000 human, mouse and rat proteins with a choice of fusion tags: Myc or FLAG[®]. All ORFs are fully sequenced and guaranteed to express.

- Expression-ready with validated tagged vectors
- Choice of fusion tags - added functionality
- Guaranteed expression - increase confidence in your results
- Insert completely resequenced before delivery

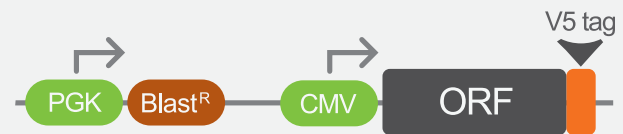


Vector schematic shows a C-terminal tag ORF. MGC premier Tagged ORF clones are available in N- or C-terminal fusions.

Lentiviral Tagged ORFs

Representing the human genome, this collection is available as V5 tagged ORFs in a lentiviral expression vector.

- Transfection or transduction delivery options
- Bacterial glycerol stock or viral particles format
- Express ORFs in primary and non-dividing cells
- Expression and sequence guaranteed



Lentiviral ORFs
available as
viral particles

Available as individual clones, gene family focused sets or entire genome

Go to www.biocat.com/mgc to find cDNA or ORF clones representing your gene of interest

(FLAG[®] is a registered trademark of Sigma-Aldrich)

Custom Solutions - Mutagenesis, Lentiviral Cloning and Packaging

Mutagenesis

Select from over 100,000 cDNA and ORF clones to create splice variants, modify domains or alter sequences to create mutants.

- Includes insertions, deletions and substitutions
- Best value for sequence variants
- Choice of expression vector including lentiviral vector option

Lentiviral Cloning and Packaging

Have any cDNA or ORF insert cloned into a lentiviral vector.

- Choice of selection marker
- Inducible or constitutive expression
- Available as bacterial glycerol stocks or ready-to-transduce lentiviral particles



Standard deliverable includes $>5 \times 10^6 - 1 \times 10^7$ TU/ml functional titer at volumes of 100-300 μ l.

Note: Size and sequence of insert can impact titers.

Find more details and order custom clones at www.biocat.com/mgc

Gene Silencing

shERWOOD-UltramiR shRNA Collections

Sensor-based shERWOOD Algorithm + UltramiR Scaffold = Best Potency and Specificity

Developed by Dr. Greg Hannon and colleagues at Cold Spring Harbor Laboratory (CSHL), new generation shRNA with shERWOOD algorithm based design and optimized UltramiR scaffold produce increased small RNA processing for more consistent and potent knockdown efficiency.

- *Consistent and potent knockdown - guaranteed**
- *Enhanced microRNA scaffold - increased small RNA processing*
- *Genome scale coverage – human, mouse and rat*

*All shRNA-mir constructs in a target gene set are guaranteed to knock down mRNA expression by >70%. For details see www.biocat.com/ultramir

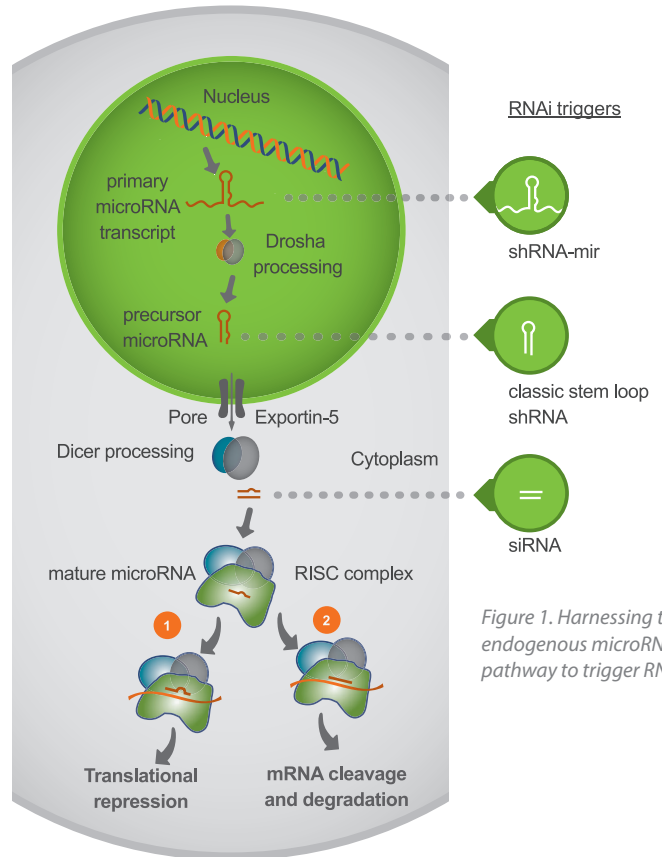


Figure 1. Harnessing the endogenous microRNA pathway to trigger RNAi.

Vector options

shERWOOD UltramiR shRNA for human, mouse and rat genomes are available in a choice of vectors, promoters and reporters.

