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:

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

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EXHT7:          ATAGAATTCTCTAGAAGCTTAATACGACTCACTATAGGG
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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.