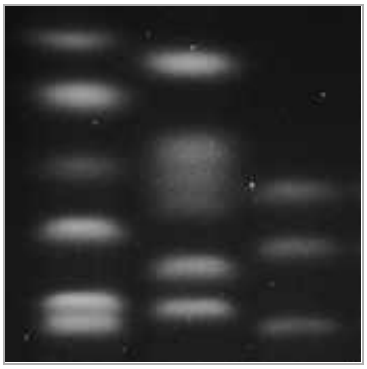

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Visualization of Yeast Chromosomes Using Clamped Homogeneous Electric Field (CHEF) Electrophoresis

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ABSTRACT

I inherited this protocol while working as a postdoctoral researcher in the laboratory of Dr. Stewart Scherer in the 1990s. Literature that influenced protocol development is listed in the References section.

This protocol describes how to isolate chromosome-sized DNA from yeast cells. The yeast cells are encased in agarose, then the cell is digested away. The result is unsheared chromosomes in an agarose plug that can be loaded onto electrophoresis gels or subjected to additional analysis such as digestion with restriction enzymes.

The protocol details two sets of electrophoresis conditions for separating the chromosomes using a BioRad CHEF-DR III variable angle electrophoresis system. Because the protocol was developed for work with *Candida albicans*, the conditions are most useful for chromosomes in the size range of approximately 0.5 to 5 Mb. The "short protocol" (Condition 1) separates the smaller *C. albicans* chromosomes (approximately 1 Mb) without running them off the bottom of the gel. The "long protocol" (Condition 2) separates the larger chromosomes (approximately 3 Mb) with approximately 0.1 Mb resolution but risks running smaller DNA molecules off the gel. We typically run samples using both electrophoresis protocols and combine the resulting information to understand the karyotype.

IMAGE ATTRIBUTION

A portion of a CHEF gel run using this protocol. Lane 1 = *Candida albicans* chromosomes; Lane 2 = *Lodderomyces elongisporus* chromosomes; Lane 3 = *Yamadazyma tenuis* chromosomes

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Protocol status: Working
This protocol is in active use and works well.

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77053

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MATERIALS

Materials

Pulsed-field gel electrophoresis system
Plug molds
Microwave and/or heating plate
Water baths
Pipettes
Pipettemen
Hemacytometer
Spatula (like those used for weighing chemicals)
Hamilton syringe (optional)
14 ml round bottom tubes
6-well tissue culture plate

Yeast strains to study
Growth medium (liquid medium and agar plates)

Low-melting-point agarose
Pulsed field certified agarose

Purified water (i.e. distilled or deionized)

0.5 M EDTA, pH 8.0
0.5 M EDTA, pH 9.5

1 M Tris-HCl, pH 8.0
1 M Tris-HCl, pH 9.5

Zymolyase
Proteinase K

50% glycerol
1 M sodium phosphate, pH 7.5
10% Sarkosyl
Tris base
Glacial acetic acid

How to Make Agarose-Embedded Yeast Chromsomes (Chrom

1 Grow the yeast strain on an agar plate, streaking for isolated colonies. Plates can be stored at

4 °C until needed.

Note

My laboratory keeps its yeast stocks frozen in 38% glycerol at -80 °C. A fresh agar plate is made from the frozen stock for each experiment. Stock plates are kept at 4 °C for no longer than 1 week.

Select a well-isolated, representative colony. Inoculate it into the liquid growth medium of your choice. Cultures can be incubated to saturation or harvested at an earlier time point, as long as a sufficient number of cells are available (see below).

Note

My laboratory typically uses YPD medium (per liter: 10 g Bacto yeast extract, 20 g Bacto peptone, 20 g dextrose). A 50-ml Erlenmeyer flask with 20 mL of liquid medium provides more than enough cells for this protocol.

Note

This protocol works well for freshly grown yeast cells, as well as for flasks of cells that sat on the lab bench for several days.

2

Prepare 1.5% Low-Melting-Point (LMP) agarose in [M] 125 millimolar (mM) EDTA, pH 8.0 .

Recipe:
Mix 10 mL of [M] 0.5 Molarity (m) EDTA pH 8.0 with 30 mL purified water.

