shERWOOD - UltramiR shRNA Collections

Enabling Discovery Across the Genome

shERWOOD - UltramiR shRNA Collections

Incorporating advances in shRNA design and processing for superior potency and specificity

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shERWOOD-UltramiR shRNA

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.

Advantages Include:

- ***Consistent and potent knockdown – 100% Guaranteed****
- ***Enhanced microRNA scaffold - increased small RNA processing***
- ***Genome scale coverage – human, mouse and rat***

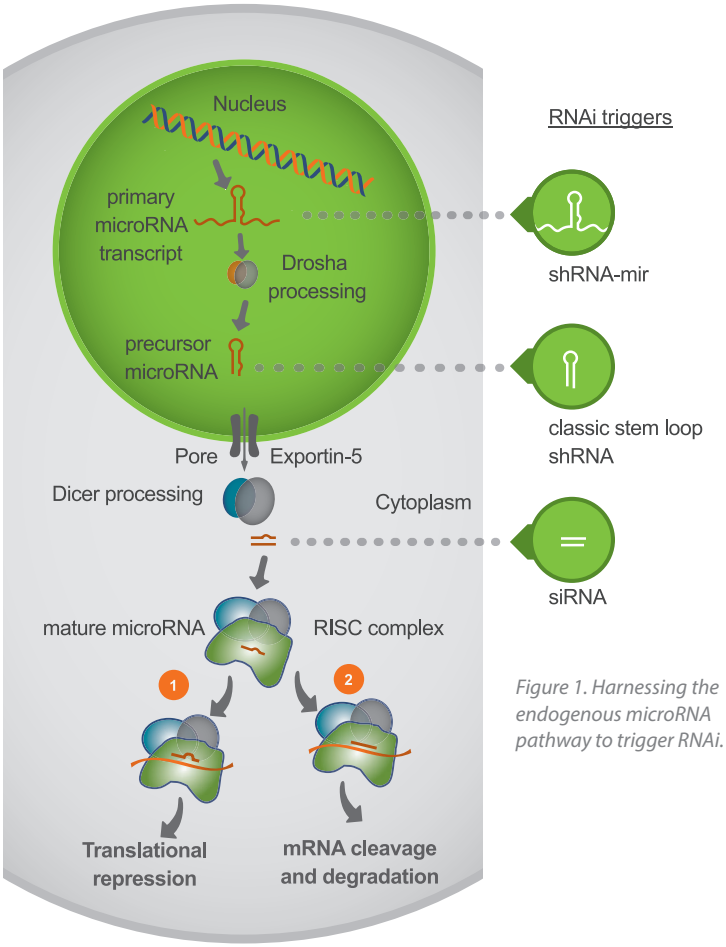


Figure 1. Harnessing the endogenous microRNA pathway to trigger RNAi.

