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siRNA Electroporation of Hydra V.1

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Works for me

[dx.doi.org/10.17504/protocols.io.6t2heqe](https://doi.org/10.17504/protocols.io.6t2heqe)[Open Hydra](#)[Adrienne Cho](#)

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

1. Vogg MC, Beccari L, Olle LI, Rampon C, Vríz S, Perruchoud C, Wenger Y, Galliot B. An evolutionarily-conserved Wnt3/ β -catenin/Sp5 feedback loop restricts head organizer activity in Hydra. Nat Commun. 2019 Jan 18;10(1):312. doi: 10.1038/s41467-018-08242-2
2. Lommel M, Tursch A, Rustarazo-Calvo L, Trageser B, Holstein TW. Genetic knockdown and knockout approaches in Hydra. Biorxiv preprint: doi: 10.1101/230300

MATERIALS TEXT

- Hydra Medium (HM) without antibiotics
- David's Dissociation Medium, filtered, pH 6.9
- 10mM HEPES pH 7.0
- siRNA
- Glass Pasteur pipette
- Eppendorf tubes
- 6-well plates
- Electroporation cuvettes

BEFORE STARTING

1. siRNA Design

- find 21 bp sequence in coding region starting with AA dinucleotide
- GC contents between 30-50% seem to be more effective than those with higher GC contents
- avoid poly(T) sequences (more than 3 nucleotides) since this serves as termination signal for RNA pol III
- design 3-4 siRNAs targeting different loci within the gene of interest a combination of several siRNAs may result in better knock down efficiencies
- check for off targets using the hydra 2.0 database

Some siRNAs work better than others to knock down gene expression, we used a mix of 3 siRNAs in the electroporations.

2. Restoration medium:

20% dissociation medium (v/v) in hydra medium

Dissociation medium

- 3.6 mM KCl
- 6 mM CaCl_2
- 1.2 mM MgSO_4
- 6 mM Na-Citrate
- 6 mM pyruvate
- 6 mM glucose
- 12.5 mM TES

adjust pH to 6.9 before adding antibiotic:

0.05 g/L rifampicin

- 1 Wash animals (fed one day earlier) 5 times with Milli-Q water and incubate for 45 minutes at 18°C
- 2 Place 20 animals into electroporation cuvette with 0.4cm gap and remove as much liquid as possible.
- 3 Add 200 μ L sterilized 10mM HEPES (pH7.0) containing 4 μ M siRNA
- 4 Tap cuvette 10 times to evenly distribute animals, leave animals for 5 minutes to allow them to relax and extend.
- 5 Set Biorad GenePulser Xcell electroporation system to the following:

Voltage: 150 V
Length: 50 ms
Number of Pulses: 2
Pulse Intervals: 0.1 s
- 6 Electroporate animals. Immediately after administering each pulse, add 500 μ L 18°C restoration medium.
- 7 Carefully transfer animals to a dish filled with restoration medium
- 8 Let animals recover in restoration medium for 48 hours.
- 9 Repeat Steps 1-8 2x.



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