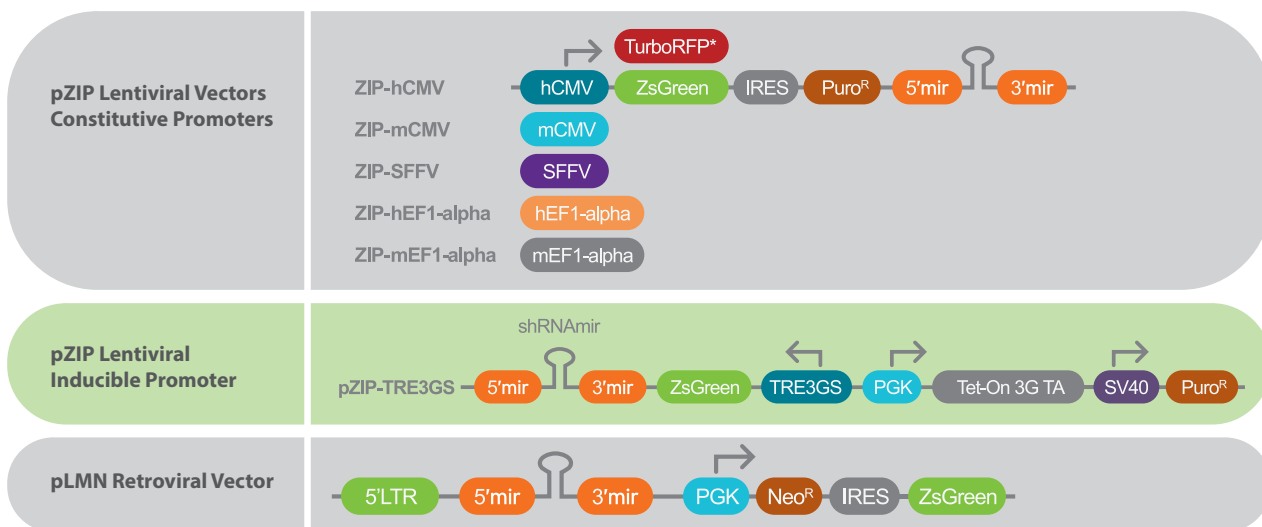


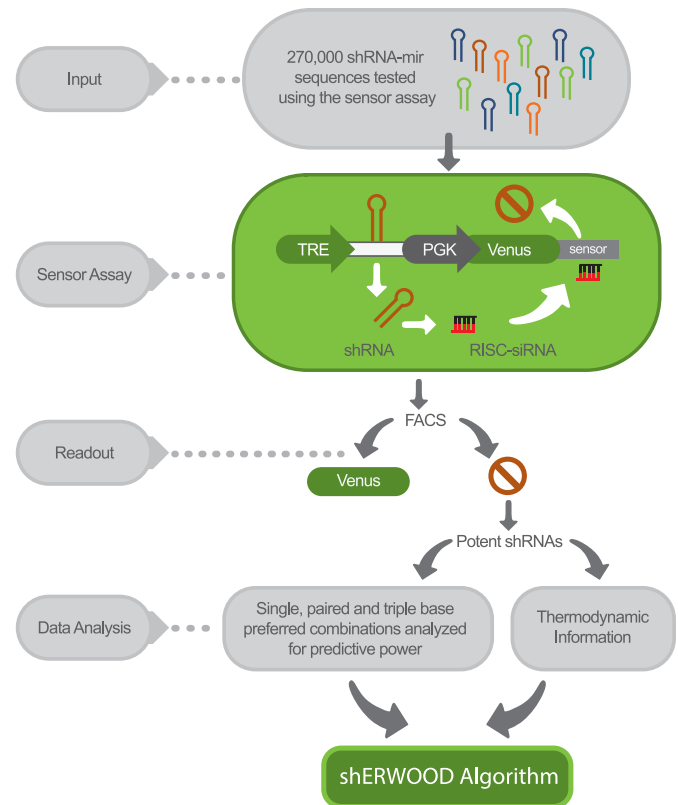
Figure 2. Vector cartoon showing multiple options with variable promoter and reporter choices. Options for the different ZIP vectors are shown vertically without repeating elements that are the same between all ZIP vectors. *TurboRFP is available for ZIP-mCMV, ZIP-hCMV and ZIP-SFFV.

shERWOOD Algorithm: Sensor-based for enhanced knockdown efficiency

A high-throughput “sensor” assay was used by the Hannon lab to test 270,000 shRNA-mir sequences for their ability to knock-down their target (or sensor) gene fused to a fluorescent reporter “Venus”. Short hairpin RNAs that effectively inhibited the expression of their gene targets in the sensor would also inhibit expression of the reporter gene, resulting in loss of fluorescence (schematic). shRNA sequences targeting every gene in the human genome were tested for potency using the sensor assay and the data on sequence requirements for the rare, potent hairpins were used to train the shERWOOD algorithm (Knott *et al.*, 2014).