Vector options

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

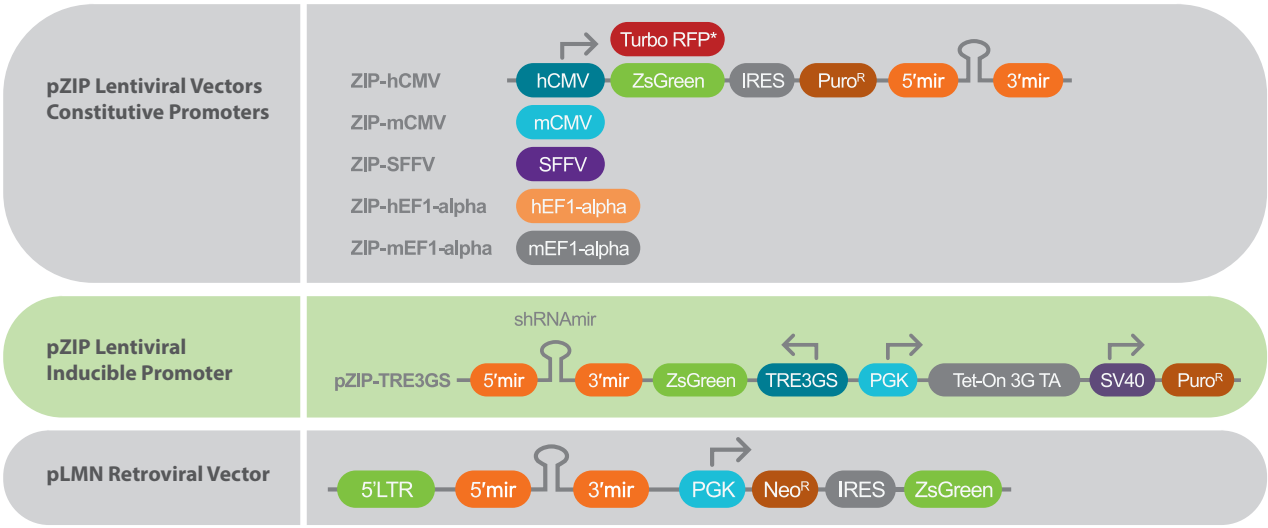
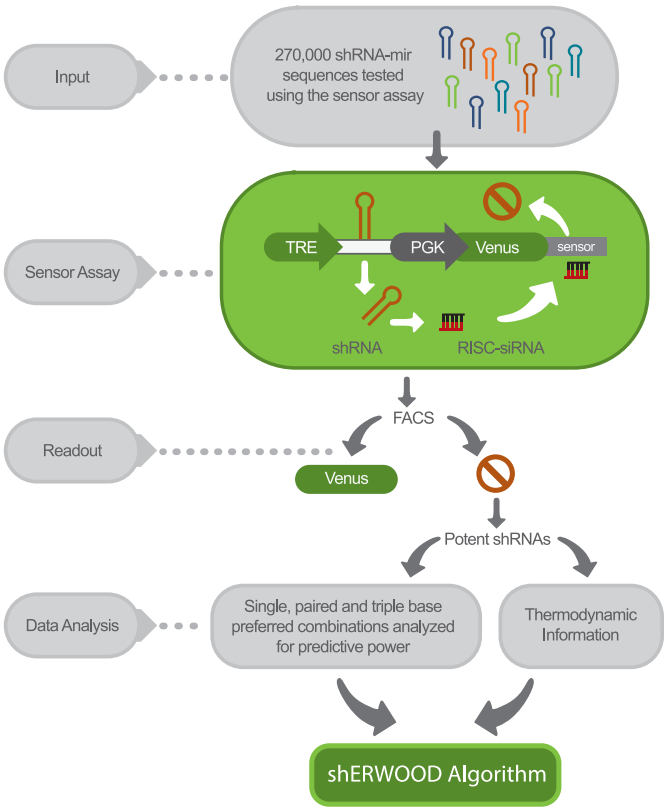


*Figure 2. Vector cartoon showing multiple options with variable promoter and reporter choices. Options for the different ZIP vectors are shown vertically without repeating elements that are the same between all ZIP vectors. *tRFP is available for ZIP-hCMV, ZIP-mCMV and ZIP-SFFV.*

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).

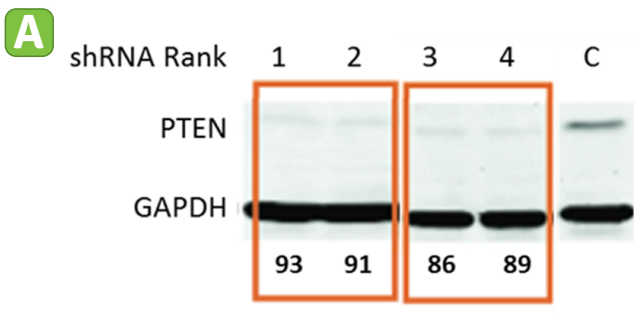


Features of the shERWOOD Algorithm

- ***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.



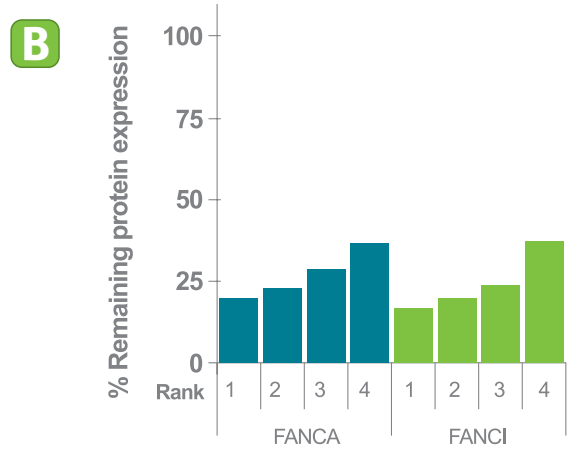


Figure 3. Western blot(A) and graph(B) showing protein knockdown produced by several shERWOOD predicted hairpins targeting 3 genes. Cells were transduced at single copy (MOI=0.3) in HEK293T (A) or U2OS (B) cells.

