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invertedClampFISH ligation

Forked from invertedClampFISH ligation

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Protocol for making invertedClampFISH probes.

Materials

MATERIALS

- T7 DNA Ligase - 750,000 units **New England Biolabs Catalog #**M0318L
- Monarch® PCR & DNA Cleanup Kit (5 µg) **New England Biolabs Catalog #**T1030
- T4 Polynucleotide Kinase - 2,500 units **New England Biolabs Catalog #**M0201L

Troubleshooting

Probe backbone phosphorylation

1 Reconstitute invertedClampFISH arms, backbones and adaptors to 400uM in nuclease free water.

Note

If the quantity of backbone oligos is very low, can reconstitute to 200uM and use twice as much for subsequent reactions.

2 combine reaction components to phosphorylate unmodified backbone oligos

- 10 µL 2x T7 DNA ligase buffer
- 1 µL 400uM backbone oligo
- 0.5 µL T4 polynucleotide kinase
- 2 µL nuclease free water

Note

If you plan to add the same invertedClampFISH arms to each backbone oligo, the phosphorylation and subsequent ligation can be done with multiple backbone oligos simultaneously. I have ligated 30 backbone probes at once, scaling up the reaction 2x-3x.

- 50 °C thermal cycler lid
- 37 °C thermal cycler
- 08:00:00 incubate PNK reaction.

Note

You probably can incubate for a shorter duration. Overnight incubation is fine too.

invertedClampFISH probe ligation

3 Combine reaction components

- 1.5 µL 400µM left arm
- 1.5 µL 400µM right arm
- 1.5 µL 400µM left adapter
- 1.5 µL 400µM right adapter

4 Heat reaction components to 95ºC for 5 minutes then cool slowly to 12ºC.

95 °C

00:05:00 on thermal cycler

12 °C cool slowly (0.1ºC/sec)

5 Bring to room temperature then add T7 DNA ligase.

25 °C Bring to room temperature

0.5 µL T7 DNA ligase

6 Mix reaction then centrifuge. Incubate at room temperature overnight.

25 °C

12:00:00 incubate overnight

Note

If any of the oligos contain a dye, incubate in the dark.

Column purify invertedClampFISH probes

7 Column purify using NEB Monarch PCR and DNA cleanup kit according to the manufacturer's instructions. Use 1 column per 10µL ligation reactions.

8 Add 30 µL nuclease free water to ligation reaction.

30 µL nuclease free water

Note

Can be scaled up or down as long as 7x volume of binding buffer is added before loading column. I like to scale the volume such that 200 µL can be loaded on each column.

9 Add 350 µL binding buffer

350 µL binding buffer

10 Apply to monarch column then spin down for 1 minute.

00:01:00 Centrifuge at 16,000 x g

11 Remove flow-through then apply 200 µL wash buffer to column and spin down.

200 µL Monarch DNA wash buffer

00:01:00 Centrifuge at 16,000 x g

12 Repeat wash with 200 µL wash buffer and spin down.

200 µL Monarch DNA wash buffer

00:01:00 Centrifuge at 16,000 x g

Note

You do not need to remove flow through in between washes.

13 Transfer column to clean centrifuge tube then spin down to remove residual wash buffer.

00:01:00 Centrifuge at 16,000 x g

14 Rotate column 180º then centrifuge again.

00:01:00 Centrifuge at 16,000 x g.

Note

Probably carry-over superstition from using Qiagen columns.

15 Transfer to clean centrifuge tube for elution. Apply 30uL nuclease-free water to column.

30 µL

Note

Can scale up or down volume to adjust final concentration. With 30 µL elution, my final concentration tends to be between 50-80ng/µL (using an absorbance constant of 33 on the nanodrop).

16 Incubate at room temperature for 5 minutes.

00:05:00 incubate at room temperature.

17 Elute probes.

00:01:00 Centrifuge at 16,000 x g.

18 Measure concentration by nanodrop.

Note

I set the absorbance constant to 33 for ssDNA.