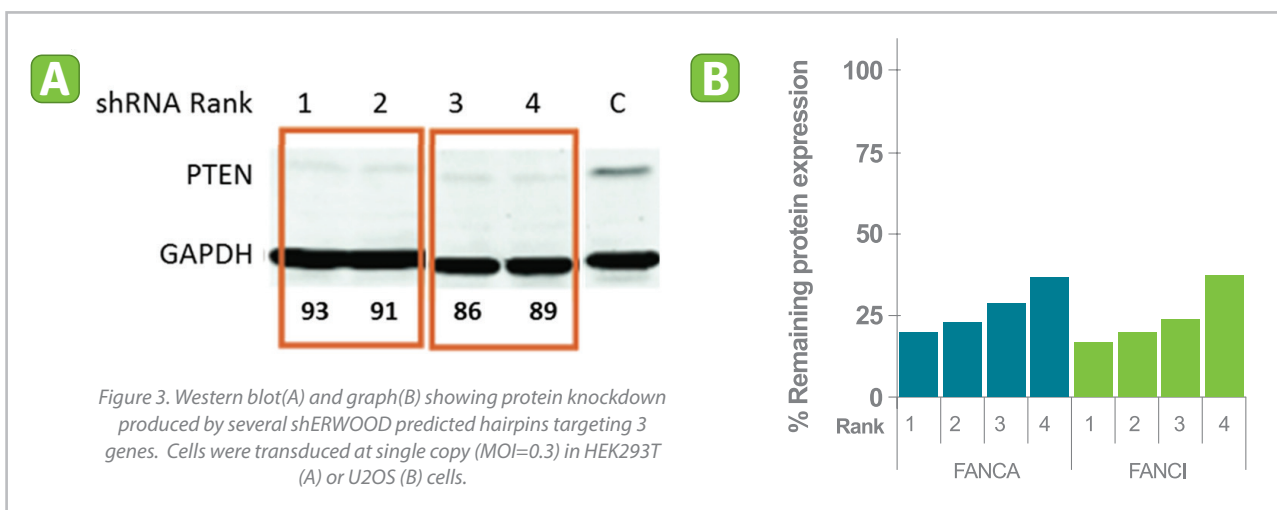
Features of the shERWOOD Algorithm

- Optimized to predict designs producing potent single copy knockdown
- All shRNA designs are scored and ranked
- Designs target all gene transcripts
- Algorithm includes filters to minimize off-target effects



shERWOOD predicted ranks correlate with potent knockdown

Western blot analysis showing protein knockdown in HEK293T or U2OS cells after single copy transductions of shERWOOD predicted shRNA sequences targeting PTEN, FANCA or FANCI. Top ranked hairpins targeting each gene produced effective and consistent protein knockdown.



Gene Silencing

UltramiR - Increased Small RNA processing = Increased Knockdown

The miR-30 scaffold has been further optimized based on conserved domains shown to be important determinants of primary microRNA processing by Drosha (Auyeung *et al.*, 2013). This enhanced microRNA scaffold increases small RNA levels presumably by improving biogenesis. When shRNA were placed into the UltramiR scaffold, mature small RNA levels were significantly increased relative to levels observed using the standard miR-30 scaffold (roughly two fold. Figure 4) This increase in small RNA processing produces a corresponding increase in knockdown efficiency (Knott *et al.*, 2014).

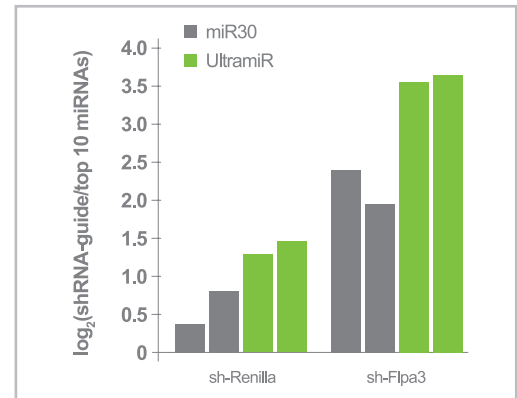


Figure 4. Relative abundances of processed guide sequences for two shRNA as determined by small RNA cloning and NGS analysis when cloned into traditional miR-30 and UltramiR scaffolds. Values represent log-fold enrichment of shRNA guides with respect to sequences corresponding to the top 10 most highly expressed endogenous microRNA.

Consistent knockdown efficiency relative to early generation shRNA

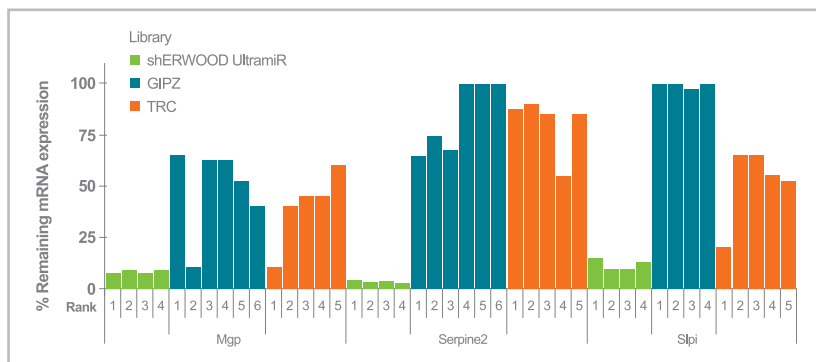


Figure 5. Knockdown efficiencies for shERWOOD UltramiR shRNA targeting mouse Mgp, Slpi and Serpine2. Mouse 4T1 cells were infected at single copy and knockdown was tested following selection of infected cells.