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 e*t al.,* 2014).

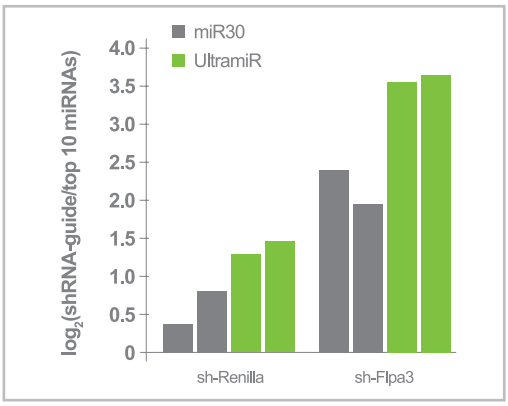


Figure 4. Relative abundances of processed guide sequences for two shRNA as determined by small RNA cloning and NGS analysis when cloned into traditional miR-30 and UltramiR scaffolds. Values represent log-fold enrichment of shRNA guides with respect to sequences corresponding to the top 10 most highly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

Consistent knockdown efficiency relative to early generation shRNA

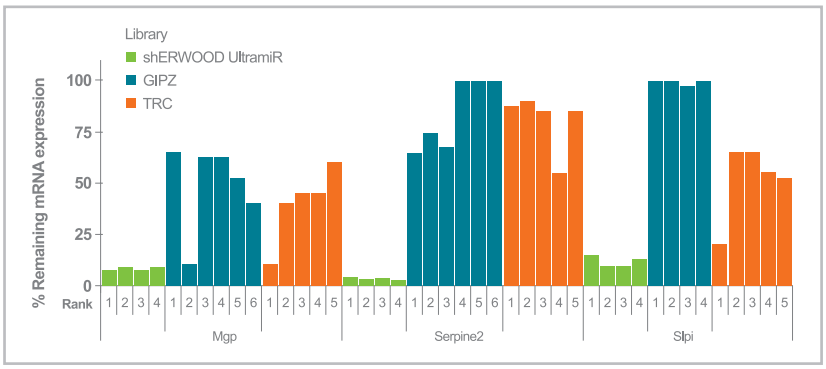


Figure 5. Knockdown efficiencies for shERWOOD UltramiR shRNA targeting mouse Mgp, Slpi and Serpine2. Mouse 4T1 cells were infected at single copy and knockdown was tested following selection of infected cells.

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 e*t 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 e*t 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.

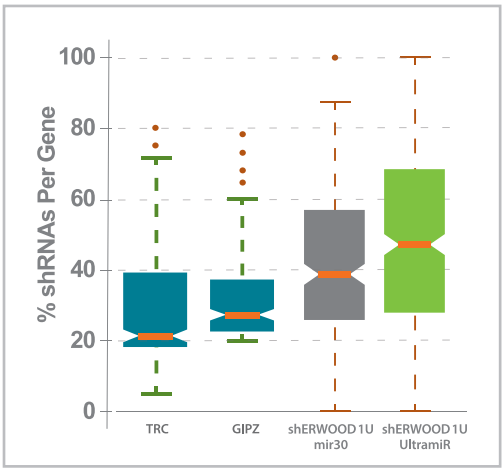


Figure 6. Percentage of shRNA targeting essential genes that depleted in each of the TRC, GIPZ, shERWOOD or shERWOOD-UltramiR shRNA screens.

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.

