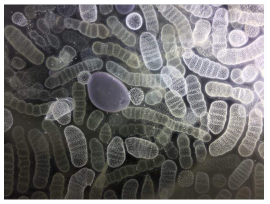
Jun 15, 2020

Collect of Collodarian (Rhizaria, Radiolaria) nuclei for genomic analyses



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We use this protocol and it's working

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Abstract

Collodaria are ubiquitous and abundant marine radiolarian (Rhizaria) protists (Biard *et al*. 2015). They occur as large colonies (a few millimeters up to 3 meters long) or as solitary specimens. Collodarians are known to play an important role in oceanic food webs both as active predators and as hosts of intracellular endosymbiotic microalgae primarily belonging to the dinoflagellate genus *Brandtodinium*. Despite their important ecological roles, very little is known about their diversity and evolution. Taxonomic delineation of collodarians is challenging and only a few species have been genetically characterized.

Most Collodaria form colonies comprising tens to hundreds of individual radiolarian cells (i.e. central capsules) embedded in a gelatinous matrix. Each central capsule contains genomic DNA of the Collodaria host while the gelatinous matrix which also contains the DNA of prey and symbionts.

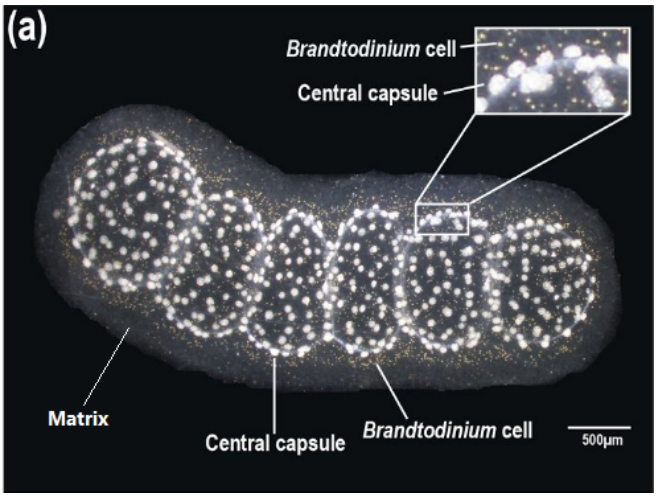

Figure 1: Cells (i.e. central capsules) are distributed in the matrix forming a well-defined compartment. Central capsules, appearing bright under the microscope, measure from 100 to 150 µm in diameter. The dinoflagellate symbionts are enclosed in cytoplasmic structures, either localized within the gelatinous matrix or closely associated to the central capsules. (from Villar *et al*. 2018)

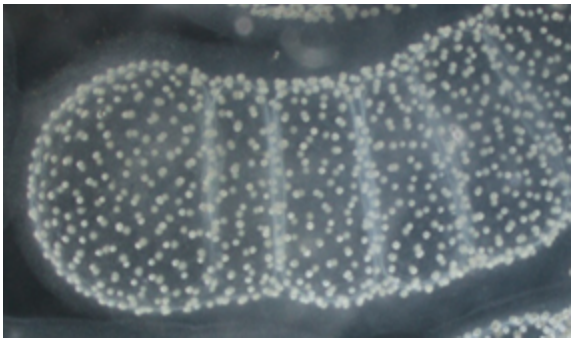

Figure 2a: 1 part of a Collodaria colony. Each cells is visible thanks to its central capsule (white dots) containing its nucleus. ©Pictures IMPEKAB - E. Bigeard - VFR2016

