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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.

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.

Troubleshooting

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

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

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.

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.

Note

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

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

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)

00:15:00

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)

00:15:00

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).

00:05:00

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).

00:05:00

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.

01:00:00

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

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

13 Add 1.5 ml OS and incubate overnight at 4°C.
18:00:00

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

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

16 Wash #1: Add 1.5ml cold 25% ethanol and incubate on ice for 10 minutes.
00:10:00

17 Wash #2: Add 1.5ml cold 50% ethanol and incubate on ice for 10 minutes.
00:10:00

18 Wash #3: Add 1.5ml cold 75% ethanol and incubate on ice for 10 minutes.
00:10:00

19 Wash #4: Add 1.5ml cold 95% ethanol and incubate on ice for 10 minutes.
00:10:00

20 Wash #5: Add 1.5ml cold 100% ethanol and incubate on ice for 10 minutes.
00:10:00

21 Wash #6: Add 1.5ml cold 100% ethanol and incubate on ice for 10 minutes.
00:10:00

22 Remove ethanol and resuspend in 1.5ml cold 100% ethanol. Leave the blocks overnight at 4°C in 100% ethanol.
18:00:00

Spurr's treatment

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).

24 Add 0.15 grams DMAE(Electron Microscopy Sciences) and stir for 20 minutes. De-gas for 1-2 hrs.
00:15:00

25 Wash blocks with 1.5ml unopened room temp 100% ethanol by incubating at room temp for 10 minutes. Repeat 4 more times.
00:30:00

26 Treatment#1: Remove ethanol and add 1.5ml of a 2:1 ratio of 100% ethanol:Spurr's reagent.
00:15:00

27 Treatment#1: Rotate vial for 15 minutes on wheel at room temperature.
00:30:00

28 Treatment#1: Allow to stand for 30 minutes at room temperature.
00:15:00

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.
00:30:00

30 Treatment#2: Allow to stand for 30 minutes at room temperature.
00:15:00

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.
00:30:00

32 Treatment #3: Allow to stand for 30 minutes at room temperature.
00:15:00

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.
00:30:00

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

04:00:00

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.
18:00:00

36 Treatment#5: Allow to stand for 30 minutes at room temperature.
18:00:00

37 Remove 100% ethanol:Spurr's reagent and add 1.5ml Spurr's reagent to vial. Incubate for 4hrs at room temperature.
04:00:00

38 Replace with 1.5ml Spurr's reagent and rotate the vial overnight on the wheel at room temperature.
18:00:00

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

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.
00:00:15

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

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

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

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

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.

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

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