

# RNAi Codex: a portal/database for short-hairpin RNA (shRNA) gene-silencing constructs

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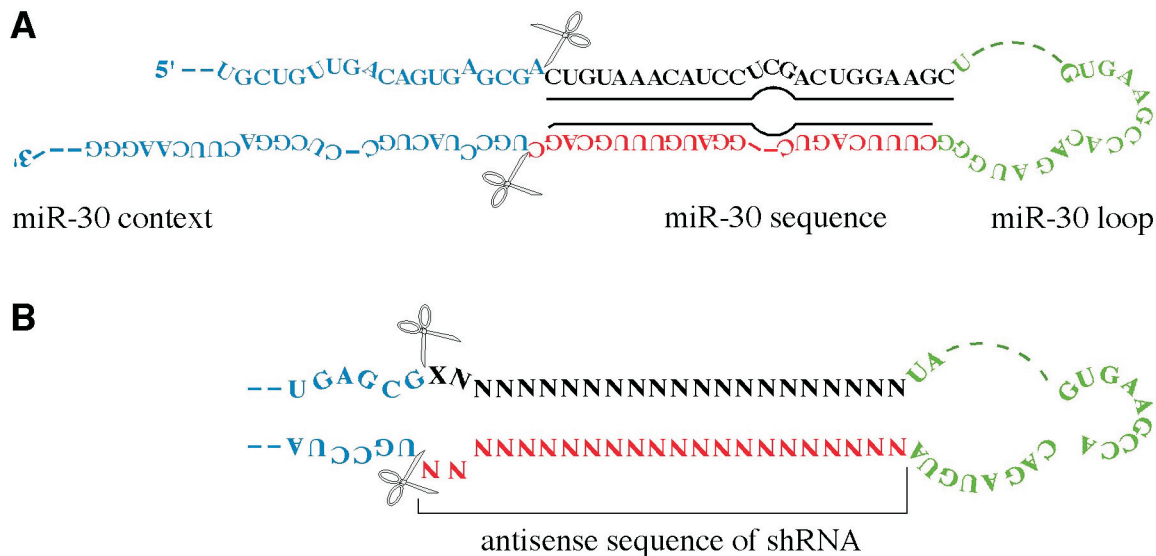
## ABSTRACT

Use of RNA interference (RNAi) in forward genetic screens is proliferating. Currently, short-interfering RNAs (siRNAs) and short-hairpin RNAs (shRNAs) are being used to silence genes to tease out functional information. It is becoming easier to harness RNAi to silence specific genes, owing to the development of libraries of readymade shRNA and siRNA gene-silencing constructs by using a variety of sources. RNAi Codex, which consists of a database of shRNA related information and an associated website, has been developed as a portal for publicly available shRNA resources and is accessible at <http://codex.cshl.org>. RNAi Codex currently holds data from the Hannon–Elledge shRNA library and allows

the use of biologist-friendly gene names to access information on shRNA constructs that can silence the gene of interest. It is designed to hold user-contributed annotations and publications for each construct, as and when such data become available. We will describe features of RNAi Codex and explain the use of the tool.

## INTRODUCTION

RNAi, or RNA interference, is the disruption of the expression of a gene by a double-stranded RNA (dsRNA), in which one strand is complementary (either perfectly or imperfectly) to a section of the gene's mRNA (1). A dsRNA can enter the cytoplasm, through the expression of a hairpin (or inverted repeats), through viral gene expression or through artificial



**Figure 1.** miR-30 based shRNA design (12). The figure shows the architecture of the constructs that are currently in RNAi Codex. The upper hairpin (A) is the primary transcript of the miR-30 miRNA. The sense and antisense strands are underlined. The lower hairpin (B) is the shRNA designed within the miR-30 context. The N's show the position of the sense and antisense strands on the hairpin. The figure has been adapted from the Open Biosystems' website (<http://www.openbiosystems.com>).

