A collection of archaeal 16S rRNA Clone-FISH cultures for probe validation in fluorescence in situ hybridization experiments

Joelie M. Van Beek; Grayson Robles; Ritesh Mewalal; Ian Blaby; Roland Hatzenpichler

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Environmental Microbiology | Announcement

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

Joelie M. Van Beek,[1] Grayson Robles,[1] Ritesh Mewalal,[2] Ian Blaby,[2] Roland Hatzenpichler[1,3]

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT We present a collection of 30 *Escherichia coli* cultures (Clone-FISH cultures), each carrying a plasmid for the heterologous expression of a (near) full-length 16S rRNA gene from 1 of 30 lineages of archaea, including 17 yet uncultured ones. We make these clones available for use as controls in fluorescence *in situ* hybridization experiments.

KEYWORDS archaea, fluorescence *in situ* hybridization, controls, microscopy, taxonomic identification, plasmids, 16S rRNA, *E. coli*, CloneFISH, microbial ecology

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, also known as 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* Clone-FISH cultures to enable validation of new probes and use as positive controls for 16S rRNA-targeted FISH experiments of diverse archaea (Fig. 1).

We selected 30 (near) full-length 16S rRNA gene sequences that together represent most of the currently uncultured archaeal diversity in nature (4) (Table 1; Table S1). Sequences were synthesized, including 30-mer vector linkers (Twist Bioscience), Gibson-assembled (NEBuilder HiF Assembly, New England Biolabs) downstream of a T7 promoter into ApaI-linearized pCR2.1-oriT plasmids (ThermoFisher Scientific), and transformed into chemically competent T7 Express *E. coli* cells (New England Biolabs) according to the manufacturer's instructions. Inserts were sequence verified by colony PCR amplification of the DNA insert using universal vector-specific primers (pCR2.1-oriT_F: TTTATGCTTCCGGCTCGTATGTTGTGTGGA and pCR2.1-oriT_R: CAAGCCAACCAGGA AGGGCAGCCCACCTAT), followed by sequencing of PCR amplicons on a PacBio Sequel II platform. Located upstream and downstream of each 16S rRNA gene insert are an initiator and terminator sequence, respectively. 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 Clone-FISH 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°C 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 reached an OD_{600} of ~0.8 before adding 100 µg/mL (final) of chloramphenicol. The cells were then incubated for an additional 3 hours. Then, 1.5 mL of culture was harvested by centrifugation, and cells were fixed in 3% paraformaldehyde in 1× PBS for 1 hour at room temperature. Cells were washed with 1× PBS before the pellet was resuspended in a 1:1 mix of absolute ethanol and 1× PBS and stored at −20°C.

Editor Kenneth M. Stedman, Portland State University, Portland, Oregon, USA

Address correspondence to Roland Hatzenpichler, rolandhatzenpichler@gmail.com.

Joelie M. Van Beek and Grayson Robles contributed equally to this article. Author order was determined by seniority.

The authors declare no conflict of interest.

See the funding table on p. 3.

