**Preparation of Yeast for Pulsed-Field Gels**

* 1. Measure optical absorbance at A600 for your yeast cell culture. Calculate the amount of yeast equal to an A600 between 2 and 4. Transfer this amount to a microcentrifuge tube.

### Pellet the cells by spinning for 2 minutes at full speed.

* 1. Remove supernatant with a P1000 pipet.
	2. Wash cells by adding 1 ml of dH2O and pipetting up and down until no clumps remain.

### Pellet the cells by spinning for 2 minutes at full speed.

* 1. Remove supernatant with a P1000 pipet.
	2. Wash cells by adding 1 ml of TE and pipetting up and down until no clumps remain.

### Pellet the cells by spinning for 2 minutes at full speed.

* 1. Remove supernatant with a P1000 pipet.
	2. Prepare 1% LMP agarose by adding 0.5 g LMP agarose to 50 ml of 0.125 M EDTA and carefully melting to homogeneity on low setting in microwave.

**CRITICAL STEP:** Ensure that agarose is completely melted before cooling to 55°C, and use within 3 min of preparation.

* 1. Add 100 µl of Zymolyase and pipetting up and down until no clumps remain. Transfer this suspension to one well of a multiwell plate.
	2. Immediately add 150 µl of 1% LMP agarose into the well and pipet up and down until no clumps remain.

**CRITICAL STEP:** Steps 11 and 12 should not take longer than 1 min.

* 1. Place the plate on ice for 5 min to allow agarose to set. Agarose plugs will solidify and adhere to the bottom.
	2. Add 150 µl of TE per well and cover the entire plate with a plate seal.
	3. Incubate for 30 min at 37°C.
	4. Add 100 µl of proteinase K/sarkosyl working solution to each well.
	5. Re-seal the plate with fresh plate seal.
	6. Incubate in a 55°C water bath for 1 hour.
	7. Remove the liquid with a P1000 pipet.
	8. Wash the plugs by adding 1 ml of room temperature dH2O to each well/plug and immediately removing.
	9. Wash plugs by adding 1 ml TE to each well then rock gently on rocking platform for 10 min.
	10. Remove the liquid with a P1000 pipet tip.
	11. Repeat steps 21 and 22.
	12. Repeat steps 21 and 22 again.
	13. During the wash steps above, weigh out 1.5 g of MegaBase agarose in a 250 ml flask, and add to 150 ml of 0.5× TBE. Microwave, checking frequently for boiling, and confirm that agarose is completely melted.
	14. When agarose has cooled to 55°C, slowly pour into gel frame and let cool for 30 min.
	15. Pour remaining 0.5× TBE into gel rig chamber.
	16. From the multiwell plate, remove liquid with a P1000 pipet tip.
	17. Incubate the plate in a 75°C water bath for 5 min.

**TIP:** Make sure the agarose plugs have melted by gently shaking the plate.

* 1. Load 20 µl of each liquid agarose plug sample with a wide bore pipette tip.
	2. Run gel at optimized parameters, for instance for *Saccharomyces*, 6 V, 60–100 s switch time, for 30 h at 15°C.
	3. After run, remove gel and place in container with 1 L 0.5× TBE buffer for staining.
	4. Add 10 µl of 10 mg/ml ethidium bromide. Mix gently on rocking platform for 30 min.
	5. Visualize chromosomes on UV transilluminator.

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