

# dsRNA prep protocol

(from Dudley et al. 2002, based on the Fire et al 1998 protocol)

## Design primers:

We usually design primers to amplify ~1kb of mostly coding sequence, usually within the first few exons, but we and others have also targeted the middle and end regions of genes, with similar results.

## PCR:

We make an *in vitro* transcription template from a two step PCR, which saves money when making multiple primers, and should increase the yield of product bearing complete T7 sites.

The first step uses a pair of 35bp primers, each designed based on 15 bases of the T7 sequence plus 20 bases of sequence from the gene of interest. Example:

	partial T7	gene of interest
forward primer	CGACTCACTATAGGG	CGATGAGGGCCTATTTATTC
reverse primer	CGACTCACTATAGGG	GAGAAAGTACACGATATAGC

The first PCR product is cleaned using the Qiagen PCR Purification kit.

The second PCR reaction uses the same primer on each end -- a primer containing the full T7 site plus a few restriction sites (for use with cloning -- EcoRI, XbaI, and HindIII):

EXHT7 :            ATAGAATTCTCTAGAAGCTTAATACGACTCACTATAGGG

## In vitro transcription:

The second PCR product is then gel purified (Qiagen Gel Extraction kit) and 1-2 micrograms of PCR product are used as template for transcription. We use the Ambion Megascript-T7 kit and get high yields, but doing it the old fashioned way by buying rNTPs, polymerase and RNase inhibitors separately and then phenol:Chloroform and EtOH precipitation works well too.

## RNA recovery:

We then run the RNA through a Qiagen PCR Purification kit (treating the RNA as if it were a PCR product) to get rid of the free nucleotides, polymerase, DNase, etc. (You can use RNA-specific columns instead, but it's not necessary). Assess concentration on a gel.

## Storage:

Mix the resulting solution with 2 volumes 100% EtOH and store at -80C. EtOH can be evaporated off in a speed-vac and dsRNA can be resuspended at desired concentration.