

Yeast RNA prep (using ABI6100+Absolute RNA wash)

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1. Grow O/N cultures of all strains in 3mL YPD at 30°C.
2. The next morning, read OD600 of 1:100 dilutions. Make sure total volume =2mL for reading on spec (dilute 20µL + 1980µL water).
3. Add appropriate amount of O/N to 5mL YPD to make the OD600= 0.1. Grow 6hrs. If doing a +/- induction, make cultures in duplicate.
4. After 6 hrs, read OD600 of 1mL of culture + 1mL water. OD600 should be ~1.0.
5. Spin cells 4min, 3000 rpm. Can freeze pellets at this point, or proceed with spheroplasting.
6. Resuspend cells in 300µL **Solution A** (1mg/mL Zymolyase) and transfer to microfuge tubes (leak-proof). Vortex and incubate 1hr at 37°C on rotating platform.
7. Spin spheroplasts 3min 6000rpm.
8. Pour off supernatants
9. Add 300µL 1X **Lysis Buffer** (ABI) and vortex.
10. Take samples to ABI6100 machine and proceed with ABI protocol below.

ABI6100 protocol: Pre-filtering and RNA prep (+Absolute RNA Wash).

-make sure to thaw on ice the Absolute RNA Wash (stored at -20°C).

-bring to equipment room sterile trays (for loading solutions), marker, notebook, timer, PCR tray tape or sealing material.

Pre-filtering

- A. Load disposables (Deep-well plate and pre-filter tray).
- B. Add samples to each well of the pre-filter tray and perform pre-filtration.

1. Add 300µL lysates to each well.
2. Collect 180sec 80% vacuum.
3. Collect 120sec 80% vacuum.
4. Observe that all material has passed through filter.
5. Repeat step 4 until all material has passed through.
6. Touch off at Collection.

- Can freeze pre-filtered lysates at this point, or proceed with RNA preparation below.

RNA prep

-load disposables (96-well optical reaction plate, splash guard, Total RNA Purification Tray).

-Tape up or seal wells that won't be used on Purification Tray.

-Move carriage to Waste position and lock it.

-Access the "absolute rna program" preconfigured method on ABI6100 (may require access code).

“absolute rna program”

<u>Description</u>	<u>Vol (μL)</u>	<u>Position</u>	<u>Time (sec)</u>	<u>Vacuum</u>	<u>Action</u>
0. Pre-wet wells with RNA Purification Wash Solution 1.	40	Waste	-	-	-
1. Add 300μL pre-filtered lysate	300	Waste	180	80	start
2. Add RNA Purification Wash Soln 1	500	Waste	180	80	start
3. a. Add Wash Solution 2	500	Waste	180	80	start
b. Add Absolute RNA Wash.	50	Waste	15min.	-	incubate
4. Add RNA Purification Wash Soln 2	400	Waste	5min + 180	80	incubate +start
5. Add RNA Purification Wash Soln 2	300	Waste	120	60	start
6. Add RNA Purification Wash Soln 2	300	Waste	120	60	start
7. Pre-elution vacuum	-	Waste	300	90	start
8. Touch Off at Waste	-	Touch Off	-	-	start
9. Add Nucleic Acid Purification Elution Soln.	100	Collection	1min + 120	40	incubate +start
10. Touch Off at Collection	-	Touch Off	-	-	touch off

Read A260 and A280 on Nanodrop Spectrophotometer (no dilution necessary).
Look for A260/A280 = 2.0 for purity.

Reagents

Solution A:

	<u>for 10mL (for 33 rxns)</u>	<u>for 15mL (for 50 rxns)</u>
0.9M sorbitol	4.5mL of 2M stock	6.75mL
0.1M EDTA ph 7.5	2mL of 0.5M stock	3mL
1mg/mL Zymolyase	10mg	15mg
14mM β-ME	1μL	1.5μL
dH2O	3.5mL	5.25mL