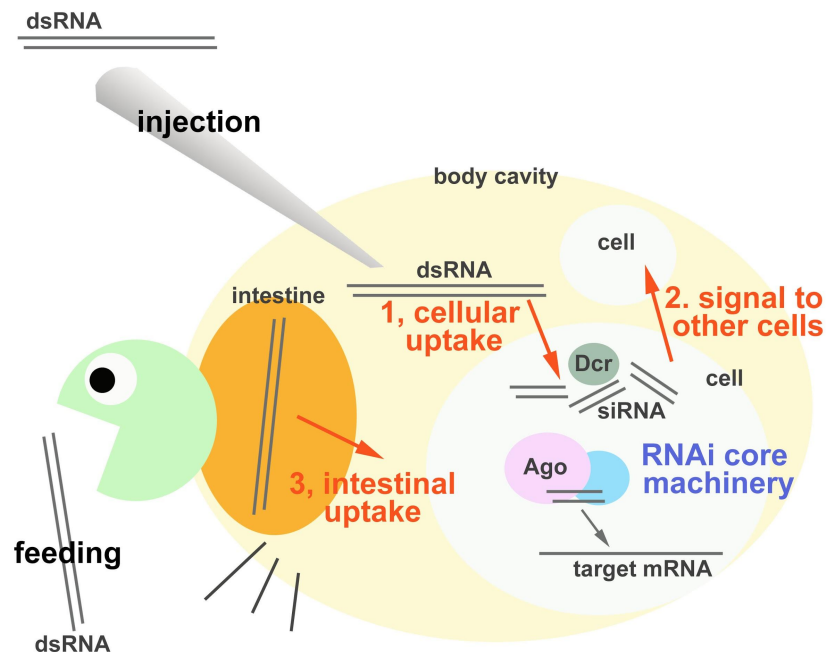


Polishing up your RNAi techniques (mainly in insects)

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The discovery of RNA interference (RNAi) has revolutionized evo-devo by enabling the analysis of loss-of-function (LOF) phenotypes in organisms in which classical genetic analysis is laborious or not possible. Since its discovery in late 90s, RNAi has been applied successfully to numerous insects and other organisms, resulting in a variety of methodologies that can trigger efficient RNAi responses. However, there are significant variations among these methodologies, which make it difficult to decide the most efficient approach to performing RNAi.

In this roundtable, we will discuss various parameters related to RNAi to be able to maximize the efficiency of your RNAi. We will also discuss potential pitfalls and obstacles you may face while you perform RNAi and how to troubleshoot them.



1) dsRNA preparation

What do you need to consider when you design dsRNA?

- dsRNA lengths. What's best? One size fits all?
- Determination of secondary structure
- Limiting OTEs. Online tools? (eg E-RNAi <http://www.dkfz.de/signaling/e-rnai3/>)
- The role of genome / transcriptome knowledge

What is the quickest and/or the most cost efficient way to make dsRNA?

- Cloning, PCR-based, or *de novo* gene synthesis (such as gBlock)?
- Two-step (unidirectional), one-step (bidirectional)?
- Choice of kits for dsRNA synthesis and clean-up

2) Delivery methods

What is the best way to deliver dsRNA in your organisms/ in your tissues?

- Systemic or non-systemic?
- Microinjection: egg, post-embryonic stages, adult
- Feeding
 - defined diets containing dsRNA
 - transgenic plants (cytosol vs. chloroplasts?)
- Soaking
- Electroporation

3) RNAi parameters

What is the general parameters for RNAi?

- dsRNA lengths? concentration?

Can we do multiple RNAi at the same time? How can we avoid competition?

- RNAi competition at the RNAi core machinery level and at dsRNA cellular uptake
- Use the same length? same concentration? sequential vs. simultaneous injection

How long does RNAi last?

- dsRNA penetrance of target tissues
- Stages
- Measuring persistence of dsRNA / siRNAs

4) Validation

What is the best method to validate RNAi?

- RT-PCR, qRT-PCR, Northern, Western
- Caveat and pitfall? (eg. mRNAs go up after RNAi even proteins go down? See Figure)