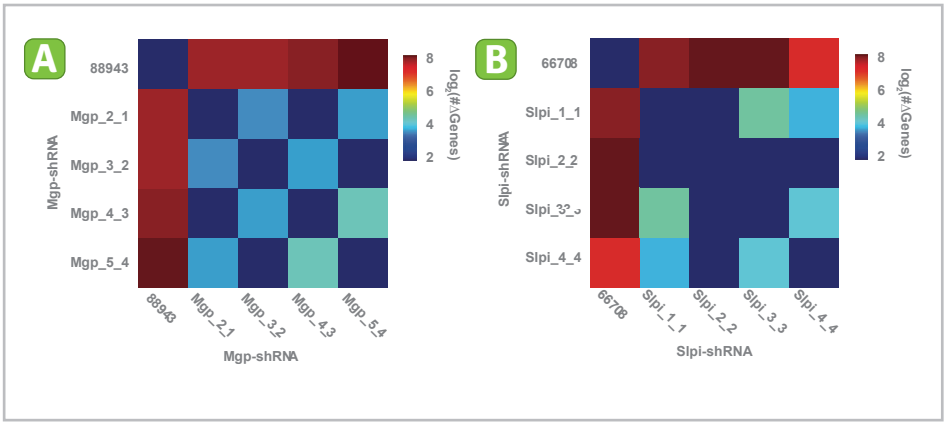


Figure 7. Heat map showing the number of differentially expressed genes (> 2-fold change and FDR <0.05) identified through pairwise comparisons of the cell lines corresponding to (A) Mgp and (B) Slpi knockdown by the shERWOOD-UltramiR selected shRNAs and the TRC shRNAs 88943 and 66708.

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 e*t al.,* 2014) have shown that off-target effects can be ameliorated by expressing the same targeting sequence in a primary microRNA scaffold (shRNA-miR).

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.

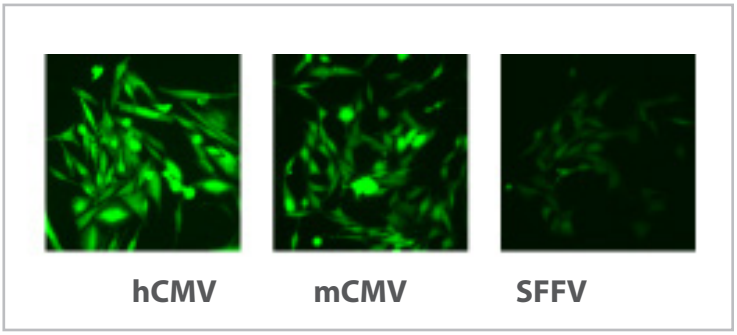


Figure 8. Visualizing expression levels from different promoters in D2.OR cells. Cells were transduced at similar titers. Fluorescence reflects expression levels of transcript which includes the shRNA-mir.

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.

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*

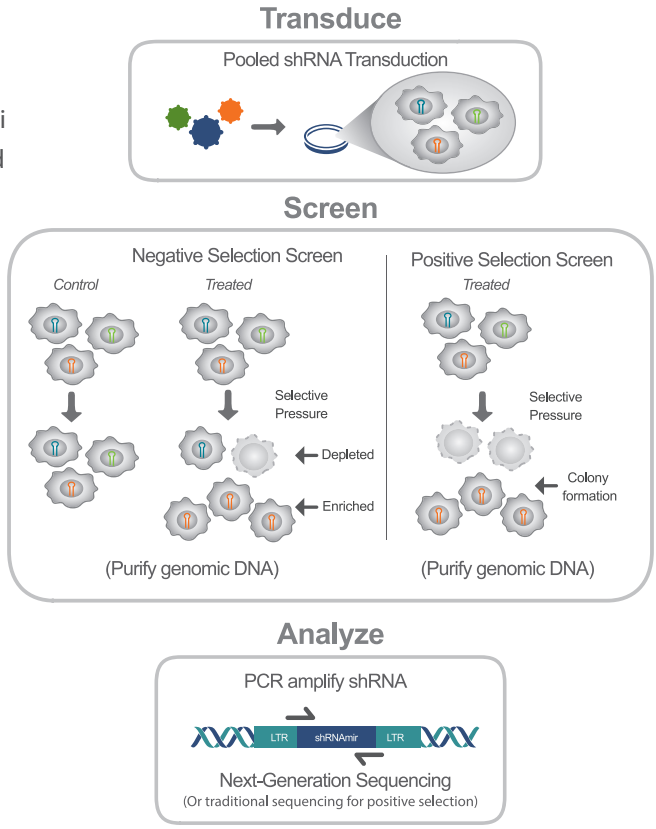


Figure 9. Schematic of pooled shRNA screening workflow. Cells are transduced. Positive or negative selection screens are performed. PCR amplification and sequencing of the shRNA integrated into the target cell genome allows the determination of shRNA representation in the population.

Simplify Your Screen with Sequencing and Deconvolution Services

Pooled shRNA Screen Genomic DNA Isolation → shRNA Amplification → NGS Library Preparation → Next Gen. Sequencing → Deconvolution for shRNA counts

Included in Service

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.