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Curated list

**Limit**  
☒ Show all hairpins  
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Date: From  To  (YYYY-MM-DD)

**Your Query :** Automatic p53 IN ALL **Limits:** Exclude "Under Construction"  
 Found 4 hairpins targeting 1 *Homo sapiens* gene, 1 *Mus musculus* gene and 1 *Rattus norvegicus* gene.  
 Displaying 1 to 4 [download all](#)

**Hairpin Status :** ■ Released ■ Release Pending ■ Under Construction ■ Withheld

Select ☒ All ☐ None

<input type="checkbox"/> 1	<span style="color: green;">■</span>	<a href="#">HP_210</a>	contact vendor: <a href="#">Open Biosystems</a>	<a href="#">comments</a> (0)
<b>Hairpin</b> TGCTGTTGACAGTGAGCGCGGAGGATTTCATCTCTTGTAATTAGTGAAGCCACAGATGTAAACAAAGAGATGAAATCCTCCATGCCTACTGCCTCGGA				
<b>Targets</b> <a href="#">NM_000546.2</a> (2120..2138)   <a href="#">TP53</a>   <a href="#">Homo sapiens</a> tumor protein p53 (Li-Fraumeni syndrome)				
<input type="checkbox"/> 2	<span style="color: green;">■</span>	<a href="#">HP_94</a>	contact vendor: <a href="#">Open Biosystems</a>	<a href="#">comments</a> (0)
<b>Hairpin</b> TGCTGTTGACAGTGAGCGCACAGTCTACTTCCCGGCATAATAGTGAAGCCACAGATGTATATGCGCGGAAGTAGACTGGCTGCCTACTGCCTCGGA				
<b>Targets</b> <a href="#">NM_011640.1</a> (1650..1668)   <a href="#">Trp53</a>   <a href="#">Mus musculus</a> transformation related protein 53				
<input type="checkbox"/> 3	<span style="color: yellow;">■</span>	<a href="#">HP_65</a>	<a href="#">comments</a> (0)	
<b>Hairpin</b> TGCTGTTGACAGTGAGCGCCCACCTACAAGTACATGTGTAATAGTGAAGCCACAGATGTATTACACATGTACTTGTAGTGATGCCTACTGCCTCGGA				
<b>Targets</b> <a href="#">NM_011640.1</a> (1224..1242)   <a href="#">Trp53</a>   <a href="#">Mus musculus</a> transformation related protein 53				
<input type="checkbox"/> 4	<span style="color: yellow;">■</span>	<a href="#">HP_587396</a>	<a href="#">comments</a> (0)	
<b>Hairpin</b> TGCTGTTGACAGTGAGCGCCGTACTCAATTTCCCTCAATATAGTGAAGCCACAGATGTATTGAGGGAATTGAGTACGTTGCCTACTGCCTCGGA				
<b>Targets</b> <a href="#">NM_030989.1</a> (552..570)   <a href="#">Tp53</a>   <a href="#">Rattus norvegicus</a> tumor protein p53				

Select ☒ All ☐ None

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**Figure 2.** Results of a search for p53. This page of results shows all the hairpins in the database that target the p53 gene in the human, mouse and rat genomes. Color codes are used to show if the constructs are released and available (green), in the process of being released (yellow), under construction (red) or withdrawn (grey). For each hairpin, the actual sequence is shown, with the sequence of the sense and antisense strands highlighted in red. The name of the mRNA sequence is linked to resources at NCBI (<http://www.ncbi.nlm.nih.gov>). There is a direct link to the vendor's order page (Open Biosystems in the cases shown here), which can be used to purchase the hairpin. The [download all](#) link allows downloading all the search results into a csv file, which can be opened in spreadsheet programs. The user can also download specific hairpins by checking the check boxes and using the [Download selected hairpins](#) button. The [Comments](#) link shows user-supplied comments as well as publications that have referenced the construct. Clicking on the hairpin name takes the user to a view that is shown in Figure 3. The hairpin could be designed against a different target gene, it will appear in the results as long as it can target the gene of interest. The search bar in the top of the figure can be used for additional searches, which can be limited by conditions such as organisms, state of hairpins, and so on. Files containing search terms, which can be symbols, definitions, names (HUGO specified names) or GO IDs, can be uploaded to the website, to search for hairpins that target the relevant genes. The [search history](#) button can be used to retrieve old search results as well as combine results from two different searches using the logical operations AND, OR, NOT or XOR. The links on the top of the page take the user to protocols from the laboratories whose constructs are in the database.

constructs that enter the cell via the cell membrane. The disruption can take the form of mRNA degradation, translational repression or transcriptional repression through epigenetic modifications (2–5).

