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Multi-round smHCR-FISH for archaea

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Abstract

Understanding archaeal gene regulation is technically challenging due to the difficulty of measuring gene expression in individual archaeal cells. Here, we develop a multi-round smHCR-FISH method to quantify the transcription of multiple genes in single cells of a methanogenic archaeon.

Materials

CRITICAL Use RNase- and DNase-free materials whenever possible.

Diethylpyrocarbonate (DEPC)-treated water, Rnase free (Ambion/Invitrogen, cat. no. 4387937)
10*PBS, Rnase free (Ambion/Invitrogen, cat. no.AM9624)
Formamide, deionized, nuclease free (Ambion/Invitrogen, cat. no.AM9342)
Sodium Chloride (Fisher Scientific, cat. no. BP358-1)
RNase Zap (Sigma, cat. no.R2020-250ml)
8% paraformaldehyde (EMS, cat. no.157-8, 10◊10ml)
Aqua-poly/mount (Polysciences, cat. no. 18606)
Ethanol, for molecular biology, absolute 500ml (Fisher chemical, cat. no. BP2818-500)
Tween 20 (Sigma, cat. no. P9416-50ml)
0.5M Na_2HPO_4 solution (Sigma, cat. no. 94046-100ml)
0.5M EDTA, PH 8.0, RNase free (Fisher Scientific, cat. no. AM9260G)
1M TRIS-HCl (ShengGong, cat. no.B548138-0500)
Dextran sulfate sodium salt (Sigma, cat. no. D8906)
20*SSC, RNase free (Invitrogen,AM9770)
Hoechst 33342 (Invitrogen, cat. no. H3570)
50*Denhardt (Fisher Scientific, cat. no. 750018)
Poly-L-Lysine (Sigma, cat. No. P4707)
HCR-FISH DNA probes with fluorescence dye labeled.
DNase I (TURBO DNase, AM2238)
popo^{TM}-1 (Invitrogen, P3580)

4-chamber glass-bottom dish (Cellvis,D35C4-20-1-N)
Zeiss LSM 880 laser scanning confocal microscope equipped with an Airyscan detector and a plan-Apochromat 63×/1.4 NA oil-immersion objective

Troubleshooting

Cell fixation:

1 For smHCR-FISH experiments, 3 mL of sampled culture was fixed with 1 mL of 8% (v/v) paraformaldehyde for
12 h at 4 °C, then washed twice with 1× PBS (10 min each), and stored in a 1:1 (v/v) mixture of ethanol and 1× PBS at -20 °C.

Multi-round smHCR-FISH and imaging

2 A 4-chamber glass-bottom dish (Cellvis, D35C4-20-1-N) was bottom-coated with poly-L-Lysine (Sigma, P4707). Fixed cells were settled onto the bottom of a well for two hours at room temperature. The dish was then mounted in the incubation system of a Zeiss LSM 880 laser scanning confocal microscope for multi-round smHCR-FISH.

3 For each round of the experiment, cells in each well were incubated in 40 mL of hybridization buffer (20 mM Tris-HCl, 25% (w/v) formamide, 250 mM EDTA, 2% (v/v) 50× Denhardt's solution, and 0.1% (v/v) Tween 20) with primary probes (0.1 mM for each gene) at 46 °C for 4 h.

4 Following hybridization, cells were washed three times (20 min each) at 48 °C in 500 mL of wash buffer (159 mM
NaCl, 20 mM Tris-HCl, 0.1% Tween 20).

5 For HCR amplification, each amplifier probe was separately dissolved in amplification buffer (5× SSC, 10% dextran sulfate, 0.1% Tween 20, 150 nM probe), heated to 95 °C for 2 min, and cooled to room temperature for 30 min to open the hairpin structure. Cells were incubated with a 20-mL-mixture of all amplifier probes at 35 °C in a humid atmosphere for 1 h.

6 After HCR amplification, cells were washed three times (10 min each) in 500 mL 4× SSC and stained with 1:10000 diluted Hoechst 33342 (Invitrogen, H3570) for 10 minutes. Following three additional rinses in 4× SSC, cells were covered by a mixture of Aqua-poly/Mount (Polysciences, 18606) and 4× SSC (~3:10 (v/v)) for imaging.

7 Image acquisition was performed using a Zeiss LSM 880 laser scanning confocal microscope equipped with an Airyscan detector and a plan-Apochromat 63×/1.4 NA oil-immersion objective.

8 After imaging, HCR-FISH probes in the cell were removed using DNase I (TURBO™ DNase, AM2238). Briefly, cells in each well were washed in 50 mL of 1× DNase buffer for 5 min and incubated with 3 mL DNase I (2 Units/mL) in 1× DNase buffer at 37 ℃ for 2 hours.

9 Following enzyme digestion, cells were washed three times (10 min each) in wash buffer at 37 ℃ and fixed in 2% PFA for 15 min at room temperature to inactivate DNase I.

10 After three more washes (10 mineach) in 4× SSC, samples were ready for the next round of the experiment. From
the second round on, since cellular DNA had been degraded in DNase I treatment, cellular RNA, instead of DNA, was stained for cell identification using popo^{TM}-1(Invitrogen,P3580).

Protocol references

1. Yamaguchi, T. et al. Rapid and sensitive identification of marine bacteria by an improved in situ DNA hybridization chain reaction (quickHCR-FISH). *Syst. Appl. Microbiol.* **38**, 400-405 (2015).
2. Choi, H.M.T. et al. Mapping a multiplexed zoo of mRNA expression. *Development* **143**, 3632-3637 (2016).
3. Morono, Y., Kubota, K., Tsukagoshi, D. & Terada, T. EDTA-FISH: A Simple and Effective Approach to Reduce Non-specific Adsorption of Probes in Fluorescence in situ Hybridization (FISH) for Environmental Samples. *Microbes Environ.* **35**, ME20062 (2020).
4. Shah, S., Lubeck, E., Zhou, W. & Cai, L. In situ transcription profiling of single cells reveals spatial organization of cells in the mouse hippocampus. *Neuron* **92**, 342-357 (2016).

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