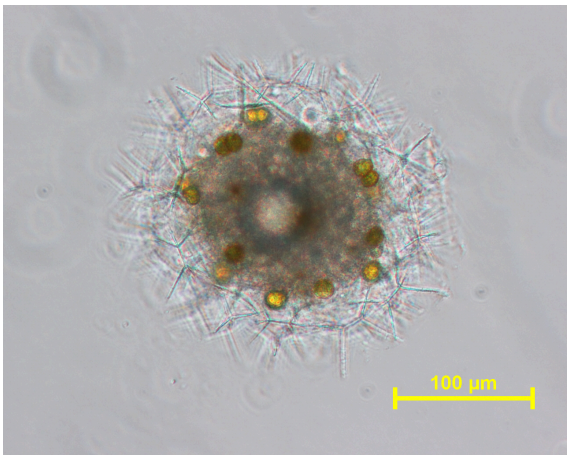

Figure 2b: Magnified view of a single central capsules with surrounding spicules (colorless) and symbionts (yellow). ©Pictures - F. Not

Some species build a shell-like skeleton aroundtheir central capsule while others have siliceous spicules, similar to those in sponges, in the matrix, and some lack mineral structures altogether. Current taxonomic classification reveals several clades : Sphaerozoidae (skeleton-less but spicule-bearing), Collosphaeridae (mix of skeleton-bearing and skeleton-less taxa), Collophidiidae (skeleton-less). The family Thalassicollidae is composed exclusively of solitary species.

This protocol describes a method for isolating central capsules containing oly the genomic DNA of the collodarian host by removing prey and symbionts through targeted dissolution of the gelatinous matrix and removal all material outside of host central capsules.

Attachments

PDF 2006 Lee et al.pdf 525KB

PDF Biard et al ISME2017... 4.1MB

PDF Biard et al Protist2... 3.4MB

PDF Biard et al Nature20... 16.5MB

Guidelines

protocol EB-BM-MO-016

Villar E, Dani V, Bigeard E, Linhart T, MendezSandin M, Bachy C, Six C, Lombard F, Sabourault C & Not F (2018). Chloroplasts of symbiotic microalgae remain active during bleaching induced by thermal stress in Collodaria(Radiolaria) doi:10.1101/263053

Biard T, Bigeard E, Audic S, Poulain J, Gutierrez-Rodriguez A, Pesant S, Stemmann L & Not F (2017). Biogeography and diversity of Collodaria (Radiolaria) in the global ocean. ISME Journal, doi:10.1038/ismej.2017.12

Biard T, Stemmann L, Picheral M, Mayot N, Vandromme P, Hause H, Gorsky G, Guidi L, Kiko R & Not F (2016). In situ imaging reveals the biomass of giant protists in the global oceans. Nature, doi:10.1038/nature17652

Biard T., Pillet L., Decelle J., Poirier C., Suzuki N. & Not F (2015). Towards an Integrative Morpho-molecular Classification of the Collodaria (Polycystinea, Radiolaria). Protist, doi:10.1016/j.protis.2015.05.002

Lee *et al*. (2007), Monitoring Repair of DNA Damage in Cell Lines and Human Peripheral Blood Mononuclear Cells, Anal Biochem. doi: 10.1016/j.ab.2007.03.016

Materials

Chemicals:
Sucrose Ref S0389 - Sigma-Aldrich
Spermine Ref S3256 - Sigma-Aldrich
Spermidine Ref 85558 - Sigma-Aldrich
NaCl Ref S9888 - Sigma-Aldrich
KCl Ref P9333 - Sigma-Aldrich
Tris HCl Ref T5941 - Sigma-Aldrich
EDTA 0.5M pH8 Ref 03690 - Sigma-Aldrich
Igepal CA-630 Ref I8896 - Sigma-Aldrich
PBS Ref P3744-12PAK - Sigma-Aldrich

Supplies:
6wells - plate Ref CC7672-7506 - Starlab
40µ sieve Ref 010198 - Dominique Dutscher
Petri dishes Ref 632191 - Dominique Dutscher

Solutions

1 Preparation of Solutions

1.1 Sucrose 3M (M = 342.3g / mol)

112.96g in 110ml water milliQ
Store at room temperature

1.2 Spermine 0.1M (M = 202.34g / mol)
Powder stored at 4 ° C.

Weigh 40.5mg in a 15ml Falcon.
then add 2ml water milliQ.

