dsRNA synthesis for zygotic RNAi

Primers:

T7	5'-TAATACGACTCACTATAGG-3'
T7-RNAi	5'-gaattgTAATACGACTCACTATAGG-3'
T7-SP6-RNAi	5'-TAATACGACTCACTATAGGatttaggtgacactataga-3'
T7-T3-RNAi	5'-TAATACGACTCACTATAGGaattaaccctcactaaaggg-3'

Objective

dsRNA is synthesised from a PCR product with T7 promoter sites on each site with the Ambion T7-Megascript Kit with a yield of >6 µg/µl

1. Template DNA with T7 sites on each end

Invitrogen TaqPCRx DNA Polymerase, Recombinant

total	100.00	Mastermix	1.30	
Distilled water to	69.50	µl	90.35	
10X PCR Buffer, Minus Mg	10.00	µl 1X	13.00	
50 mM MgCl2 / H2O	3.00	µl 1.5 mM	3.90	
dNTP (2 mM)	10.00	µl 0.2 mM each	13.00	
T7-RNAi	3.00	µl 0.3 µM each	3.90	
T7-SP6-RNAi	3.00	µl 0.3 µM each	3.90	
Template DNA	1.00	10ng/ul		
Taq DNA Polymerase (5 U/µl)	0.50	µl 0.025 units / ul	0.65	
		total	128.70	ul
		aliquot	99.00	

PCR: 4x 25µl for each synthesis

1x	5’	3'	94˚C
35x	30”	94˚C	
	30”	58˚C	(for T7-RNAi & T7-SP6-RNAi primers)
	1’	72˚C	(1min / 1kb length)
1x	5’	72˚C	

Combine to 100ul, take out 5ul and run on 1% agarose gel

2. dsRNA synthesis with Ambion Megascript kit

ATP	8	µl
CTP	8	µl
GTP	8	µl
UTP	8	µl
10x buffer	8	µl
PCR template (1ug)	26	µl
H2O (RNase free)	6	µl
enzyme mix	8	µl

3. Use LiCl precipitation (no DNase treatment necessary)
   - Add 120µl RNase free H2O
   - Add 120µl LiCl Solution
   - Chill at -20C for >30min
   - Spin at 13000RPM for 15min
   - Wash with 70%EtOh (DEPC H2O)
   - Spin at 13000RPM for 5min
   - Dilute in 40µl 1x injection buffer

4. Aligning of dsRNA and 6µg-µl aliquots
   - Align dsRNA with heat block from 95-100°C->50°C
   - spec and run on gel (1:8 dilution: use 0.5µl dsRNA and add 3.5µl RNase free H2O)
   - fill up to 6 µg/µl
   - aliquots at 2µl and store at -20°C