Received 9 October 2025
Accepted 16 October 2025
Published 17 November 2025

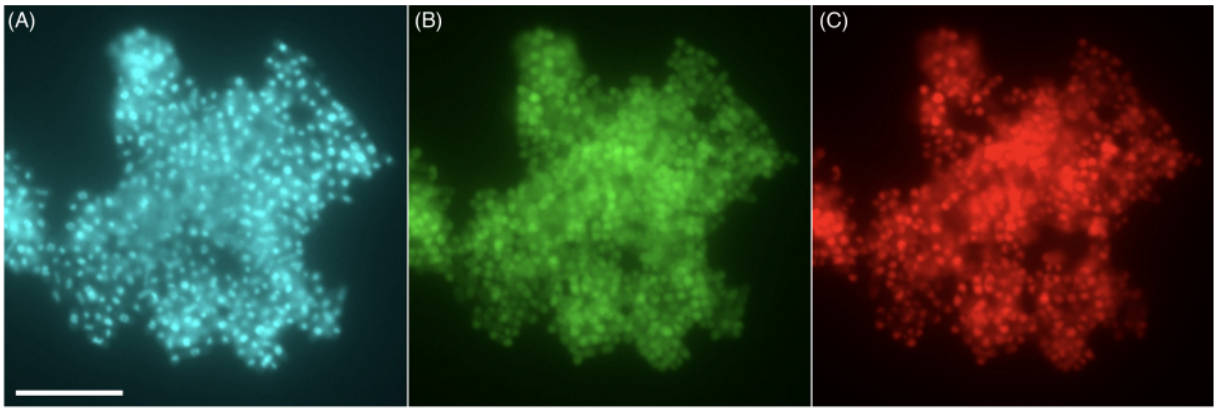

FIG 1 Clone-FISH cells expressing both bacterial and archaeal 16S rRNA. (A) DAPI, DNA stain. (B) EUB338, binding the 16S rRNA of *E. coli*. (C) Arch915, binding the 16S rRNA of a member of the yet uncultured phylum Hydrothermarchaeota. Scale bar is approximately 10 µm.

TABLE 1 List of archaeal Clone-FISH cultures and their phylogenetic affiliations[a]

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	Methanosuratincolia	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

[a]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 Table S1 (https://figshare.com/articles/dataset/Table_S1_for_Van_Beek_et_al_2025_Microbiology_Resource_Announcements/29361158/2).

Culture aliquots were inspected by FISH using bacterial (EUB338 [5]) and either archaeal (Arch915 [6]) or lab-internal FISH probes to confirm the expression of the archaeal rRNA in *E. coli*. Hybridizations using archaea-targeted probes of cultures in which transcription had not been induced, as well as hybridizations with probe NON-EUB338 (7), were used as negative controls.

ACKNOWLEDGMENTS

This work was supported through award DE-SC0025661 by the U.S. DOE Biological and Environmental Research program to R.H. and a DOE Joint Genome Institute DNA Synthesis Community Science Program award to Tim McDermott and R.H. The work (proposal: DOI: https://doi.org/10.46936/10.25585/60001212) conducted by the U.S. Department of Energy Joint Genome Institute (https://ror.org/04xm1d337), a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy, operated under Contract No. DE-AC02-05CH11231. J.V.B. was partially supported by NSF Research Traineeship Program award 2125748.

All opinions expressed in this paper are the authors' and do not necessarily reflect the policies and views of the DOE.

AUTHOR AFFILIATIONS

[1]Department of Microbiology and Cell Biology, Thermal Biology Institute, Center for Biofilm Engineering, Montana State University, Bozeman, Montana, USA
[2]Department of Energy Joint Genome Institute, Berkeley, California, USA
[3]Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana, USA

AUTHOR ORCIDs

Roland Hatzenpichler http://orcid.org/0000-0002-5489-3444

FUNDING

Funder	Grant(s)	Author(s)
U.S. Department of Energy	DE-SC0025661	Roland Hatzenpichler
National Science Foundation	2125748	Joelie M. Van Beek

AUTHOR CONTRIBUTIONS

Joelie M. Van Beek, Formal analysis, Investigation, Visualization, Writing – review and editing | Grayson Robles, Formal analysis, Investigation, Visualization | Ritesh Mewalal, Formal analysis, Investigation, Writing – review and editing | Ian Blaby, Formal analysis, Investigation, Writing – review and editing | Roland Hatzenpichler, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

Plasmids have been deposited at AddGene (https://www.addgene.org/Roland_Hatzenpichler/). Clone-FISH 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.

REFERENCES

1. Azevedo N, Almeida C. 2021. Fluorescence *in situ* hybridization (FISH) for microbial cells: methods and concepts. Springer Nature.
2. Amann R, Fuchs BM. 2008. Single-cell identification in microbial communities by improved fluorescence *in situ* hybridization techniques. Nat Rev Microbiol 6:339–348. https://doi.org/10.1038/nrmicro1888

3. Schramm A, Fuchs BM, Nielsen JL, Tonolla M, Stahl DA. 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. https://doi.org/10.1046/j.1462-2920.2002.00364.x
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. https://doi.org/10.1038/s41564-020-0715-z
5. Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56:1919–1925. https://doi.org/10.1128/aem.56.6.1919-1925.1990
6. Stahl D, Amann R. 1991. Development and application of nucleic acid probes in bacterial systematics, p 205–248. In Nucleic acid techniques in bacterial systematics. John Wiley & Sons Ltd.
7. 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. https://doi.org/10.1002/cyto.990140205