invertedClampFISH ligation

Benjamin Emert

Abstract

Protocol for making invertedClampFISH probes.

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Materials

- T7 DNA Ligase - 750,000 units M0318L by New England Biolabs
- Monarch® PCR & DNA Cleanup Kit (5 µg) T1030 by New England Biolabs
- T4 PNK M0201L by New England Biolabs

Protocol

Probe backbone phosphorylation

Step 1.

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

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

Probe backbone phosphorylation

Step 2.

combine reaction components to phosphorylate unmodified backbone oligos

AMOUNT
10 µl Additional info: 2x T7 DNA ligase buffer
AMOUNT
1 µl Additional info: 400uM backbone oligo
AMOUNT
0.5 µl Additional info: T4 polynucleotide kinase

AMOUNT
2 µl Additional info: nuclease free water
TEMPERATURE
50 °C Additional info: thermal cycler lid
TEMPERATURE
37 °C Additional info: thermal cycler

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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.

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

invertedClampFISH probe ligation
Step 3.

Combine reaction components

AMOUNT
1.5 µl Additional info: 400µM left arm
AMOUNT
1.5 µl Additional info: 400µM right arm
AMOUNT
1.5 µl Additional info: 400µM left adapter
AMOUNT
1.5 µl Additional info: 400µM right adapter
invertedClampFISH probe ligation
Step 4.

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

TEMPERATURE
95 °C Additional info:
TEMPERATURE
12 °C Additional info: cool slowly (0.1ºC/sec)
invertedClampFISH probe ligation
Step 5.

Bring to room temperature then add T7 DNA ligase.

AMOUNT
0.5 µl Additional info: T7 DNA ligase
TEMPERATURE
25 °C Additional info: Bring to room temperature
invertedClampFISH probe ligation

Step 6.

Mix reaction then centrifuge. Incubate at room temperature overnight.

TEMPERATURE
25 °C Additional info:

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

Column purify invertedClampFISH probes
Step 7.

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

Column purify invertedClampFISH probes
Step 8.

Add 30 µL nuclease free water to ligation reaction.

AMOUNT
30 µl Additional info: nuclease free water

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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.

Column purify invertedClampFISH probes
Step 9.

Add 350 µL binding buffer

AMOUNT
350 µl Additional info: binding buffer

Column purify invertedClampFISH probes
Step 10.

Apply to monarch column then spin down for 1 minute.

Column purify invertedClampFISH probes
Step 11.

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

AMOUNT
200 µl Additional info: Monarch DNA wash buffer

Column purify invertedClampFISH probes
Step 12.

Repeat wash with 200 µL wash buffer and spin down.

AMOUNT
200 µl Additional info: Monarch DNA wash buffer

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

Column purify invertedClampFISH probes
Step 13.

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

Column purify invertedClampFISH probes
Step 14.

Rotate column 180º then centrifuge again.

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Probably carry-over superstition from using Qiagen columns.

Column purify invertedClampFISH probes
Step 15.

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

AMOUNT
30 µl Additional info:

NOTES
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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).

Column purify invertedClampFISH probes
Step 16.

Incubate at room temperature for 5 minutes.

Column purify invertedClampFISH probes
Step 17.

Elute probes.

Column purify invertedClampFISH probes

Step 18.

Measure concentration by nanodrop.

NOTES

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I set the absorbance constant to 33 for ssDNA.