

Population scale nucleic acid delivery to *Caenorhabditis elegans* via electroporation

(Supplementary Information)

Anastasia S. Khodakova^{1,2}, Daniela Vidal Vilchis¹, Dana Blackburn¹, Ferdinand Amanor², Buck S. Samuel^{1,2}

¹*Alkek Center for Metagenomics and Microbiome Research and Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston TX USA*

²*SMART Program, Baylor College of Medicine, Houston TX USA*

Supplementary data provided with the manuscript:

- **Table S1** - Strains used in this study
- **Table S2** - Primers used for genotyping of worms
- **Table S3** - Viability of worms (N2) after electroporation at L4 stage
- **Figure S1** - Body lengths of BIG0107 worms, electroporated at different conditions
- **Figure S2** - Body lengths of BIG0106 worms electroporated and compared to controls
- **Figure S3** - Fluorescent Z-stack imaging of BIG0106 worms electroporated with *gfp*-dsRNA
- **Figure S4** - Fluorescent Z-stack imaging of BIG0107 worms electroporated with *gfp*-dsRNA
- **Figure S5** - Comparison of body lengths of electroporated and untreated VC1119 [*sid-2(gk505) III*] worms.
- **Figure S6** - Electroporated VC1119 [*sid-2(gk505) III*] worms with *pos-1*-dsRNA at L4 stage
- **File S1. Custom Matlab scripts for image analysis.**

The file contains two Matlab scripts for image analysis used throughout the research, additional files required for the scripts (map.mat ; bformatlab folder) and exemplary fluorescent images (in .nd2 format) of "Control", "Partially silenced" and "Completely silenced" populations of worms after electroporation with *gfp*-dsRNA.

- Categorical counting script.

The script was used to count the number of worms belonging to the following three categories of worms in population: partial silencing, complete silencing, and no silencing. As an input the script accepts .nd2 image files taken on Nikon fluorescent microscope. The file should have resolution of 6964×6964 pixels and two channels (bright field and fluorescence field). During script execution, the script provides user with hints on what to do during each stage. Briefly, on a bright field image first manually select worms by mouse clicking, when finished click once inside the square in left upper corner; next go on fluorescent image and select worms corresponding to one category, by clicking, when finished click once inside the square in left upper corner, then continue selection of worms from the other category, again when finished click once inside the square in left upper corner; at the end you will see the number of worm in each of three categories and a total number of worms. As an output the script generates a table with a number of worms falling into each category.

- Single channel worm selection script.

The script was used for measuring average fluorescence intensity of the worm (or multiple worms) selected on the image. As an input the script accepts .nd2 image files taken on Nikon fluorescent microscope. The file should have resolution of 6964×6964 pixels and two channels (bright field and fluorescence field). During script execution, the script provides user with hints on what to do during each stage. The output file of the script is a table containing a number of rows corresponding to the selected worms. Each row for each particular worm includes the following information: worm area (Column B), average fluorescence normalized to the worm's area (Column C), average background intensity (Column F) and average fluorescence intensity with subtracted background intensity (Column H). Average background intensity is measured on area with no worm. The script also generates a separate folder where the images for bright field, fluorescent field and binary masks for each worm are saved.

- **File S2. Videos of live worms with Dpy and Rol phenotypes.**

The file contains three video files (in .mp4 format). The video files demonstrate representative F1 worms with Dumpy and Roller phenotypes observed after electroporation of *dpy-10* gRNA in the population of YA EG9888 worms with Cas9 expression in the germline.

- **File S3. Illumina amplicon sequencing data of *dpy-10* loci in Dpy and Rol worms.**

The file contains NGS sequencing files (in .fastq.gz format) including:

- Dumpy_R1.fastq.gz
- Dumpy_R2.fastq.gz
- Roller_R1.fastq.gz
- Roller_R2.fastq.gz

The files are Illumina 2x250 pair-end sequencing datasets of *dpy-10* PCR amplicons obtained from the single worms with Dumpy and Roller phenotypes.

- **File S4.xlsx**

The file contains additional information and raw data including:

- Table S4.1 (Sheet 1) - raw data Figure 1b; animals viability testing after electroporation at L1 stage under different electroporation conditions.
- Table S4.2 (Sheet 2) - raw data for Figure 1c; measurement of worms' lengths after electroporation at different conditions.

- Table S4.3 (Sheet 3) - raw data for Figure 2a; measurement of GFP fluorescence intensity per worm.
- Table S4.4 (Sheet 4) - raw data for Figure S1a; measurement of worms' lengths after electroporation at different conditions.
- Table S4.5 (Sheet 5) - raw data for Figure 3c; measurement of GFP fluorescence intensity per worm.
- Table S4.6 (Sheet 6) - raw data for Figure S1b-d; measurement of worms' lengths after electroporation.
- Table S4.7 (Sheet 7) - raw data for Figure 3c; measurement of measurement of GFP fluorescence intensity per worm.
- Table S4.8 (Sheet 8) - raw data for Figure S2; measurement of worms' lengths after electroporation.
- Table S4.9 (Sheet 9) - raw data for Figure 4a; measurement of worms' lengths after electroporation at different dsRNA concentrations.
- Table S4.10 (Sheet 10) - raw data for Figure 4d; measurement of worms' lengths after electroporation at different dsRNA concentrations.
- Table S4.11 (Sheet 11) - raw data for Figure S5; measurement of worms' lengths after electroporation.
- Table S4.12 (Sheet 12) - raw data for Figure S6; progeny counting after worms electroporation with pos-1-dsRNA.