Add 0.6 g Low-Melting-Point agarose. Heat in a microwave or on a heating plate to dissolve the agarose.

Since water will evaporate during this process, add purified water back to adjust the volume of the solution to 40 mL . Since adding water decreases the temperature of the agarose solution, it may be helpful to have a bottle of purified water pre-warmed for this purpose.

The final agarose solution should be cooled to 37 °C prior to use in Step 7 below.

Note

Low-melting-point (LMP) agarose is not the regular agarose that typically is used to run electrophoresis gels. LMP agarose can be remelted at 65 °C, which is below the melting temperature for double-stranded DNA.

Note

Only a small amount of the agarose solution is needed per recipe. The bottle of LMP agarose/EDTA can be stored at room temperature and remelted when another set of chromosome plugs is made.

Note

The recipe above is for 1.5% LMP agarose, which results in a final plug of 0.75% agarose (see below). Alternatively, plugs can be made using a 1% Low-Melting-Point agarose stock (0.5% agarose final concentration), but the 0.5% agarose plugs are fragile and more-difficult to handle.

Note

Some protocols may mention use of EDTA, pH 7.5. This reagent is difficult to make because of the pH-dependent solubility of EDTA. EDTA, pH 8.0 works fine for this protocol.

3 Count the yeast cells using a hemacytometer.

For "1x" plugs, use 2 x 10^8 cells total for a single recipe; for "2x" plugs, use 4 x 10^8 cells total.

Note

"1x" plugs are sufficient for visualizing chromosomes to understand the species karyotype and for Southern blotting of the agarose gel. The photo that is on the title page of this protocol used "1x" plugs.
"2x" plugs are more useful for procedures like random breakage mapping or restriction enzyme digestion of chromosomal DNA.

4

Remove the culture volume required for the desired number of cells. Collect the cells by centrifugation. Wash the cells twice in [M] 50 millimolar (mM) EDTA, pH 8.0.

5

Resuspend cells in 200 µL [M] 50 millimolar (mM) EDTA, pH 8.0.

Since the presence of the yeast cells increases the overall volume in the microfuge tube, measure the true volume using a Pipetteman. Set the Pipetteman for a larger volume than expected, pull up the entire volume into the pipette tip and carefully dial the Pipetteman back until the liquid is just at the end of the pipette tip. Read the Pipetteman volume and make a note of it for Step 7 (below).

Safety information

Be careful not to shoot the cell suspension out of the end of the pipette tip, especially if working with pathogens.

6

Prepare the following Zymolyase-containing solution:

	A	B	C
	Final solution	**Stock**	**Vol. needed for 250 µl**
	~ 50% glycerol	50% glycerol	250 µl
	2.5 mg/ml Zymolyase (yeast lytic enzyme)	Powder	0.625 mg
	10 mM sodium phosphate, pH 7.5	1 M	2.5 µl

Mix well with a Pipetteman to ensure that the Zymolyase powder is dissolved.

Add 15 µL of Zymolyase-containing solution to the cell suspension and mix by pipetting.

7 30m

Note the volume for each sample you are preparing. Total volume = (EDTA + cell volume measured in Step 5) + 15 µL Zymolyase-containing solution from Step 6.

Make a 1:1 mix of the cell preparation with the LMP agarose. In other words, add an equal amount of LMP agarose (from Step 2) to the tube containing the cells. Mix well and immediately distribute into plug molds.

> Note
>
> Two kinds of plug molds are available from Bio-Rad. Thicker plugs (which give good resolution of *Saccharomyces cerevisiae* chromosomes but are too thick to resolve larger *Candida albicans* chromosomes sharply) can be made using catalog number 170-3713 plug molds. Thinner plugs can be made with a reusable mold (catalog number 170-3622) and are ideal for resolving *C. albicans* chromosomes. The image at the start of this protocol was from plugs made with the thinner mold.

Allow filled molds to cool at 4 °C for at least 00:30:00.

8

Remove the solidified agarose plugs from the molds.

When using the thinner mold, final plugs are larger than the electrophoresis gel well. The plugs can be cut into equal thirds using a razor blade. Working on a chilled glass plate keeps the plugs solid during the process of cutting them.