Preparation instructions: This product is soluble in water (50 mg / ml), yielding a clear, colorless to light yellow solution.
Storage / Stability: Store at 2-8 ° C.
Solutions of spermine free base are readily oxidized. Solutions are most stable when prepared in degassed water and stored in frozen aliquots, under argon or nitrogen gas.

1.3 Spermidine 0.05M (M = 145.25g / mol)
Liquid stored at room temperature.

Weigh 87.15mg in a 15ml Falcon.
then add 12ml water milliQ.

Preparation instructions: Spermidine is soluble in water (50 mg / ml), ethanol, and ether.
Storage / Stability: Spermidine is very hygroscopic and air sensitive. A solution can be formed for storage by dissolving 1.45 g in 10 ml of water and then sterilizing with a 0.22 µm filter.

Store this solution as single-use aliquots at -20 ° C for no longer than one month.

1.4 4M NaCl (M = 58.44g / mol)

Weigh 0.47g of NaCl.
Add 2ml of milliQ water.
Store at room temperature.

1.5 KCl 5M (M = 74.5513 / mol)

Weigh 2.6g of KCl.
Add 7ml of milliQ water.
Store at room temperature.

1.6 Tris HCl 1M pH8 (M = 157.60 / mol)

Weigh 15.76g of Tris HCl.
Make up to 100ml of milliQ water.
Tamp to pH8.
Store at room temperature.

1.7 EDTA 0.5M pH8 (M = 292.24 / mol)

Weigh 15g of EDTA.
Add 50ml of milliQ water.
Add NaOH pellets until a pH of 8 is reached.
QSP 100ml of water milliQ.
Filter on 0.2µm.
Store at room temperature.

1.8 For 500ml of Lysis Buffer Solution

Product	Initial concentration	Final concentration	volume
Sucrose	3M	0.3M	50ml
KCl	5M	60mM	6ml
NaCl	4M	15mM	1.875ml
Tris HCl pH8	1M	60mM	30ml
Spermidine	0.05M	0.5mM	5ml
Spermine	0.1M	0.15mM	750µl
EDTA	0.5M	2mM	2ml
Igepal CA-630			2.5ml

	Water milliQ			Qsp 500ml

Table 1: Recipes for Lysis Buffer Solution

1.9 For 500ml of Wash Buffer solution

	Product	**Initial concentration**	**Final concentration**	**volume**
	Sucrose	3M	0.3M	50ml
	KCl	5M	60mM	6ml
	NaCl	4M	15mM	1.875ml
	Tris HCl pH8	1M	60mM	30ml
	Spermidine	0.05M	0.5mM	5ml
	Spermine	0.1M	0.15mM	750µl
	EDTA	0.5M	2mM	2ml
	Water milliQ			Qsp 500ml

Table 2: Recipes for Wash Buffer Solution

Selection of colonies

2 Sort several colonies (maximum 20) according to their morphological type (segmented round shape, segmented straight form, non-segmented, purple color, blue dots, etc.) assuming it corresponds to a single species. Observe using a stereomicroscope and take pictures in wide field and zoom.

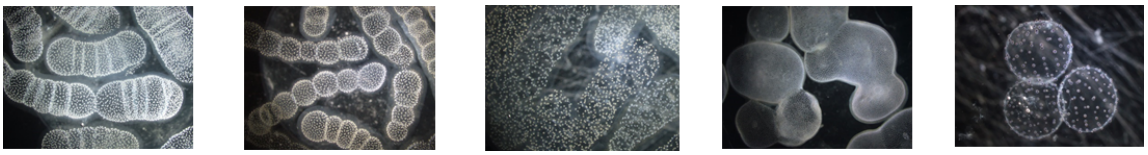

Figure 3 : Pictures from 5 different types of colladarians (Types A; B; C; D purple colonies & E colonies with blue dots) - ©Pictures IMPEKAB - E. Bigeard - VFR2016

Lysis of colonies

3 In a 6-well plate, deposit 8 ml of Lysis solution per well.
Add a 40µm diameter sieve previously rinsed with milliQ water per well. Place about 10 medium sized colonies (1.5 - 2 cm) per sieve. Incubate for 30 minutes at room temperature.
Take a sieve from a well, place it in a 90mm diameter petri dish containing Lysis solution.
Tap the sieve and shake circles with the sieve in the BP.
Repeat several times until the matrix breaks up and is released from the sieve (about 3 minutes).