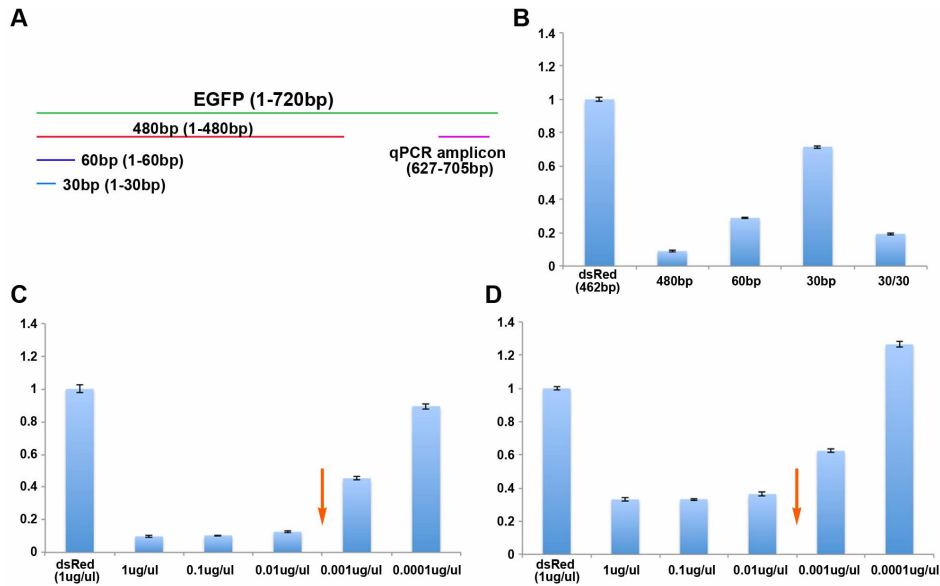
How can we assess OTE?

- Control experiments?
- Two non-overlapping fragments

5) Species differences

What are the factors that contribute to the difference in RNAi efficiency among organisms?

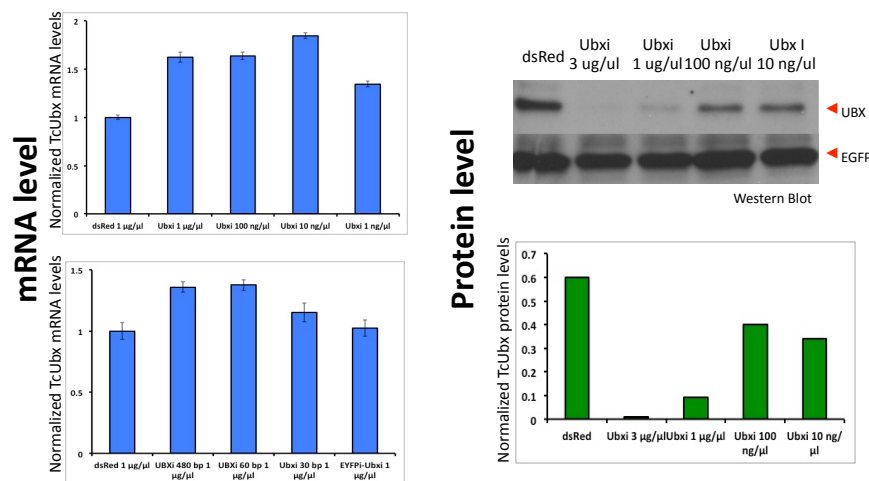
Supplementary Figures



Reduction of EGFP mRNA by various sizes and concentrations of EGFP dsRNA.

(A) Location of EGFP dsRNAs and qPCR amplicon relative to the EGFP gene. (B) Reduction of EGFP mRNA induced by various sizes of EGFP dsRNA. (C) Reduction of EGFP mRNA induced by various concentrations of EGFP 480 bp dsRNA. (D) Reduction of EGFP mRNA induced by various concentrations of EGFP 60 bp dsRNA. (Miller et al. PLoS ONE 2012)

Gradual reduction of Ubx protein level (but not the mRNA level?) by Ubx RNAi



Tomoyasu lab unpublished data

Successful taxa

- Holometabola
 - *Drosophila* (non-systemic)
 - other Diptera
 - beetles (*Tribolium*, *Tenebrio*, *Onthophagus*, etc.)
 - hymenoptera (*Nasonia*, honeybees)
 - Lepidoptera (*Manduca*, *Bombyx*, *Epiphyas*.)
- Hemiptera
 - milkweed bug (*Oncopeltus*)
 - water strider (*Gerris*)
 - *Rhodnius*, *Triatoma*
 - aphids (*Acyrtosiphon*, *Myzus*)
 - whitefly (*Bemisia*)
 - psyllids (*Diaphorina*, *Bactericerca*)
 - human body louse (*Pediculus humanus*)
 - planthoppers (*Nilaparvata*, *Sogatella*, *Laodelphax*)
- Orthoptera (*Gryllus*, *Locusta*, *Schistocerca*, *Allonemobius*, *Modicogryllus*)
- Cockroaches (*Blattella*, *Leucophaea*, *Diploptera*, *Periplaneta*)
- Termites (*Reticulitermes*, *Cryptotermes*)
- *Thermobia*
- Non-insect arthropods
 - spider
 - harvestmen
 - tick