



Genetic tools for the sea anemone Aiptasia

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Abstract

This is a collection of protocols for use of genetic tools in the small sea anemone Aiptasia (*Exaiptasia diaphana*). The protocols give step-by-step guides for production of genetic constructs (plasmid DNA, capped mRNA and shRNA), electroporation into zygotes and techniques for subsequent analysis of the larvae. They also include updated guidelines for general Aiptasia husbandry and spawning set-ups.



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Design and in vitro transcription of shRNA with the NEB HiScribe™ T7 Quick High Yield RNA Synthesis Kit

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RNA extraction from Aiptasia larvae with the Zymo Research Quick-DNA/RNA Microprep Plus Kit

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Fixation and immunohistochemical staining of Aiptasia larvae

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Fixation of Aiptasia larvae for observation of native fluorescence of fluorescent proteins

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Exposure of electroporated Aiptasia larvae to Symbiodiniacean algae

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Template production for mRNA in vitro transcription by PCR

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RNA agarose gel electrophoresis with bleach gels

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RNA agarose gel electrophoresis with RNA sample buffer

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Purification of in vitro transcribed RNA with the Zymo Research RNA Clean & Concentrator™-25 Kit



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Purification of in vitro transcribed RNA with the Zymo Research Quick-RNA Miniprep Kit

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🔗 Aiptasia spawning and embryo and larvae handling - Pringle lab

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🔗 Embedding living larvae in low-melting agarose for imaging and recovery thereafter

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Isolation of genomic DNA from Aiptasia polyps

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🔗 General Aiptasia husbandry - Pringle Lab

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Electroporation of Aiptasia zygotes

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In vitro transcription of mRNA with the NEB HiScribe™ SP6 Quick RNA Synthesis Kit and subsequent enzymatic capping

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Plasmid DNA concentration for electroporation into Aiptasia zygotes via Amicon® Ultra Centrifugal Filters

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Production and purification of plasmid DNA with the Zymo Research ZymoPURE™ II Plasmid Maxiprep kit

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NAME

Production and purification of plasmid DNA with the Zymo Research ZymoPURE™ II Plasmid Gigaprep kit

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