Embedding yeast colonies for light and electron microscopy

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Abstract

The following is a modification of a Scherz R., 2001 method for colony embedding. The protocol described here is from:

Sarah Piccirillo, *et. al.* The Rim101p/PacC Pathway and Alkaline pH Regulate Pattern Formation in Yeast Colonies (2010)
Genetics 184:707-716; doi:10.1534/genetics.109.113480

Please see the full manuscript for additional details.

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Guidelines

To embed colonies for sectioning, we modified a previously described method (Scherz et al. 2001), with a major change being the substitution of Spurr's reagent as embedding medium.

Original method:
SCHERZ, R., V. SHINDER and D. ENGELBERG, 2001 Anatomical analysis of Saccharomyces cerevisiae stalk-like structures reveals spatial organization and cell specialization. J. Bacteriol. 183: 5402–5413.

Protocol

Step 1.
Incubate approximately 300 colonies on agar medium for the indicated time.

Step 2.
Remove an isolated colony (1-2 mm in diameter) and a small amount of the underlying agar medium, and place on a microscope slide

Step 3.
Place several drops of 2% agar (42°C) on a microscope slide, and immediately place the colony on the agar and then place several drops of agar on top of the colony and allow to solidify.

Step 4.
Trim the resulting agar block with a razor blade, and place in a 3.5 ml borosilicate screw-cap vial (Fisher 03-339-21B) containing 1.5ml 2%paraformaldehyde/2%glutaraldehyde.

NOTES
Saul Honigberg 30 Sep 2015

All subsequent incubations and washes use 1.5 -2.0 ml and are performed in the same vial.

Step 5.
Fix colonies by incubating for 7 days at 4°C

Step 6.
Wash #1: Wash agar blocks on ice by incubating for 15 minutes with 1.5 ml of 0.15M sodium cacodylate (pH 7.2)

DURATION
00:15:00

Step 7.
Wash #2: Wash agar blocks on ice by incubating for 15 minutes with 1.5 ml of 0.15M sodium cacodylate (pH 7.2)

DURATION
00:15:00

Step 8.
Wash #3: Wash agar blocks on ice by incubating for 5 minutes with 1.5ml OS buffer (100 mM KH_2PO_4, 10 mM $MgCl_2$, pH 6.0).

DURATION
00:05:00

Step 9.
Wash #4: Wash agar blocks on ice by incubating for 5 minutes with 1.5ml OS buffer (100 mM KH_2PO_4, 10 mM $MgCl_2$, pH 6.0).

DURATION
00:05:00

Step 10.
If sections will be used for electron microscopy, add 1% OsO_4 in OS to vials to cover the agar blocks and incubate on ice in a chemical fume hood for 1 hr. Otherwise skip to step 13.

DURATION
01:00:00

Step 11.
Dispose of 1% OsO_4 in hazardous waste.
WashA: Wash with 1.5ml OS buffer by incubating on ice for 10 minutes.

Step 12.
WashB: Wash with 1.5ml OS buffer by incubating on ice for 10 minutes.

Step 13.
Add 1.5 ml OS and incubate overnight at 4°C.

DURATION
18:00:00

Step 14.
WashA: Wash blocks with 1.5ml cold water by incubating on ice for 10 minutes.

Step 15.
WashB: Wash blocks with 1.5ml cold water by incubating on ice for 10 minutes.

Step 16.
Wash #1: Add 1.5ml cold 25% ethanol and incubate on ice for 10 minutes.

DURATION
00:10:00

Step 17.

Wash #2: Add 1.5ml cold 50% ethanol and incubate on ice for 10 minutes.

DURATION
00:10:00

Step 18.
Wash #3: Add 1.5ml cold 75% ethanol and incubate on ice for 10 minutes.

DURATION
00:10:00

Step 19.
Wash #4: Add 1.5ml cold 95% ethanol and incubate on ice for 10 minutes.

DURATION
00:10:00

Step 20.
Wash #5: Add 1.5ml cold 100% ethanol and incubate on ice for 10 minutes.

DURATION
00:10:00

Step 21.
Wash #6: Add 1.5ml cold 100% ethanol and incubate on ice for 10 minutes.

DURATION
00:10:00

Step 22.
Remove ethanol and resuspend in 1.5ml cold 100% ethanol. Leave the blocks overnight at 4°C in 100% ethanol.