The combination of the shERWOOD algorithm and UltramiR scaffold consistently produces potent shRNA. Knockdown efficiencies of shERWOOD-UltramiR hairpins were benchmarked against existing TRC and GIPZ early generation shRNA-mir hairpins targeting 3 different genes. shERWOOD-UltramiR designs produced very potent and consistent knockdown relative to available TRC and GIPZ hairpins targeting the same genes (Knott *et al.*, 2014).

More potent shRNA per gene enables superior hit stratification

To benchmark the shERWOOD algorithm design against the early generation TRC and Hannon Elledge (GIPZ) shRNA designs, the Hannon lab (Knott *et al.*, 2014) performed a large scale screen using each of these designs to target 2200 genes that were likely to impact growth and survival based on gene ontology. Inclusion as a hit required that at least 2 shRNA for that gene were depleted. The box plot shows the average percentage of shRNA per gene that were scored as hits. The data shows that the shERWOOD 1U designs produce a higher percentage of potent shRNA per hit compared to early generation design. This makes for more confidence in screen hits and ultimately fewer false positives and negatives from shRNA screens.

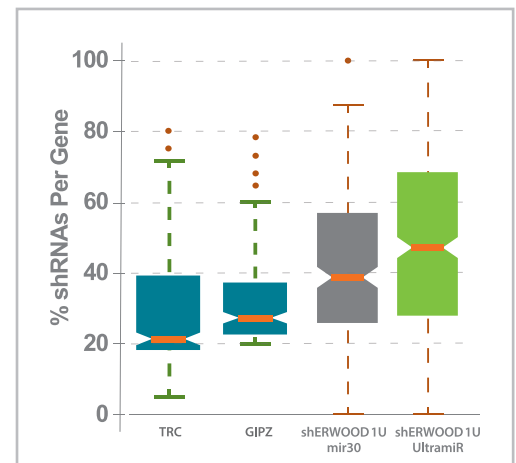


Figure 6. Percentage of shRNA targeting essential genes that depleted in each of the TRC, GIPZ, shERWOOD or shERWOOD-UltramiR shRNA screens.

Improved specificity versus classic stem loop shRNA

Knockdown specificity of the shERWOOD-UltramiR shRNA was assessed using RNA-seq on all cell lines expressing shERWOOD-UltramiR or TRC shRNA targeting Slpi and Mgp. Less than 25 genes were altered in their expression (fold change > 2 and FDR < 0.05) between two cell lines silenced with shERWOOD-UltramiR. Over 500 genes are altered in the line where Mgp has been silenced using the TRC constructs, and approximately 250 are altered in the line expressing the TRC Slpi-shRNA.

This is consistent with other publications showing classic stem loop shRNA can cause significant off-target effects and toxicity. Several reports (Beer *et al.*, 2010, Castanatto *et al.*, 2007, Pan *et al.*, 2011, Baek *et al.*, 2014, Knott *et al.*, 2014) have shown that off-target effects can be ameliorated by expressing the same targeting sequence in a primary microRNA scaffold (shRNA-miR).

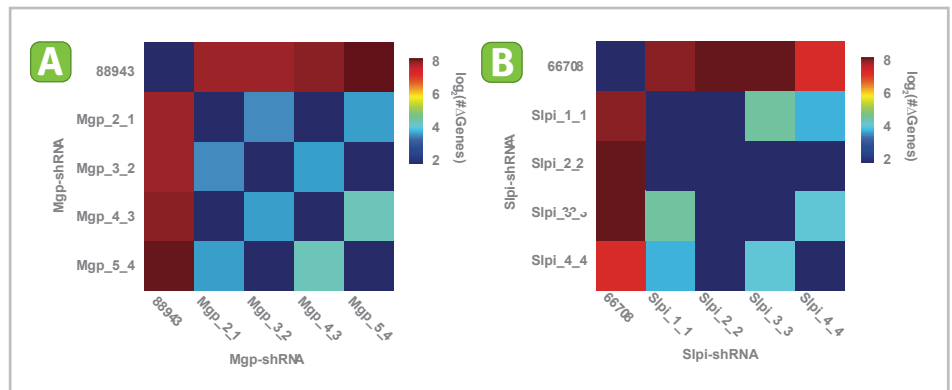


Figure 7. Heat map showing the number of differentially expressed genes (> 2-fold change and FDR < 0.05) identified through pairwise comparisons of the cell lines corresponding to (A) Mgp and (B) Slpi knockdown by the shERWOOD-UltramiR selected shRNAs and the TRC shRNAs 88943 and 66708.

