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# siRNA Electroporation of Hydra V.1

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**Manuscript citation:**

1. Vogg MC, Beccari L, Olle LI, Rampon C, Vriz 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



**Protocol status:** Working
**We use this protocol and it's working**

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## Materials

- 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 start

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 interestà 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 cuvetter with 0.4cm gap and remove as much liquid as possible.

3 Add 200 µL sterilized 10mM HEPES (pH7.0) containing 4µM 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 µL 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.