The introduction of large dsRNA into mammalian cells results in a general response (interferon or protein kinase PKR response) that leads to cell death (6). It was discovered that shorter dsRNA (<29 nt) can be used to bypass this response (7). Short-interfering RNAs (siRNAs) are short dsRNA with 2 nt 3' overhangs and a 5' phosphate group that mimic the product of Dicer activity. They can get incorporated directly into the RNAi silencing complex (RISC) resulting in silencing activity (8). This is a popular method of silencing genes in cells.

Another method of inducing RNAi is to insert hairpin constructs into the genome using vectors, which can then be stably expressed (9). The expressed hairpins are processed by Drosha and exported to the cytoplasm, where Dicer acts on them to create siRNAs, which then get incorporated into the RISC.

These constructs are called short-hairpin RNAs (shRNAs) (9). shRNAs can also be chemically synthesized and introduced into the cytoplasm (10,11), but in this case it is important to mimic the product of Drosha, which has a 2 nt 3' overhang. It is also possible to place the antisense strand in the context of a known microRNA (miRNA) hairpin. miRNAs are naturally occurring genes that play a role in switching genes on and off during development (2). The Hannon–Elledge library of shRNA constructs uses the context of the miR-30 miRNA, as shown in Figure 1 (12).

Both siRNAs and shRNAs allow gene silencing and operate through the same pathways. The design principles involved in both are similar, in terms of ensuring that the appropriate strand from the dsRNA gets incorporated in the RISC (13,14). Both can result in off-target effects, in which genes that share partial homology with either strand of the dsRNA get silenced (15,16). Unfortunately, it is difficult to make accurate quantitative predictions of these effects (17). Thus, annotating the shRNA constructs with functional information

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 or upload Choose File no file selected  
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 Homo sapiens  
 Mus musculus  
 Rattus norvegicus

Curated list select group Limit  
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 Exclude "Under Construction" & "Withheld" hairpins Date: From To (YYYY-MM-DD)

**Hairpin Status :** Released Release Pending Under Construction Withheld

ID HP\_210 Open Biosystems: v2HS\_217, v2HS\_93615

Validation Sequence Verified

Vector pSM2

Mature product GAGGATTCATCTCTTGTGA

Hairpin TGCTGTTGACAGTGAGCGCGAGGATTCATCTCTTGTATTAGTGAAGCCACAGATGTAATACAAGAGATGAAATCCTCCATGCCTACTGCCTCGGA

Comments	Quality	PubMed	Submitted by	Date
Add Comment				

**Related Links** Target gene(s)  
 TP53 tumor protein p53 (Li-Fraumeni syndrome) [Homo sapiens] Actions  
 NM\_000546.2 Start position = 2120

Design Protocol

Vector Information

Purchase shRNAs

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**Figure 3.** This page contains information on each hairpin. It can be reached from the page shown in Figure 2, by clicking on the hairpin link. This page shows comments and publications and other information regarding the hairpin construct. It also has links to protocols, vectors and to vendors. The *Actions* link pops-up a window that allows accessing other information such as homologs of the target gene in other species through the *find homologs* link and the *visualize* link allows visualizing the alignment of the constructs to the genomic region along with mRNA and expressed sequence tags in that region. The result of clicking on the *visualize* link is shown in Figure 4. A registered user can use the *Add Comment* link to annotate the hairpin with comments and publications. New users can register by clicking on the *Add Comment* link. Comments can only be selected from a controlled vocabulary so that it is machine-readable and allows statistical analysis of the dataset. Publications that reference a construct can also be added to the comments using uids from PubMed (<http://www.ncbi.nlm.nih.gov>). The controlled vocabulary will be expanded, based on user-feedback.

is useful as there is no reliable method that *a priori* predicts the performance of the shRNA construct under actual biological conditions.

