High Throughput DNA Extraction – Filter Based

Reagents

96-well Filter Plates
96-well Collection Plates
Yeast Cell Samples
Lyticase Buffer
Proteinase K Digestion Buffer
Binding Buffer
Wash Buffer 1
Wash Buffer 2
Elution Buffer

Protocol

1. Harvest cells (maximum 5 x 10^7) by centrifuging for 10 min at 4000 x g. Discard supernatant.
2. Resuspend the pellet in 600 l Lyticase buffer with freshly added beta-mercaptoethanol and lyticase. Incubate at 37°C for 30 min. Note: Lysis time and yield will vary from sample to sample, depending on the cell number and species processed.
3. Pellet the spheroplasts by centrifuging for 10 min at 4000 x g
4. Resuspend the spheroplasts in 180 l Proteinase K Digestion Buffer.
5. Add 20 l proteinase K. Mix thoroughly by vortexing, and incubate at 55°C for 45 minutes Vortex occasionally during incubation to disperse the Sample. Lysis time varies depending on the species and amount of yeast processed. Lysis is usually complete in 45 min. Supposedly the, samples can be lysed overnight; I have not tested this.
6. Vortex for 15 s. Add 200 l Binding Buffer to the sample, and mix thoroughly by vortexing.
7. Then add 200 l ethanol (100%), and mix again thoroughly by vortexing. It is essential that the sample, Binding Buffer, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Binding Buffer and ethanol can be premixed and added together in one step to save time when processing multiple samples. A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the procedure.
8. Pipet the mixture from step 6 (including any precipitate) into the 96-well filter columns placed in a Collection plate. Centrifuge at 4000 rpm for 1 min. Discard flow-through and collection tube. Discard flow through.
9. Add 500 l Wash Buffer 1, and centrifuge at 4000 rpm for 1 min Discard flow-through.
10. Add 500 l Wash Buffer 2, and centrifuge at 4000 rpm for 1 min Discard flow-through.
11. Centrifuge at 4000 rpm for 1 min to dry membrane. Discard flow-through and switch to new collection plate.
12. Add 100 uL Elution Buffer let sit for 1-2 minutes. Centrifuge at 4000 rpm for 1 min. Save flow through and measure using SYBR Green Assay. Store at -20 C.

Reagent Recipes

Lytiase Buffer – 100 mL

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>1 M Sorbitol</td>
<td>40 mL 2.5 M Sorbitol Solution</td>
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<tr>
<td>10 mM EDTA pH 8.0</td>
<td>2 mL 0.5 M EDTA pH 8.0 Solution</td>
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<tr>
<td>Sterile Milli-Q H2O</td>
<td>58 mL</td>
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<tr>
<td>14 mM beta-Mercaptoethanol</td>
<td>Add fresh. 0.6 uL Stock per sample</td>
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</tbody>
</table>
200 U Lyticase Add fresh. 4 uL of 25 U/uL stock solution

Proteinase K Digestion Buffer – 50 mL
30 mM Tris –HCL pH 8.0 1.5 mL 1 M Tris-HCL Solution
10 mM EDTA pH 8.0 1 mL 0.5 M EDTA pH 8.0 Solution
1% SDS 5 mL 10% SDS Solution
Sterile Milli-Q H2O 42.5 mL

Binding Buffer – 50 mL
6 M GuHCL 37.5 mL 8 M GuHCL solution
0.2 M NaCl 6 mL 5 M NaCl Solution
Bring pH to 5.0 using HCL and NaOH
Bring Volume to 50 mL with sterile Milli-Q H2O

Wash Buffer 1 – 100 mL
2 M GuHCL 25 mL 8 M GuHCL solution
56% Ethanol 56 mL 100% Ethanol
Sterile Milli-Q H2O 19 mL

Wash Buffer 2 – 100 mL
0.1 M NaCl 2 mL 5 M NaCl Solution
10 mM Tris –HCL pH 8.0 2 mL 1 M Tris-HCL Solution
70% Ethanol 70 mL 100% Ethanol
Sterile Milli-Q H2O 26 mL

Elution Buffer – 100 mL
10 mM Tris –HCL pH 8.0 1 mL 1 M Tris-HCL Solution
1 mM EDTA pH 8.0 0.2 mL 0.5 M EDTA pH 8.0 Solution
Sterile Milli-Q H2O 97.8 mL