

RNAi Vector feeding protocol **for distal tip cell (DTC) migration studies in *C. Elegans***

Introduction

RNAi is an effective method for analyzing gene function in *C. elegans* that often phenocopies loss-of-function phenotypes [1]. In RNAi, double stranded RNA (dsRNA) introduced into larvae or adults activates an enzymatic pathway that eliminates endogenous RNAs homologous to the dsRNA [2]. Potent and persistent RNAi silencing in *C. elegans* results from secondary amplification of small amounts of the initial RNAi trigger by RNA dependent RNA polymerases [3, 4]. RNAi can be induced in *C. elegans* using one of four methods: injecting *in vitro* synthesized dsRNA into the body cavity of the animal (injection RNAi), soaking in a solution of dsRNA (soaking RNAi), feeding animals bacteria engineered to express dsRNA (feeding RNAi), or through creation of transgenic animals that express dsRNA from DNA arrays maintained within cells (hairpin RNAi)[5, 6]. This protocol is slightly modified from [7], and can be used for candidate or broad RNAi screens in *C. elegans* [8, 9].

Materials

Reagents:

NGM Plates: NaCl, peptone, agar, MgSO₄, CaCl₂, cholesterol, KPO₄ (see recipe [10]) + IPTG and carbinicillin

LB and ampicillin

Agarose and Glass slides

Lots of Petri Dishes

22x50mm glass coverslips

Equipment:

4 to 6X (x10) dissecting scope, 20-40X (x10) light microscope with Nomarski optics. Autoclave, shaker, incubators (37°C, 23°C), microwave.

Time Taken

The entire protocol takes 9 days, but very little time is required on most of the days. The amount of time on each day depends on the number of genes screened at one time. Generally, a few minutes to several hours is required on each day.

Procedure

Day 1.

Streak out culture from desired library well on LB/Amp plate (40 µg amp/ml) (optional).

Day 2.

1. Culture bacteria containing each RNAi clone in 1 ml LB medium containing 40 µg/ml ampicillin overnight (Table 1 shows vectors used in this particular study – link to RNAi primer info file).
2. Chunk a starved nematode plate and allow starved nematodes to recover on fresh *E.coli* OP50-seeded NGM plates for two days (until Day 4 of experiment).

Day 3.

In the 24 well format, 20 μ l of each culture was spotted in a single well of a 24-well plate containing NGM agar, 6 mM IPTG and 25 μ g/ml carbenicillin. In the 6 cm format, spread out 150 μ l of culture. I usually make duplicate plates, so I have a plate ready to score a second generation.

Day 4.

Release eggs from gravid hermaphrodites on the plates chunked on Day 2 using alkaline hypochlorite solution. Following washes in M9 buffer, transfer eggs to plates seeded with *E. coli* HT115(DE3) bacteria expressing double-stranded RNA (dsRNA) (Seeded Day 3) and incubated at 23°C. (For detailed protocol, see Hope (1999))

Day 5.

Take the day off.

Day 6.

1. Analyze animals with light microscopy.

Primary screen: Score the animals for clear patches using a dissecting microscopy. This is approximately 48h after hatching; animals should be young adults.

Secondary screen: Mount the animals on agar pads using 0.08M sodium azide as an anesthetic. View DTC migration using 40X Nomarski microscopy (see figure below - RNAi scheme).

2. Transfer 5 young adults to new plates seeded with RNAi bacteria.

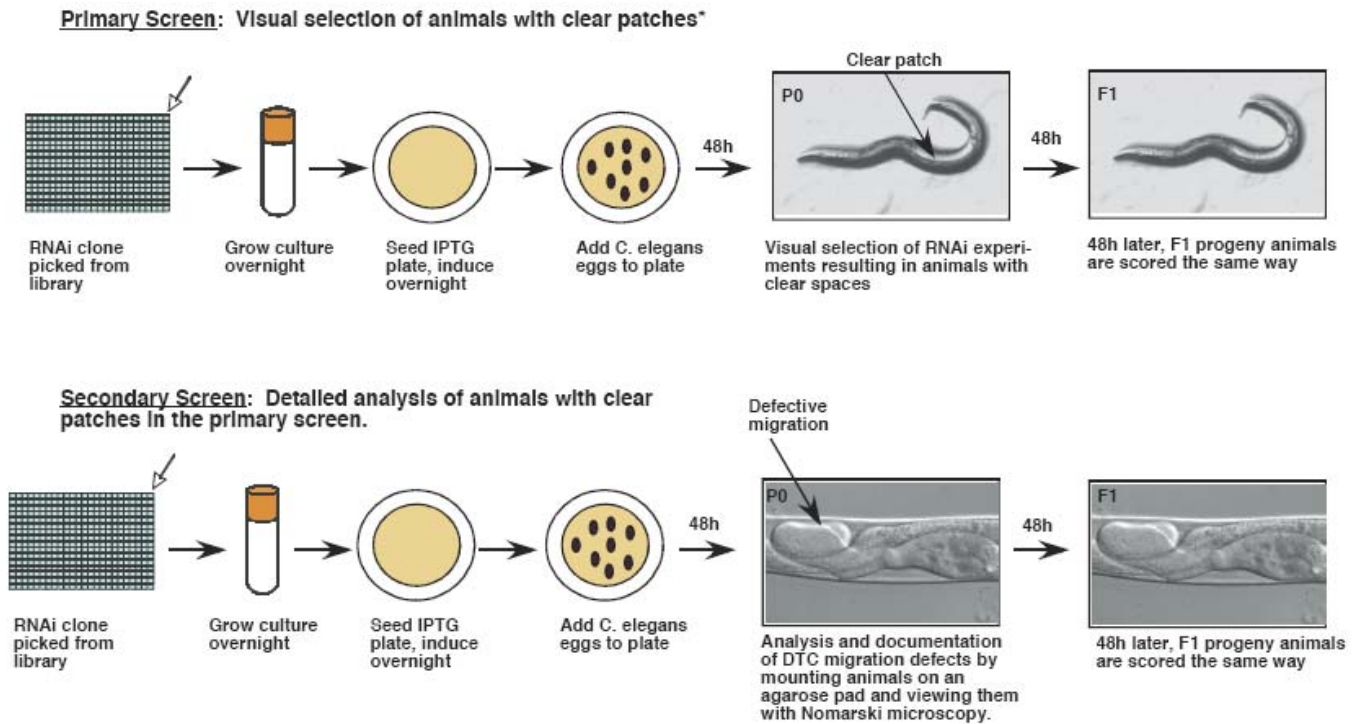
Day 7.

Take the day off.

Day 8.

Analyze young adult progeny animals as described for Day 6. If the worms aren't old enough (likely), analyze them on **Day 9**.

Figure 1. RNAi scheme



References

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