Determine the optimal promoter for your cell line

Mammalian promoters may differ in expression level or be silenced over time depending on the target cell line. Variation in expression level can affect fluorescent marker expression as well as knockdown efficiency. For cell lines where the optimal promoter is unknown, the ZIP promoter testing kit includes ready-to-use lentiviral particles expressing ZsGreen from three different promoters (human CMV, murine CMV or SFFV). The fluorescent marker and shRNA are on the same transcript allowing a quick visual assessment of expression efficiency.

The Promoter selection kit can be used to test shRNA expression in hard to transfect cells to select the promoter that produces optimal expression. Target gene sets or pooled shRNA libraries can be ordered in a choice of promoters, reporters and vectors.

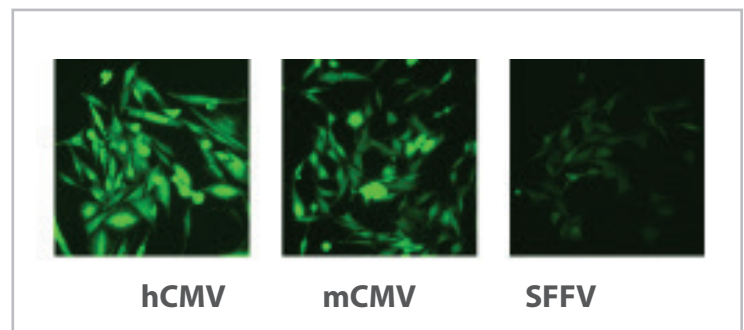


Figure 8. Visualizing expression levels from different promoters in D2.OR cells. Cells were transfected at similar titers. Fluorescence reflects expression levels of transcript which includes the shRNA-miR.

How to order

Visit www.biocat.com/ultramir and insert a keyword, a gene symbol or a gene ID for your gene of interest into the search tool. shERWOOD-UltramiR shRNA are available for human, mouse and rat genomes and can be purchased to target individual genes, gene families and pathways or the genome. Pooled shRNA screening libraries are also available targeting gene families, pathways, custom gene lists or the genome. Bacterial glycerol stock or lentiviral particle formats are provided.

Gene Silencing

shERWOOD-UltramiR pooled shRNA screening libraries

shERWOOD-UltramiR pooled shRNA screening libraries combine superior knockdown efficiency with optimized shRNA processing and a stringent equimolar pooling process to create a powerful pooled RNAi screening reagent. Equimolar pooling limits shRNA drop out and biased results, while new generation designs provide robust knockdown allowing more consistent and sensitive hit detection. Lentiviral pooled screening libraries are available targeting the whole genome, gene families, pathways or your custom gene list.

- More potent shRNA per gene – decrease false positive and false negative results
- Every clone is sequence verified – eliminate unwanted background from mutations introduced in chip-based pools
- Equimolar pooling process – reduces variation between samples. Over 90% are within 5 fold of each other

Optimize your pooled shRNA library

- Choice of promoter for optimal shRNA expression
- Plasmid DNA or high titer lentiviral particles
- In vitro or in vivo mini-pool format
- Pool deconvolution and analysis

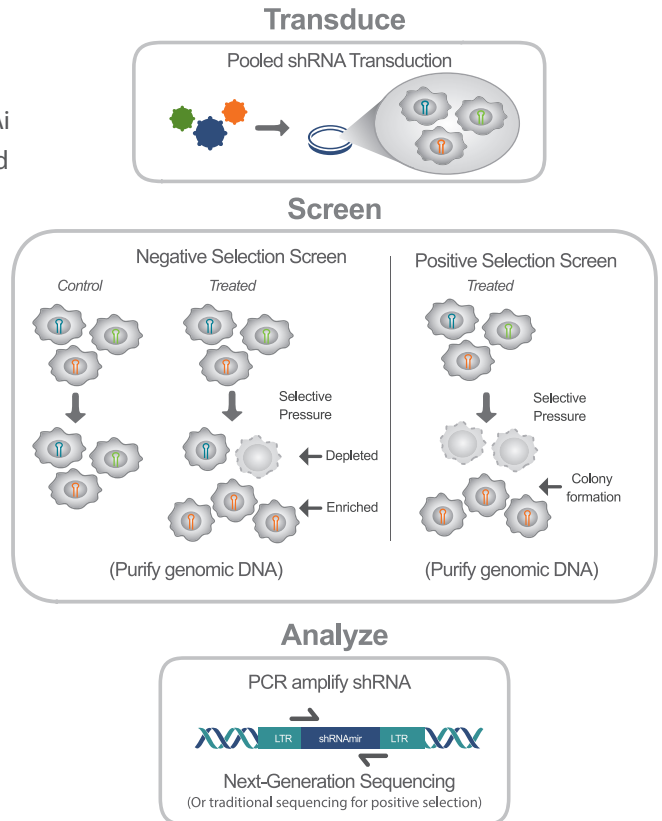


Figure 9. Schematic of pooled shRNA screening workflow. Cells are transduced. Positive or negative selection screens are performed. PCR amplification and sequencing of the shRNA integrated into the target cell genome allows the determination of shRNA representation in the population.