Plugs will be incubated overnight at 30 °C for the Zymolyase digestion to occur. Plugs can be placed into tubes, or into the wells of a 6-well tissue culture plate. It is easier to handle the plugs if they are placed into the wells of a 6-well tissue culture plate.

> Note
>
> Each tissue culture well can contain the entire set of plugs from a given species/strain. The recipe described above fills three of the thinner plug mold wells. When cut into thirds, the recipe yields 9 plugs for each species/strain. The recipe can be scaled up if more plugs are needed.

Plugs will be incubated in a Tris/EDTA solution that is made by mixing:
0.2 ml 1 M Tris-HCl, pH 8.0
20 ml 0.5 M EDTA, pH 8.0

Add several ml of the solution to each well in the tissue culture plate. Distribute the plugs into the wells as needed for your experiment. Use a flat spatula (e.g. like those used for weighing

chemicals) to lift each plug and release it into the tissue-culture-plate well. Make sure the plugs are covered with the buffer and add a little extra to accommodate moderate evaporation during incubation.

Incubate at 30 °C overnight without agitation.

9 Remove the tissue-culture plate from the incubator and pipette off the incubation buffer. Wash the plugs three times in [M] 0.5 Molarity (M) EDTA pH 9.5 (15-30 min per wash). Agitate the plate gently during washing.

> **Note**
>
> Make sure to handle plugs gently to avoid breaking them during this procedure.

10 30m The plugs will next be subjected to Proteinase K digestion, which is more efficient at a higher temperature. The plugs should be placed into a round-bottom tube with a cap that can limit evaporation. We use 14-ml round-bottom tubes like Nunc catalog number 150268, but many other choices would work well.

Proteinase K Digestion Solution Recipe:
Since Proteinase K is expensive, prepare only the amount of this solution that is needed for the number of plugs being prepared.

	A	B	C
	Final solution	**Stock**	**Vol. needed for 5 ml**
	~ 2.5 mg/ml Proteinase K	Powder	12.5 mg
	~ 1% Sarkosyl	10%	0.5 ml
	~ 0.5 M EDTA pH 9.5	0.5 M	5 ml
	~ 10 mM Tris-HCl, pH 9.5	1 M	50 µl

Mix the proteinase K digestion solution well and distribute a small amount into each tube. Carefully place the plugs into the tube. All plugs from the same species/strain can be placed in a single tube. Add more digestion solution to ensure that the plugs are covered completely. Place the cap on the tube tightly.

Incubate Overnight at 50 °C.

11 Carefully pour the plugs from the tube into a well on a 6-well tissue culture plate. Wash plugs

three times with [M] 50 millimolar (mM) EDTA pH 8.0 working similarly to Step 9.

12 Store plugs at 4 °C in [M] 50 millimolar (mM) EDTA pH 8.0. Plugs may be stored in the tissue culture plate, but evaporation of buffer occurs over time. Place them in a capped tube or check the plates at routine intervals to ensure that the plugs do not dry out.

Note

We have stored plugs successfully for years without loss of quality.

Separating Yeast Chromosomes on a Pulsed-Field Electroph.. 5d

13 The CHEF system in my laboratory includes the variable-speed pump for buffer recirculation and the cooling module.

Two sets of running conditions are presented below. These conditions are for use on a BioRad DR-III device. Modifications will be needed for other instruments. Both sets of conditions use 1x TAE buffer.

The running conditions were developed for use with *C. albicans* chromosomes. The diploid *C. albicans* has eight chromosome pairs that range in size from chromosome 1 (3.2 Mb) to chromosome 7 (0.95 Mb). Chromosome R (that encodes variable rDNA copy numbers) is large, migrating in the size range of chromosome 1.

Condition 1 will resolve all but chromosomes 1 and R, leaving room on the gel for chromosome fragments smaller than chromosome 7.

Condition 2 will separate chromosomes 1 and R but runs chromosome 7 to near the bottom of the gel.

14 Prepare the buffer and agarose gel.

Separation of chromosome-sized DNA is best accomplished using **Pulsed Field Certified Agarose** (e.g. Bio-Rad catalog number 162-0137).

