Product Description

The use of RNAi as a genetic tool in Drosophila is made easier through the availability of the Drosophila RNAi library 2.0, a set of over 8100 dsDNA templates ready for *in vitro* transcription. Each construct is a dsDNA consisting of 200-800bps of exonic sequence from an individual gene. The construct was amplified from genomic DNA using a nested PCR process to ensure specificity. T7 promoter sequences were then added to enable RNA synthesis when used in an *in vitro* transcription reaction. Open Biosystems provides you with 10µls of each DNA template for your IVT reaction to generate up to 600 micrograms of RNA. Thus, you can perform hundreds of assays with a single gene or a collection of genes in a rapid and effective manner.

The Drosophila library 2.0 is provided in RNAse/DNAse free 96-well PCR plates. Each well contains undiluted product of the PCR reaction. 10µl +/-2ul of template DNA is supplied for in each well.

Storage

The product should be stored -20°C. **To ensure the maximum yield of RNA in the IVT reactions, it is important that the tips, plates and tubes utilized to store and manipulate the template DNA are RNAse free.**
RNA interference (RNAi) is a cellular mechanism mediated by double-stranded RNA (dsRNA) to regulate the expression of genes and potentially replication of viruses. The initial discovery of RNAi took place in 1998 in *Caenorhabditis elegans* and was soon identified in diverse organisms including Drosophila, Arabidopsis, hydra, and trypanosomes. The introduction of double-stranded RNA into a variety of organisms triggers a sequence-specific response, called RNA interference or post-transcriptional gene silencing, resulting in the degradation of homologous mRNA and the production of a phenotype characteristic of the loss of specific gene expression. In the case of Drosophila, these phenotypes are created by injection of the larvae with dsRNA, engineering a strain to carry DNA containing an inverted repeat (hairpin) of the gene or by culturing Drosophila cells and transfecting with dsRNA. The resulting phenotypes coincide with a reduction in the level of specific mRNAs. The transfected or injected cells contain a nuclease activity that specifically degrades transcripts homologous to the dsRNA. dsRNA is readily digested by the enzyme Dicer into ~21 nucleotide small interfering RNAs (siRNAs) possessing 2 nucleotide 3’ overhangs. A member of the RNase III family of dsRNA-specific ribonucleases, Dicer cleaves dsRNA in an ATP-dependent manner (Figure 1). The siRNA duplexes bind to RNA induced silencing complex (RISC) and becomes active through an ATP-dependent unwinding of the siRNA duplex. RISC then identifies the homologous mRNA by base pairing and cleaves the mRNA at approximately 12 nucleotides from the 3’ terminus of the siRNA.

![RNAi pathway diagram](image-url)
**In vitro transcription using Drosophila RNAi constructs**

Each dsDNA construct encompasses 200-800 bps of exonic sequence of an individual gene, the average size being 500 base pairs. The construct contains bi-directional T7 promoter sequences that enable RNA synthesis when used in an *in vitro* transcription (IVT) reaction. Open Biosystems provides enough dsDNA to successfully generate substantial quantities of dsRNA in a single IVT reaction. dsRNA for 5 randomly picked constructs was generated using a commercially available IVT kit. The protocol used and results of the reactions are outlined below.

Five constructs of different sizes were picked for IVT using the the MEGAscript™ T7 kit (Cat#1334) from Ambion (Table 1).

Table 1: List of five constructs from the Drosophila RNAi collection that was used in the IVT reactions

<table>
<thead>
<tr>
<th>Samples</th>
<th>Gene Name</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CHORD</td>
<td>462</td>
</tr>
<tr>
<td>2</td>
<td>Rotated abdomen</td>
<td>627</td>
</tr>
<tr>
<td>3</td>
<td>Lrr47</td>
<td>635</td>
</tr>
<tr>
<td>4</td>
<td>CG6181</td>
<td>601</td>
</tr>
<tr>
<td>5</td>
<td>Fmr1</td>
<td>424</td>
</tr>
</tbody>
</table>

Two microliters of the DNA construct was used in a 20 μl IVT reaction. The reaction was incubated at 37°C for 4 hours. Next, the RNA was annealed by heating the IVT reaction at 90°C for 4 minutes and then cooling slowly to room temperature. The DNA template was then removed by DNaseI digestion. The dsRNA generated was purified by filtration on Montage PCR96 Filter Plate (Millipore, MANU 030 10) and eluted in RNase-free 10 mM TRIS buffer (pH 7).
Results

Each sample (1/20 dilution) was analyzed on a 2% non-denaturing Agarose-TBE gel (Figure 2).

![Image of gel with lane descriptions](image)

**Figure 2**: Lanes 1 to 5 – RNA samples 1 to 5 generated using MEGAscript™ kit; Lane 1 – 1kb DNA Ladder.

*Note: As dsRNA templates may have a secondary structure, higher molecular weight bands may be present on a non-denaturing gel.*

The RNA yield obtained using the IVT kit ranged between 70 and 116 μg (Table 2). With the 10 μl of construct provided close to 600 μg of RNA could be generated. The 260/280 ratio of the dsRNA samples tested was above 1.8.

**Table 2: RNA yield from 20 μl IVT reactions using MEGAscript™ T7 kit.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>RNA yield from 20μl reaction, μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>116</td>
</tr>
<tr>
<td>2</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>108</td>
</tr>
<tr>
<td>4</td>
<td>116</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
</tr>
</tbody>
</table>

Most protocols that involve microinjections, cell culture or transfection of RNAi in *Drosophila* require anywhere between 0.1ug to 30ug of dsRNA. Thus, *Drosophila* RNAi constructs offered by Open Biosystems, should generate sufficient quantities of RNA for numerous assays. Figure 3 shows an example of a phenotype generated by RNAi of the gene pebble (*pbl*).
Abnormal cytokinesis in pbl RNAi phenotypes

Figure 3: Drosophila S2 cells 72 hours after the addition of dsRNA to the pebble gene; note the large polyploid cells (a). CON dsRNA targeting the intron of the white gene did not produce any phenotype (b). DNA-blue, Nuclear envelope-green, Filamentous actin- red

Downloading Collection Data

The dmRNAi product page contains files for download in Excel format. This data provides each constructs location in the delivered PCR plates and CG numbers for reference to FlyBase.

Useful websites:
Flybase: A database of the Drosophila Genome http://flybase.bio.indiana.edu
The Berkeley Drosophila Genome Project http://www.fruitfly.org
Drosophila RNAi Screening Center http://www.flyrnai.org
References


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