Simplify Your Screen with Sequencing and Deconvolution Services



Need help with pooled screen deconvolution? Let us analyze data from your genomic DNA samples.

- Simplify your screen with our sequencing service including library preparation, NGS and deconvolution
- Optimized protocols and reagents for shRNA specific sequencing
- Quick turnaround with 6-8 weeks from receipt of samples to delivery of data.
- Cost effective with multiplex sequencing to minimize costs.

References:

Knott et al., 2014. *Molecular Cell* 56, 1-12. Castanotto et al., 2007. *Nucleic Acids Res.* 35(15):5154-51.; McBride et al., 2008. *PNAS* 105; 15,5868-5873. Auyeung et al., 2013. *Cell* 152:844-858. Beer et al., 2010. *Mol Ther* 18(1):161-170.; Pan et al., 2011. *FEBS Lett.* 6;585(7):1025-1030. Baek et al., 2014. *Neuron* 82, 1255-1262.

transEDIT™ CRISPR-Cas9 Reagents

Optimized gRNA designs, versatile vectors and flexible formats for efficient gene editing

transEDIT CRISPR-Cas9 lentiviral reagents provide powerful tools for genome editing, offering optimized gRNA designs cloned into a choice of expression vectors and formats for engineering specific gene knockouts.

transEDIT reagents include lentiviral expression vectors containing specific gRNA targeting your gene of interest in various formats:

- (1) Single or paired gRNA plus Cas9 in an all-in-one configuration
- (2) Single or paired gRNA expression vectors for co-delivery with a Cas-9 nuclease or nickase expression vector.

Cas9 nuclease and nickase expression vectors are available with different selectable markers and fluorescent reporters for efficient selection.

- *Single or paired guide RNA CRISPR strategies for gene editing*
- *All-in-one or single guide RNA delivery - including inducible Cas9*
- *Multiple vectors to enable dual or triple selection for enhanced efficiency*