Both sets of gel running conditions described below use 1x Tris-Acetate-EDTA (TAE) buffer. TAE buffer is made at a 50x concentration and diluted to 1x just before use.

50x TAE Recipe

	A	B	C

	A	B	C
	Final solution	**Stock**	**Amount needed (per liter)**
	2 M Tris	Tris base	242 g
	1 M Acetic Acid	Glacial acetic acid	57.1 ml
	0.05 M EDTA, pH 8.0	0.5 M EDTA, pH 8.0	100 ml
		Purified water	to 1 L total volume

Prepare the gel by dissolving the correct amount of Pulsed Field Certified Agarose in 1x TAE buffer. For example, the protocol called Condition 1 uses a 0.8% agarose gel. Preparing 150 ml of agarose will fill the Bio-Rad gel tray with a little extra left over. The leftover agarose is used to seal the gel wells after the plugs are loaded.

To prepare 150 ml of 0.8% agarose in 1x TAE buffer, weigh 1.2 g Pulsed Field Certified Agarose and dissolve it in 150 ml 1x TAE buffer. Heat in a microwave or on a heating plate. A stir bar may be used on the heating plate. Cool the agarose to less than 60 °C before pouring the gel to avoid warping the gel tray.

Pulsed field gels work best when poured on a level surface. Place the gel tray onto a leveling platform and use a bubble level to adjust. Pour the cooled agarose into the gel tray and place the comb so the teeth are closest to the frame of the gel tray. Placing the comb in the opposite orientation shortens the running length of the gel.

Allow the gel to solidify at room temperature.

15 Add 1x TAE to the gel box. Start the recirculation pump and the cooling module. Select either 14 °C (Condition 1) or 12 °C (Condition 2) depending on the protocol you would like to run. It takes a little time for the buffer to reach the selected temperature so set up the gel box before loading the gel.

16 Load your plugs into the gel wells. This step takes a little practice. You will likely break some plugs before you can load them into the gel cleanly and without bubbles. It helps to add 100 µL of 1x TAE (running buffer) to the gel well, which fills it approximately half-way. Plugs tend to slide into the well more easily with the buffer present. Filling the well completely with 1x TAE tends to repel the plug from entering the well.

Once plugs are loaded, use the flat spatula to ensure that the plug is "flush" with the leading edge of the gel well. In other words, manipulate the plug so that its face meets the leading edge of the gel well without air bubbles or gaps. This step also takes practice. You can also use a Hamilton syringe to remove air bubbles from the well.

Once the plugs are positioned correctly, use the remaining Pulsed Field Certified Agarose/1x TAE solution to seal the plugs into the wells. This step prevents the plugs from floating away during the electrophoresis step.

17 Place your gel into the gel box. Allow the gel to equilibrate to the temperature of the buffer before starting the electrophoresis protocol. 5d

Set up the CHEF power supply using parameters for either Condition 1 or Condition 2:

Condition 1: 0.8% agarose gel run at 14 °C at 3 V/cm with a 106-degree angle. Running time is 50:00:00 with a 120-480 sec linear ramp.

Condition 2: 0.6% agarose gel run at 12 °C at 2.5 V/cm with a 120-degree angle. Running time is 24:00:00 with a 120-300 sec linear ramp followed by 46:00:00 with a 420-900 sec linear ramp.

Examine the depth of the buffer in the gel box. Ideally, the buffer should just cover the top surface of the gel. Drain excess buffer from the box.

Start the power supply and ensure that the gel is running. If you drained too much buffer in the previous step, for example, there may not be bubbles coming from the electrodes in the box. Note the milliamps at the start of the run. We typically see values of 150 mA at the start of a run; the value increases as the run progresses. If the reading is too high, try draining more buffer from the gel box. Bio-Rad literature discusses this point in detail, suggesting that the mA reading is also affected by water quality or buffer preparation problems.

Monitor the run as it progresses. Ensure that there are no leaks, that the buffer level in the gel box is still acceptable, and that the chiller unit is maintaining the desired temperature.

When the run is completed, drain the buffer from the gel box and rinse the box/tubing with water in preparation for storage or future use.