Title

A collection of archaeal 16S rRNA CloneFISH cultures for probe validation in fluorescence *in situ* hybridization experiments

Running title

Archaeal 16S rRNA CloneFISH cultures

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Abstract

We present a collection of 30 *Escherichia coli* cultures (CloneFISH cultures), each carrying a plasmid for the heterologous expression of a (near) full-length 16S rRNA gene from one of 30 lineages of archaea, including 17 yet uncultured ones. We make these clones available for use as controls in FISH experiments.

Manuscript text

rRNA-targeted fluorescence *in situ* hybridization (FISH) is a key technique in microbial ecology but is under-utilized because of the lack of positive controls with which newly developed probes can be tested (1,2). This limitation can be overcome by FISH of rRNA gene clones *a.k.a.* Clone-FISH (3). Unfortunately, the traditional Clone-FISH technique can be cumbersome or prohibitively expensive for individual labs. Here, we offer the scientific community a collection of 30 *Escherichia coli* CloneFISH cultures to enable validation of new probes and use as positive controls for 16S rRNA-targeted FISH experiments of diverse archaea.

Plasmid construction for CloneFISH was inspired by the original CloneFISH protocol (3) but featured modifications stemming from the unavailability of gene synthesis technology at the time of the original publication and the use of an updated plasmid backbone. We selected 30 (near) full-length 16S rRNA gene

sequences that together represent most of the currently uncultured archaeal diversity in nature (4), including most major lineages within the four archaeal superphyla (Table 1; Table S1). 17 lineages represented by these cultures have yet not been cultured; four lineages are currently represented by only one culture.

Sequences were synthesized (Twist Bioscience), Gibson-assembled downstream of a T7 promoter into ApaI-linearized pCR2.1-oriT plasmids, and transformed into chemically competent T7 Express *E. coli* cells (New England Biolabs) according to manufacturer instructions. DNA constructs were sequence-verified (Pacific Biosciences). Located upstream and downstream of each 16S rRNA gene insert are an initiator and terminator sequence, respectively. We found that these sequence extensions only minimally affect 16S rRNA secondary structure (5). Each plasmid carries resistance genes against the antibiotics ampicillin and kanamycin/neomycin. To heterologously express archaeal 16S rRNA genes in *E. coli* we used a modified version of the original CloneFISH protocol (3). An overnight culture of the *E. coli* culture was inoculated into LB medium containing either 100 µg/mL ampicillin or 100 µg/mL kanamycin (final concentrations). Cells were grown at 37 ºC at 200 rpm shaking until they had reached an optical density (OD_{600}) of 0.3-0.4. Then, heterologous transcription was initiated by adding 1 mM (final) isopropyl-*β*-D-thiogalactopyranoside to induce transcription of the archaeal rRNA. Cells were incubated until they had reached an OD_{600} of ~0.8 before 100 µg/mL (final) chloramphenicol was added and cells incubated for an additional three hours. Then, 1.5 mL of culture were harvested by centrifugation and cells fixed in 3% paraformaldehyde in 1x PBS for 1 hour at room temperature. Cells were washed with 1x PBS before the pellet was resuspended in a 1:1 mix of absolute ethanol and 1x PBS and stored at -20 ºC.

Culture aliquots were inspected by FISH using bacterial (EUB338, (6)) and either archaeal (Arch915, (7)) or lab-internal FISH probes to confirm expression of the archaeal rRNA in *E. coli*. Cultures in which transcription had not been induced (using Arch915) and NON-EUB338 (8) (using *E. coli* expressing archaeal rRNA) were used as negative controls (Figure 1).

Data availability

CloneFISH cells that had heterologously expressed archaeal 16S rRNA before chemical fixation with 3% paraformaldehyde or glycerol stocks are available for free upon request from the lab of the corresponding author (R.H.). The requester is asked to cover shipping costs.