DURATION
18:00:00

Spurr’s treatment

Step 23.
Make Spurr's reagent by stirring slowly under chemical fume hood 5 grams ERL4221, 4 grams DER736 and 13 grams NSA for 20 minutes(Electron Microscopy Sciences).

Spurr’s treatment

Step 24.
Add 0.15 grams DMAE(Electron Microscopy Sciences) and stir for 20 minutes. De-gas for 1-2 hrs.

DURATION
00:15:00

Spurr’s treatment

Step 25.
Wash blocks with 1.5ml unopened room temp 100% ethanol by incubating at room temp for 10 minutes. Repeat 4 more times.

DURATION
00:30:00

Spurr’s treatment

Step 26.
Treatment#1: Remove ethanol and add 1.5ml of a 2:1 ratio of 100% ethanol:Spurr's reagent.

DURATION
00:15:00

Spurr’s treatment

Step 27.

Treatment#1: Rotate vial for 15 minutes on wheel at room temperature.

DURATION

00:30:00

Spurr’s treatment

Step 28.

Treatment#1: Allow to stand for 30 minutes at room temperature.

DURATION

00:15:00

Spurr’s treatment

Step 29.

Treatment#2: Remove 100%ethanol:Spurr's reagent and add 1.5ml of a 2:1 ratio of 100% ethanol:Spurr's reagent. Rotate vial for 15 minutes on wheel at room temperature.

DURATION

00:30:00

Spurr’s treatment

Step 30.

Treatment#2: Allow to stand for 30 minutes at room temperature.

DURATION

00:15:00

Spurr’s treatment

Step 31.

Treatment #3: Remove 100% ethanol:Spurr's reagent and add 1.5ml of a 2:1 ratio of 100%ethanol: Spurr's reagent. Rotate vial for 15 minutes on wheel at room temperature.

DURATION

00:30:00

Spurr’s treatment

Step 32.

Treatment #3: Allow to stand for 30 minutes at room temperature.

DURATION

00:15:00

Spurr’s treatment

Step 33.

Treatment #4: Remove 100% ethanol: Spurr's reagent and add 1.5ml of a 1:1 ratio of 100%ethanol:Spurr's reagent. Rotate vials for 15 minutes on wheel at room temperature.

DURATION

00:30:00

Step 34.

Treatment #4: Allow to stand for 30 minutes at room temperature.

DURATION

04:00:00

Step 35.

Treatment#5: Remove 100% ethanol:Spurr's reagent and add 1.5ml of a 1:1 ratio of 100%ethanol: Spurr's reagent. Rotate vials for 15 minutes on wheel at room temperature.

DURATION

18:00:00

Step 36.

Treatment#5: Allow to stand for 30 minutes at room temperature.

DURATION

18:00:00

Step 37.

Remove 100% ethanol:Spurr's reagent and add 1.5ml Spurr's reagent to vial. Incubate for 4hrs at room temperature.

DURATION

04:00:00

Step 38.

Replace with 1.5ml Spurr's reagent and rotate the vial overnight on the wheel at room temperature.

DURATION

18:00:00

Step 39.

Replace with 1.5ml Spurr's reagent and rotate the vial until late afternoon on the wheel at room temperature.

Step 40.

Remove the Spurr's reagent and replace with 1.5ml freshly made Spurr's reagent. Rotate the vial overnight on the wheel at room temperature.

DURATION

00:00:15

Step 41.

The next day replace with 1.5ml Spurr's reagent and rotate until the following day on the wheel at room temperature.

Step 42.

The next day replace with 1.5ml Spurr's reagent and rotate until the following day on the wheel at room temperature.

Step 43.

Place each agar block in a mold with 0.2ml Spurr's reagent and incubate at 60°C for four hours.

Step 44.

Top off the molds with Spurr's reagent and incubate at 60°C for 3 days.

Step 45.

Collect sections(0.5u) from the central region of the colony in a drop of dH20 on a glass slide. Dry slide on a 52°C heat block.

Step 46.

Stain sections with 1% toluidine blue,1%Sodium Borate for 5-15 seconds.

Step 47.

Wash slide under a stream of dH20. Dry on heat block. Cover in Permount(Fisher SP15-100). Examine by light microscopy.