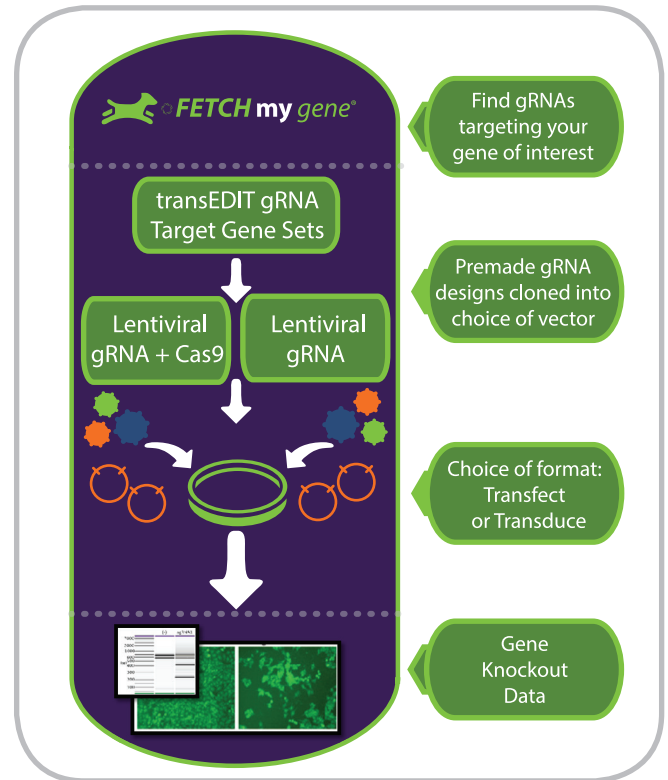


Figure 1. Schematic showing transEDIT CRISPR Cas9 for easy gene editing

transEDIT vector options for optimal guide RNA and Cas9 Expression and Selection

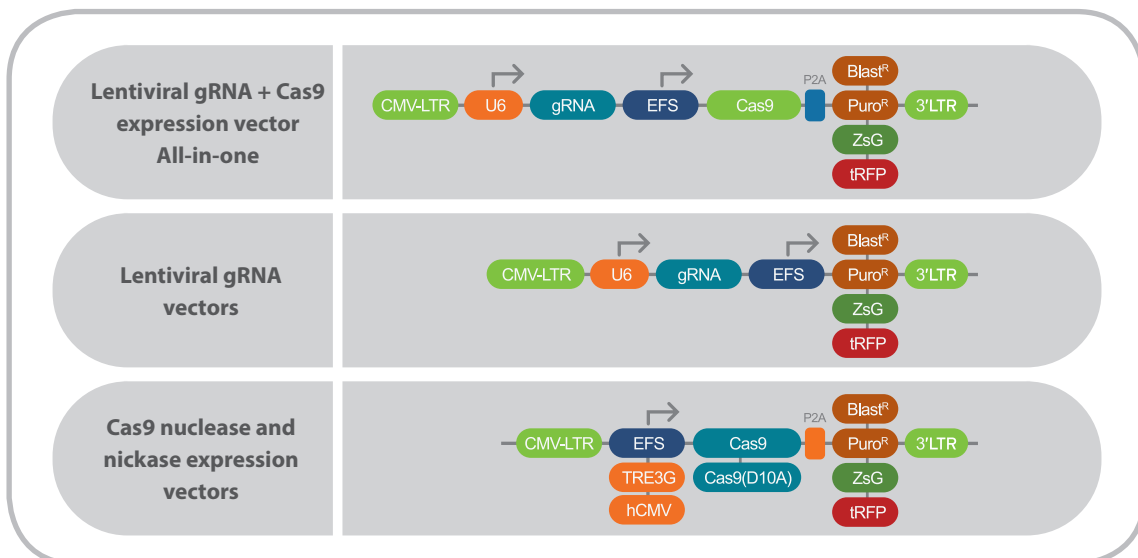


Figure 2: Lentiviral expression vectors for guide RNA and Cas9 showing different promoter, reporter and selectable marker options

Gene Editing

Detecting targeted double-stranded breaks in DNA

transEDIT lentiviral gRNA and Cas9 all-in-one expression vectors targeting DYRK1A and TP53 were transduced at low copy in HEK293T cells and surveyor assay used to detect percentage of indel frequency.

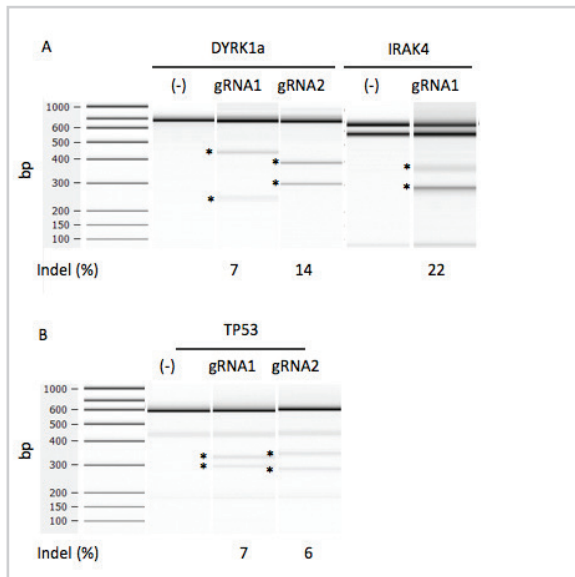


Figure 3. Surveyor assay for indel frequency analysis. A. HEK293T cells transduced with pCLIP-All targeting DYRK1A and IRAK4 B. Cas9 expressing HEK293T cells transduced with pCLIP-All targeting tp53. (*indicated expected fragment sizes)

Selection Provides Greater Genome Editing Efficiency

The level of Cas9 endonuclease expression has been shown to affect the frequency of generating genome-edited clones. Vector delivery and expression are critical determinants of genome editing efficiency. The ability to select for cells with high Cas9 expression results in a higher indel frequency in the population. All transEDIT Cas9 expression vectors include selection markers for enrichment.

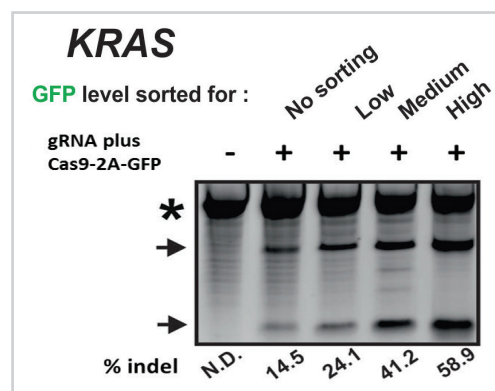


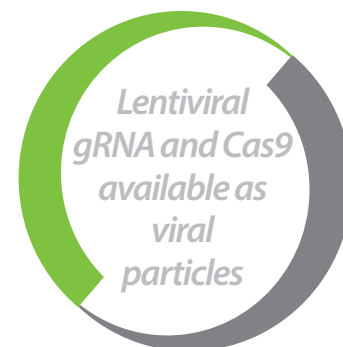
Figure 4. Fluorescent marker linked to Cas9 expression enables the selection of cells with high indel frequency. GFP expression was directly linked to Cas9 expression via P2A peptide. FACS was used to bin cells into low, medium and high expression of the fluorescent marker. Indel frequency was measured using the CEL-I Surveyor assay and the percentages are shown at the bottom of each lane. Cells enriched for the highest fluorescence expression showed the highest indel frequency. Adapted from Nucleic Acids Res. 2014;42(10):e84.

How to order

Simple - visit www.biocat.com/transedit and insert a keyword, a gene symbol or a gene ID for your gene of interest into the search tool.

Flexible - select the standard vector and format of your choice for your species of interest. Need more than the standard option? Please contact us for additional vector, promoter, selection markers and formats for single, paired nickase.

Fast - receive your ready-to-use transEDIT CRISPR-Cas target gene set to quickly start your gene editing experiment.