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References

1. Azevedo N, Almeida C. 2021. Fluorescence In Situ Hybridization (FISH) for Microbial Cells: Methods and Concepts. Springer Nature.
2. Amann R, Fuchs B. 2008. Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques. Nat Rev Microbiol 6, 339-348.
3. Schramm A, Fuchs B, Nielsen J, Tonolla M, Stahl D. 2002. Fluorescence in situ hybridization of 16S rRNA gene clones (Clone-FISH) for probe validation and screening of clone libraries. Environ Microbiol 4, 713-720.
4. Baker BJ, De Anda V, Seitz KW, Dombrowski N, Santoro AE, Lloyd KG. 2020. Diversity, ecology and evolution of Archaea. Nat Microbiol 5:887–900.
5. Mathews D, Disney M, ChildS J, Schroeder S, Zuker M, Turner D. 2004. Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. Proc Natl Acad Sci U S A 101, 7287-7292.
6. Amann R, Binder B, Olson R, Chisholm S, Devereux R, Stahl D. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56, 1919-1925.
7. Stahl D, Amann R. 1991. Development and application of nucleic acid probes in Nucleic acid techniques in bacterial systematics. John Wiley & Sons Ltd 205-248.
8. Wallner G, Amann R, Beisker W. 1993. Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. Cytometry 14, 136-143.

Table 1. List of archaeal CloneFISH cultures and their phylogenetic affiliations. The Column 'Cultured representative?' indicates whether any member of that archaeal lineage has been obtained in (pure or enrichment) culture. For more details and 16S rRNA sequence information see TableS1 (https://doi.org/10.6084/m9.figshare.29361158.v1).

Clone #	Clone name	Representative of lineage	Cultured representative?	Superphylum
01	AenigO16	Aenigmarchaeota	No	DPANN
02	Caldisub	Aigarchaeota	Yes	Thermoproteota
03	AltiSM1	Altiarchaeota	No	DPANN
04	Atabey1	Atabeyarchaeia	No	Asgard
05	BathyJZ7	Bathyarchaeota	Yes	Thermoproteota
06	BrockGD2	Brockarchaeota	No	Thermoproteota
07	Culex-027	Culexarchaeia	No	Thermoproteota
08	DiapAR10	Diapherotrites/Iainarchaeota	No	DPANN
09	Freya1	Freyarchaeia	No	Asgard
10	GeoOSPB1	Geoarchaeota	No	Thermoproteota
11	GtJdFR14	Geothermarchaeota	No	Thermoproteota
12	HadesN21	Hadesarchaea	Yes	Euryarchaeota
13	HeiAB125	Heimdallarchaeota	Yes	Asgard
14	HelMeg19	Helarchaeota	No	Asgard
15	HyJdFR18	Hyrothermarchaeota	No	Euryarchaeota
16	LCB3	Korarchaeia	Yes	Thermoproteota
17	Loki-LSSM13-B	Lokiarchaeia	Yes	Asgard
18	MarsG2c2	Marsarchaeota	No	Thermoproteota
19	Mthermo1	Methanobacteria	Yes	Euryarchaeota
20	LCB24	Methanoglobus	Yes	Euryarchaeota
21	LCB70	Methanomethylicia	Yes	Thermoproteota
22	Mhalo	Methanomicrobia	Yes	Euryarchaeota
23	YNP3N	Methanonezhaarchaeia	Yes	Thermoproteota
24	Nitgar	Nitrososphaeria	Yes	Thermoproteota
25	UMGIII	Pontarchaea	No	Euryarchaeota
26	Thalmedi	Poseidoniales	No	Euryarchaeota
27	Z7ME17	Theionarchaea	No	Euryarchaeota
28	MlumB10	Thermoplasmata	Yes	Euryarchaeota
29	Thor-LSSM13-A	Thorarchaeia	No	Asgard
30	Woese1	Woesearchaeia	No	DPANN

Figure 1. CloneFISH cells expressing both bacterial and archaeal 16S rRNA. (A) DAPI, DNA stain. (B) EUB338, binding the 16S rRNA of *E. coli*. (C) Arch915, binding the 16S rRNA of a member of the yet uncultured phylum Hydrothermarchaeota. Sale bar is approximately 10 μm.