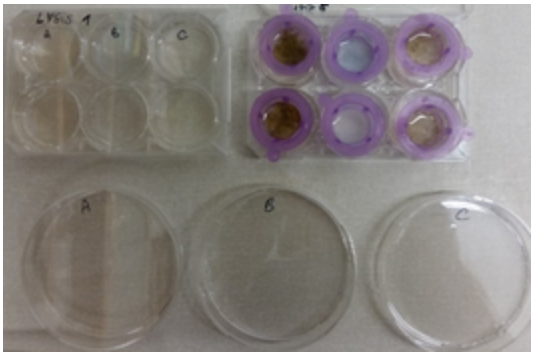

Figure 4: Plates containing lysis buffer and sieves with colonies and BP containing wash buffer - ©Pictures IMPEKAB - E. Bigeard - VFR2016

For purple colonies and those with blue dots, just one lysis will suffice. For other types, perform with a second lysis as below.
In a 6-well plate, deposit 8 ml of Lysis solution per well. Place the sieve containing the colonies at the beginning of lysis.
Incubate again for 30 minutes at room temperature.

Washing of central capsules

4 Remove the sieve from the well and place it in the petri dish containing Wash solution.
Tap the sieve and shake circles with the sieve in the BP.

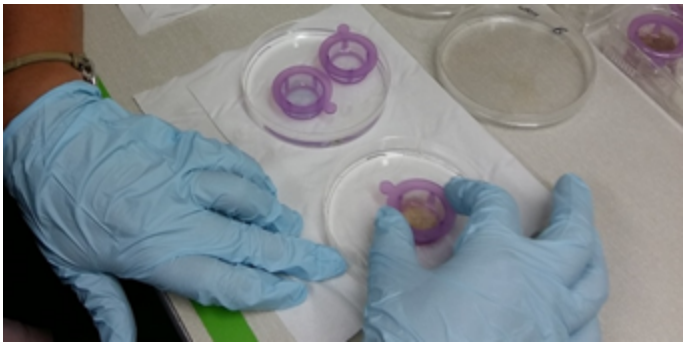

Figure 5 : Washing of collodarian central capsules - ©Pictures IMPEKAB - E. Bigeard - VFR2016

Washing of nuclei present in the sieve.

Repeat several times until the matrix is completely removed.
Observe and control with a binocular loupe (capsule size with respect to the pores, cleanliness of the sample, etc.).
Take pictures If OK.

Rinsing & Concentration of nuclei

5 Rinse the capsules in 1x PBS solution or in 0.2µm filtered and autoclaved seawater in a 90mm diameter BP.

Remove the capsules from the sieve by rinsing it with 0.2µm filtered seawater or 1x PBS solution.
Place the capsules in a 1.5ml microtube (or 2ml microtube if necessary).
Centrifuge at 1000 rcf 10 minutes.
Remove the supernatant.

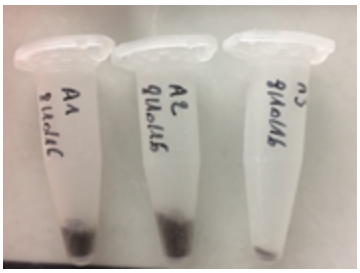

Figure 6: Pellet of central capsules - ©Pictures IMPEKAB - E. Bigeard - VFR2016

Flash freezer.
Storage at -80 ° C.

If using of sea water in the above steps, it is better to rinse with PBS before the DNA extraction to remove salt:
Rinse with 1x PBS solution by depositing 500 µl of PBS solution into the microtube.
Tap gently.
Centrifuge at 1000rccf 10 minutes.
Remove the supernatant.
Repeat once if necessary.

Analyses

6 The DNA extraction method will be published under a separate protocols.io (in collaboration with Genoscope).