A central repository of shRNA constructs is essential since such a resource can act as a clearinghouse that can track results, identify patterns in shRNA performance and allow users to locate constructs from a variety of sources. RNAi Codex (<http://codex.cshl.org>) fulfills this role, though, at present, there is scant published information on the performance of specific shRNA constructs in the public domain. Our website and the associated database enable users to locate constructs from these libraries and purchase them from commercial vendors. We will explain our resource and give detailed instructions on the use of this tool.

## MATERIALS AND METHODS

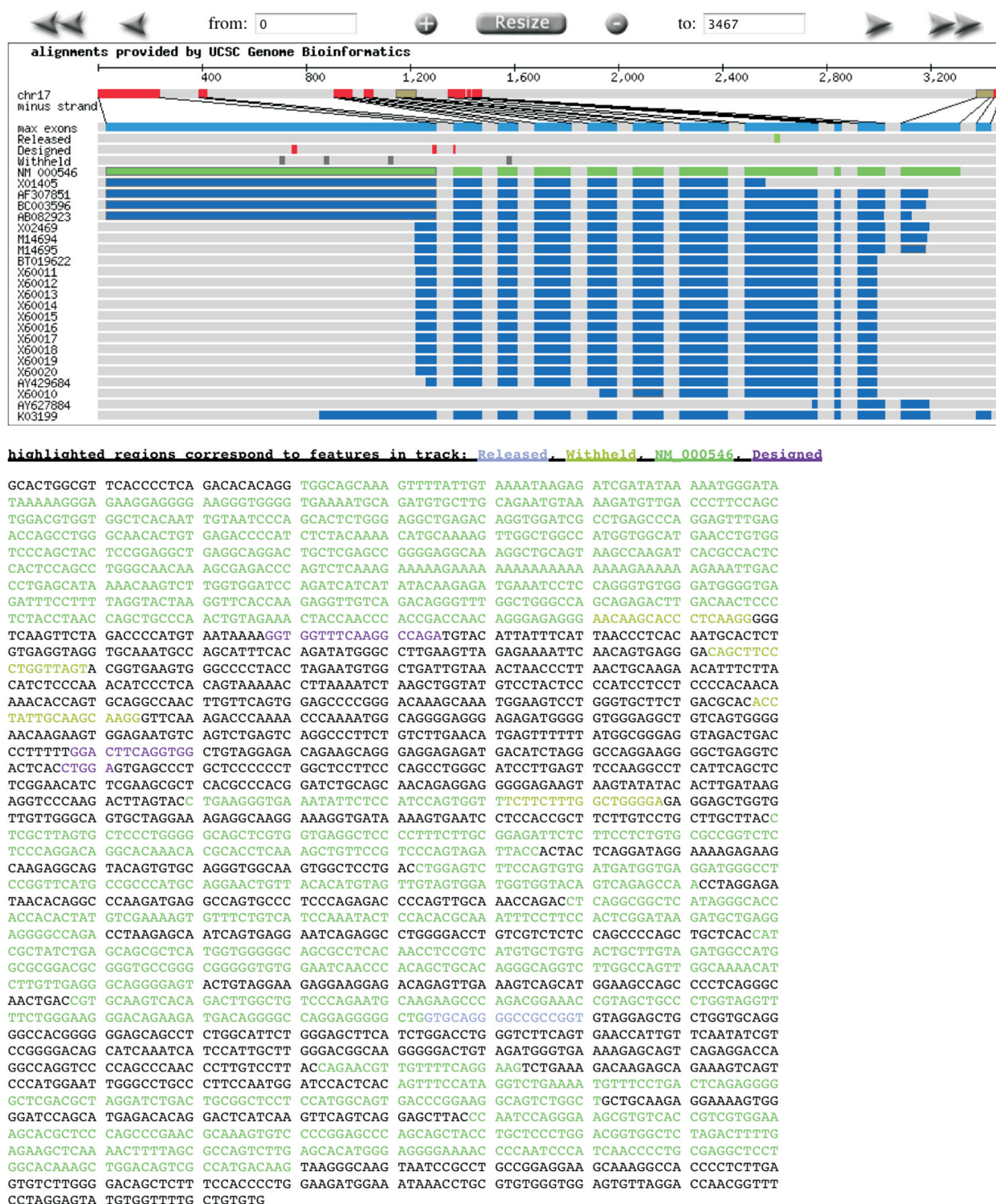
We built a database of shRNA constructs from the Elledge-Hannon collection (18). There are other collections (19), but these are not yet in the public domain. Each construct has associated with it several pieces of information such as the gene, the target sequence on the gene and the actual sequence of the construct. The database holds all this information.