FETCH my gene®

FETCH *my* gene® search tool is designed to help you easily find shRNA-mir clones for your gene of interest and confirm your results using the gene information provided. Use gene accession, gene symbol or gene ID to begin your search.

1. Search

Input your search term into FETCH *my* gene®.

2. Choose product type

Quickly filter product type using tabs.

3. Confirm gene and product

Validate your gene of interest with additional gene information provided.

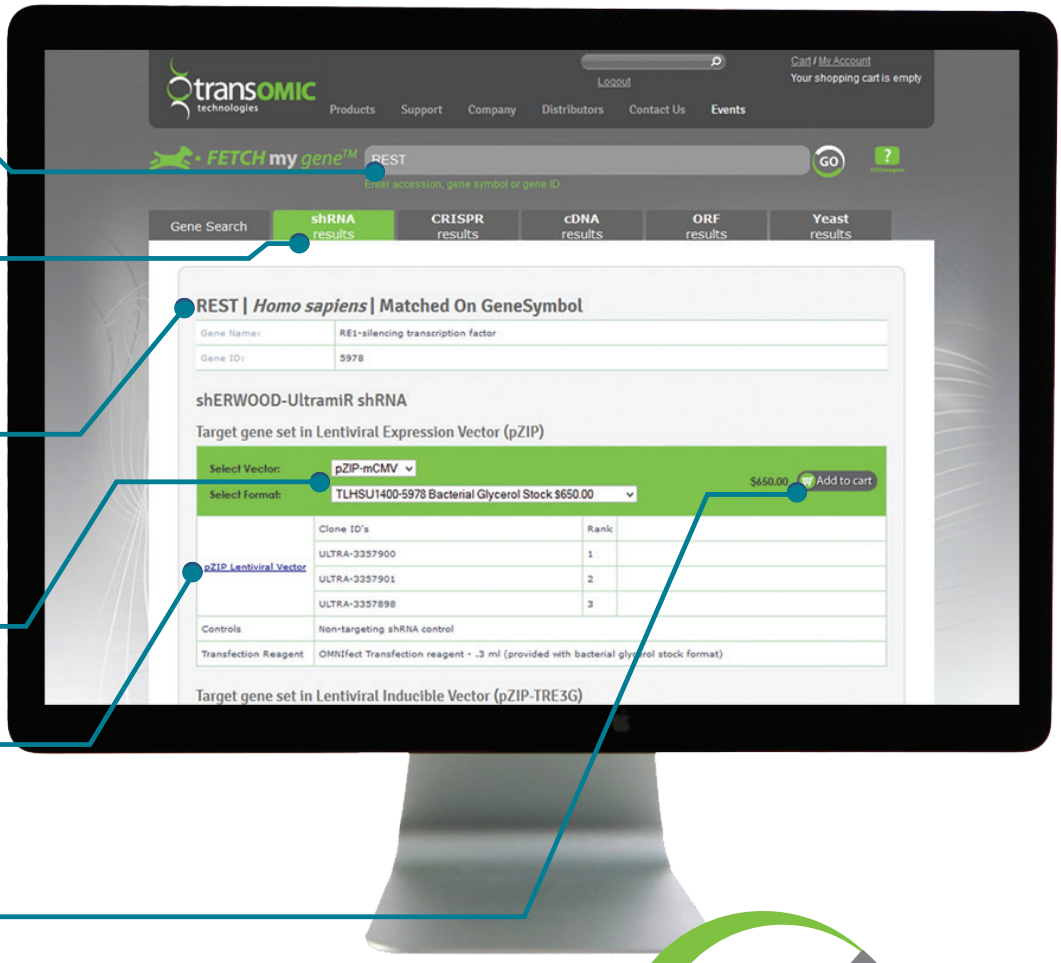
4. Select vector and format

Use drop down to select your vector and format.

Link to vector and clone information page.

5. Add to cart

Click add to cart to purchase or continue shopping.



Ordering shRNA-mir reagents:

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. Lentiviral, inducible lentiviral and retroviral vectors can be purchased in several promoter/reporter configurations. Bacterial glycerol stock or lentiviral particle (10^6 - 10^9 TU/ml) formats are provided.

For more information and to order shRNA products, come to ***www.transomic.com*** or email us at ***info@transomic.com.***

100% guaranteed knockdown

All shRNA-mir constructs in a target gene set are guaranteed to knockdown mRNA expression >70%. Cell line of choice should show target gene expression and demonstrate gene knockdown using positive and negative controls.

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.

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