January 2014

RNAi Handbook

From dsRNA synthesis
to RNAi Treatment

NSF/EDEN Grant number IOS # 0955517

Required Equipment

Sample	
Attached gemmules in a 12 wells plate	
Solutions	
Strekal's medium	1mL/well/day
Strekal's medium (for wash)	2mL/well
Primers stock solutions (100µM)	2µL/reaction
Sponge cDNA	1µL/gene
3M Sodium Acetate (pH 5.2)	2.2µL/reaction
Isopropanol	22µL/reaction
RNAlater® solution	200µL/treatment
Kits	
GoTaq® Green Master Mix (or any similar Kit)	
Cloning Kit	
QIAquick® Gel Extraction Kit (or any similar Kit)	
QIAquick® PCR purification kit (or any similar Kit)	
QIAprep® Spin Miniprep Kit (or any similar Kit)	
Promega T7 RiboMAX™ Express RNAi System	
Qiagen RNeasy® Mini Kit (or any similar Kit)	
Invitrogen Super Script® III Reverse Transcriptase	
Tools	
Thermocycler	
Incubator	

Primers design

Several primers will be required to synthetize the dsRNA and check the knockdown by qPCR :

- One pair to target the 5' end of the mRNA (product size ~200pb)
- Primers as above with a partial T7 sequence (5'CGACTCACTATAGGG…3')
- Full T7 primer (5'ATAGAATTCTCTAGAAGCTTAATACGACTCACTATAGGG3')
- One primer pair targeting the 3' end of the mRNA (product size ~100pb)

dsRNA synthesis

1. **Perform a regular PCR using primers targeting 5' end of your gene of interest (PCR 0).**
   For example using GoTaq® Green Master Mix

Mix	
GoTaq	25µL
Fw-Primer	2µL
Rv-Primer	2µL
cDNA (~200ng)	1µL
H20	20µL

Cycles		
95°C	5'	
95°C	15s	x30
55°C	30s	
72°C	30s	
72°C	5'	

2. **Check 2µL of PCR product on a 1% agarose gel.**

3. **Purify DNA using QIAquick® PCR purification kit.**
   Or running all the PCR product and purify it with QIAquick Gel Extraction Kit depending on gel profile.

4. **Proceed with a plasmid ligation (e.g., TOPO cloning) and a bacterial transformation.**

5. **After checking colonies by PCR, carry out an overnight cell culture and extract plasmid with QIAprep® Spin Miniprep Kit.**
   Sequence it in order to confirm the insert.

6. **Perform a PCR as in step 1 with primers containing the partial T7 extension and the miniprep solution (or the PCR 0 product) as template (PCR 1).**

7. **As previously, run 2µL of PCR product and purify DNA.**

8. **Perform a PCR as in step 1 with Full T7 primers and DNA from step 7 as template (PCR 2).**

9. **One more time, check 2µL of PCR product and purify DNA.**

10. **Quantify DNA concentration of PCR 2.**
    Need about 1µg for dsRNA synthesis.

11. **Prepare the T7 RiboMAX™ mix as following:**

Mix	
RiboMAX™	10µL
PCR 2 product	1µg
Nuclease-Free Water	xµL
Enzyme Mix, T7 Express	2µL
Final Volume	20µL

12. **Incubate at 37°C for 30 minutes.**

13. **Remove DNA template by adding 1µL freshly diluted Rnase Solution and 1µL RQ1 Rnase-Free Dnase. Then, incubate 30 minutes at 37°C.**
    According to the protocols supplied by Promega**.**

14. **Purify dsRNA with 2.2µL of 3M Sodium Acetate (pH 5.2) and 22µL of isopropanol.**