Contact info@biocat.com to ask about custom cloning gRNAs and generating lentiviral vector particles

Gene Expression

www.biocat.com/mgc

SPECIAL

MGC *premier* cDNA Clones **15% OFF*** | MGC *premier* ORF Libraries **Special Price***

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Keyword | Cat.No. | Symbol | Gene ID | Uniprot

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 Catalyze Your Success.

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Product Groups

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- ELISA Kits
- Proteins
- Cell-based Assays
- Stem Cell Research
- Metabolism Assays
- microRNA Analysis
- Epigenetics
- PCR
- Cloning
- Viral Systems
- Tissue-specific Analysis
- Reporter Assays
- Diabetes
- Fluorescent Proteins
- Sample Preparation
- Next Generation Sequencing
- Protein Expression
- Transcription Factors

MGC premier cDNA Clones

Largest Collection of Full-Length cDNA Clones


Full length, sequence-verified cDNA clones

MGC premier cDNA clones from transOMIC provide the highest sequence quality and confidence when purchasing pre-made full length cDNA clones.

- Ideal for native protein expression
- Full insert sequence easily available
- Best value on the market today

Based on the Mammalian Gene Collection (MGC) developed by the National Institutes of Health (NIH) with rigorous sequence analysis resulting in less than 1 error in 50,000 bp (MGC Project Team 2009). All MGC premier cDNA clones are backed by a 100% guarantee to be an exact match to the published sequence.

Full length, sequence-verified cDNA clones are available for human, mouse, rat, bovine, Xenopus and zebrafish genomes. These collections are available as individual cDNA clones, rearrays of cDNA clones for gene families and



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Find your cDNA Clone

Keyword | Cat.No. | Symbol | Gene ID

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- MGC_premier Human cDNA Sets
- Yeast Knockout Collections

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- Protein Expression
- Transcription Factors

shERWOOD-UltramiR shRNA


Targeting Human, Mouse and Rat Genomes

shERWOOD-UltramiR Highlights

- shERWOOD algorithm design for superior knockdown performance
- UltramiR scaffold for increased shRNA processing and potency
- Minimized off-target effects
- All transcripts for a gene are targeted for the most complete knockdown
- Every shRNA is guaranteed to knockdown its intended target*

Superior knockdown with shERWOOD design

shERWOOD-UltramiR shRNA reagents are next generation vector-based RNAi triggers designed using the proprietary shERWOOD algorithm developed and validated in Dr. Gregory Hannon's laboratory at Cold Spring Harbor Laboratory (Knott et al., 2014). An alternate microRNA scaffold "UltramiR" has been optimized for increased shRNA processing and potency based on new information on the key determinants for primary microRNA processing (Auyeung et al., 2013). The shERWOOD algorithm is based on the functional testing of over 250,000



Find shERWOOD-UltramiR shRNA

Keyword | Symbol | Gene ID

Technical Details

- Manuals
- Product Brochure

Controls

- shERWOOD-UltramiR shRNA Negative Controls
- shERWOOD-UltramiR shRNA Positive Controls

Genomics Resources

- cDNA_ORF & shRNA Clones

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Keyword | Cat.No. | Symbol | Gene ID | Uniprot

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- Stem Cell Research
- Metabolism Assays
- microRNA Analysis
- Epigenetics
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- Cloning
- Viral Systems
- Tissue-specific Analysis
- Reporter Assays
- Diabetes
- Fluorescent Proteins
- Sample Preparation
- Next Generation Sequencing
- Protein Expression
- Transcription Factors

transEDIT CRISPR/Cas9


Lentiviral Vectors | Flexible Markers | Tet-Inducible Option

Ready-to-go CRISPR Kits covering > 67,000 Genes

- All-in-one or single guide RNA delivery - including inducible Cas9
- Multiple vectors to enable dual or triple selection for enhanced efficiency
- Single or paired guide RNA CRISPR/Nickase strategies

transEDIT CRISPR/Cas9 lentiviral reagents provide powerful tools for genome editing, offering optimized gRNA designs cloned into a choice of expression vectors and formats for engineering specific gene knockouts. The vectors are designed to select for high gene editing efficiency using direct indicators of Cas9 expression from fluorescent or selection markers as well as efficient delivery as plasmid or lentiviral particles. In addition, complementary markers for co-selection of Cas9 and gRNA ensure efficient expression of both components necessary for Cas9 based genome editing. transEDIT reagents include lentiviral expression vectors containing specific gRNA targeting your gene of interest in various formats:

- Single or paired gRNA plus Cas9 in an all-in-one configuration



Find Your Gene to be Edited

Keyword | Symbol | Gene ID

transEDIT Technical Details

- Product Brochure
- Manuals

Other CRISPR/Cas9 Reagents

- Cas9 SmartNuclease Vectors
- Cas9 SmartNuclease Lentiviral Vectors
- CRISPR/Cas9_pCAS-Guide Vectors
- Homologous Recombination Vectors

Genomics Resources

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