In addition, the database can also accept annotations of constructs by using a controlled vocabulary to log experiences from experiments as well as links to publications that reference the construct.

A problem with such databases is that it is difficult to locate appropriate constructs using names that might not be familiar to the database. To solve this problem, we previously built an extensive name translation service, GeneSeer (<http://geneseer.cshl.org>) (20), which allows the use of familiar names to identify corresponding silencing constructs. In addition, the system also allows searching for constructs using sequences. Functional groups of genes, such as *kinases*, *phosphatases*, *cancer-1000* and so on, have been annotated with the help of expert curators. This allows the creation of collections of shRNA constructs (mini-libraries) that can silence functional groups. The system is familiar with Gene Ontology (21) terms, user-friendly names such as p53 and names from other databases such as Swiss-Prot/TrEMBL (22) and HUGO (23).

The RNAi Codex checks the mature sequence of the hairpins against the gene of interest, and returns all the hairpins that can target the gene, even if they were initially designed against a different gene. This is a very useful feature since





**Figure 4.** This figure shows the result of using the visualize link from the page shown in Figure 3. This is created using the program Light Weight Genome Viewer (lwgv) which can be downloaded from our website (<http://lwgv.sourceforge.net>). The top track is the genomic strand whereas the second track shows the exons in this region. The next three tracks show the hairpin designs from three categories (released, designed and withheld) and the bottom tracks show the alignments of expressed sequence tags to the genomic region. Below the tracks, the sequence of the region is shown, with the exons and the hairpins highlighted.

there are several constructs that can simultaneously silence genes from the mouse and human genomes.

We built a website that allows easy access to these resources and presents the results in a user-friendly manner. Figure 2

shows the results of a search conducted on RNAi Codex. Users can access all the data in the database (Figure 3). In addition, information from external sources is also shown, such as mapping information consisting of a view of the

mRNA and the position of the constructs on the mRNA (Figure 4). It is also possible to contact commercial vendors and purchase the shRNA constructs through the website (Figures 2 and 3).

The website also allows annotation of constructs, and addition of references to publications (Figure 3). This adds utility to the database since it is impossible for a single laboratory to verify the functional status of every construct or even a substantial portion of the constructs.

## RESULTS

The database holds data for constructs targeting three organisms, the *Homo sapiens*, *Mus musculus* and *Rattus norvegicus* genomes. It currently holds 82 450 constructs targeting 31 039 human genes, 73 562 constructs targeting 30 381 mouse genes and 26 611 constructs targeting 15 410 rat genes. There are 6885 constructs that simultaneously target 5569 genes in both, the mouse and human genomes.

The RNAi Codex website (<http://codex.cshl.org>) acts as the primary means of accessing the database. Bulk downloads of data are also allowed from the website. We use three figures (Figures 2–4) to explain use of the system and the features available on the website. Figure 2 shows the result of searching RNAi Codex for constructs that silence the gene, p53, and also explains how to search for constructs. Figure 3 shows how information on each individual hairpin can be obtained. Figure 4 shows how the website allows visualization of the alignments of the antisense sequences with the mRNAs.

## DISCUSSION

RNAi Codex is a unique resource for shRNA constructs, which will allow researchers worldwide access to information as well as allow purchase of the constructs. The underlying GeneSeer service (<http://geneseer.cshl.org>) (20) is undergoing constant improvement, which in turn improves functioning of the RNAi Codex, making searches easier and more accurate. We plan to incorporate data from other libraries that are coming online and also encourage user participation in annotating their experiences with specific constructs. Unfortunately, there is scant experimental data on shRNA constructs. We plan to use a human curator, along with user-contributions, to help in entering such data (and related publications), when they become available. The annotation of constructs will allow building models to predict the performance of shRNA constructs and help improve designs. In addition, RNAi Codex will also help track publications in the field.

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*Conflict of interest statement.* None declared.

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