

In vitro transcription using *Drosophila* RNAi constructs

***Drosophila* RNAi Collection**

Open Biosystems offers the *Drosophila* RNAi Collection version 1.0 (Cat#RDM1189); a collection of over 7,200 *Drosophila* dsDNA constructs for fast and consistent generation of dsRNA. Each dsDNA construct encompasses the exonic region 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 dsDNA to successfully generate sufficient quantities of dsRNA in a single IVT reaction. Using five randomly picked constructs from our collection with two different kits available on the market, IVT reactions were performed. The protocols used and results of the reactions are outlined below.

In Vitro Transcription Reactions

Five different constructs ranging from 300 to 700 base pairs in size were picked for IVT using the RiboMAX™ T7 RNA production system (Cat#P1300) from Promega and 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

Samples	Gene Name	Size
1	Inhibitor of Apoptosis 2	635
2	Cyclin A	653
3	Unknown	399
4	Decay	311
5	Son of sevenless	722

Three micro liters of the DNA construct was used in a 20 µl IVT reaction using the RiboMAX™ T7 RNA production system. The reaction was incubated at 37°C for 4 hours. This was followed by DNase digestion to remove the template. The RNA generated was then precipitated with sodium acetate and 100% ethyl alcohol and resuspended in nuclease-free water.

The five samples were then tested using the MEGAscript™ T7 kit. However, this time only 1 µl of the DNA construct was used in a 20 µl reaction. Following DNase digestion, the samples were treated as above, except for a lithium chloride precipitation of the RNA. In both cases, the manufacturer's protocols were followed. All samples were purified using MEGAclean™ purification kit (Cat#1908) from Ambion.

One micro liter of a 10x dilution of the samples was loaded on a 2 % Agarose-TBE gel (Figure. 1).

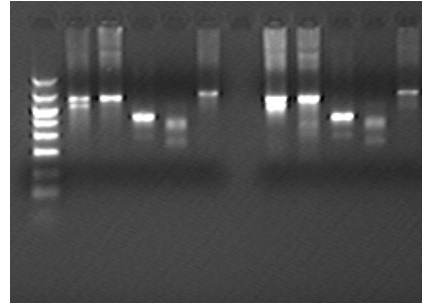


Figure 1: Lane 1 – 1kb DNA Ladder, Lanes 2 to 6 – RNA samples 1 to 5 generated using RiboMAX™ kit, Lanes 7 to 11 – RNA samples 1 to 5 generated using MEGAscript™ kit.

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

Results

A spectrophotometric analysis of the purified RNA indicated yields comparable to those estimated from the gel. The RNA obtained using either kit ranged between 21 and 67 µg. With the 10 µl of construct provided with every purchase, close to 700 µg of RNA could be generated. The variation in yield is both template and kit dependent (Table 3). The 260/280 ratio of the dsRNA samples tested was above 1.8.

Table 3: RNA yield from 20 µl IVT reactions using two different kits.

Samples	Yield from RiboMax™ Kit ug/20ul reaction (Using 3 µl template)	Yield from MegaScript™ Kit ug/20ul reaction (Using 1 µl template)
1	66	64
2	63	66
3	21	61
4	45	54
5	64	69

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.

References:

1. S. M. Hammond, E. Bernstein, D. Beach, G. J. Hannon, *Nature* 404, 293 (2000).
2. Worby et al., *Science's STKE* p11 (2001)