15. **Incubate overnight at -20°C**

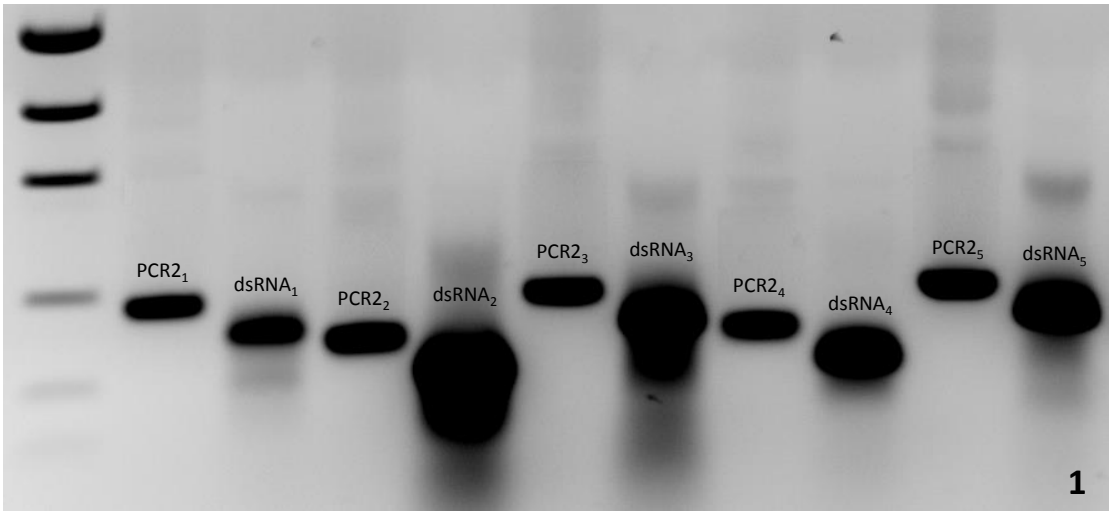
16. **Spin at top speed for 10 minutes.**

17. **Remove supernatant and wash pellet with cold 70% ethanol.**

18. **Remove ethanol, air dry the pellet for 15 minutes and resuspend dsRNA in 50µL of Nuclease-Free Water.**

19. **Quantify dsRNA solution and run 2µL of both dsRNA and PCR 2 products.**
    A shift should appear between the PCR 2 product and dsRNA migrations (see picture 1).

20. **Aliquot and store at -20°C.**



RNAi Treatment

1. **Check that gemmules are at the desire stage of development and take pictures.**

2. **Dilute dsRNA in 1mL of new Strekal's medium (10µg/mL final concentration).**

3. **Well by well, quickly but gently replace de medium with the medium containing the dsRNA.**

4. **Change all the solutions every 24h.**
   Also in control wells.

5. **Take pictures as often as required.**

6. **Remove dsRNA after 48h and wash sponges two times with 1 mL of Strekal's medium.**

7. **Using a 1mL pipette, collect sponges in a 1.5mL microcentrifuge tubes combining like treatments in the same tube.**

8. **Spin 3 minutes at top speed, remove liquid and add 200µL of RNAlater ® and store at -20°C until used.**

9. **Extract mRNA using Qiagen RNeasy® Mini Kit with a step of DNA degradation and quantify.**

10. **Synthesize corresponding cDNA from 125ng of mRNA as template using Invitrogen Super Script® III Reverse Transcriptase.**

11. **Check gene expression shutdown by qPCR using primers targeting the 3' end of the mRNA.**

April Hill, *Department of Biology, University of Richmond, Richmond, VA, USA (ahill2@richmond.edu)*

Quentin Schenkelaars, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (quentin.schenkelaars@univ-amu.fr)*

Fernando Gomez, *Department of Biology, University of Richmond, Richmond, VA, USA (fgomez@richmond.edu)*

Chelsea Hall, *Department of Biology, University of Richmond, Richmond, VA, USA (chelsea.hall@richmond.edu)*

Laura Fierro, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (laura.fierro@univ-amu.fr)*

Emmanuelle Renard, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (emmanuelle.renard@univ-amu.fr)*

Carole Borchiellini, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (carole.borchiellini@univ-amu.fr)*

NSF/EDEN Grant number IOS # 0955517