# Washing and preparing K. Phaffii cryo stocks ready for transformation

## Introduction

As our previous experiments have proved to show, *K. phaffii* cells can be washed and prepared for electroporation, and be put in the freezer for storage until the electroporation can occur. This is a great way of saving time, since the washing step itself takes at least half a day.
This protocol is based on a protocol adapted from Adrian and provided by Louise, called "Transformation into K. phaffii: Electroporation"

## Materials

- Pipettes
  - P5000
  - P1000
- Reagents
  - YPD
  - 100 mM LiAc, 10 mM DTT, 0.6 M sorbitol, and 6 mM Tris HCl pH 8.5
  - 1M sorbitol

## Procedure

### Preparation of *K. phaffii* cultures

1. Inoculate 4 mL of YPD with a single cell colony of *K. phaffii* GS115 ΔKU70 and incubate O/N at 30°C

   Note: The inoculation should be done early afternoon, since the ΔKU70 strains grows slower than a wild type
2. Transfer 500 uL from the pre-culture to a 50mL falcon tubes with 25mL YPD
3. Incubate for 5 hours at 30°C and 200RPM

### Preparation of Master Mix for the washing

4. Mix the necessary amount according to the table below

**Master Mix recipe**

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | Reagent | Volume for 500 mL Mastermix [mL] | Volume per transformation reaction [µL] | Mastermix volume for 8x8mL [µL] | | | |
| 2 | Sorbitol 1M | 300 | 4800 | 42240 | | Number of reactions | 8 |
| 3 | LiAc 1 M | 50 | 800 | 7040 | | Safety margin (%) | 10 |
| 4 | Tris HCl pH 8.5 1M | 3 | 48 | 422.4 | | | |
| 5 | DTT | 5 | 80 | 704 | | | |
| 6 | sterile dH20 | 142 | 2272 | 19993.6 | | | |
| 7 | Total volume [mL] | 500 | 8 mL | 70.4 mL | | | |

5. CRITICAL ADD THE DTT JUST BEFORE ADDING THE MASTER MIX TO THE CELL PELLETS

## Washing of *K. phaffii cells*

6. Pellet cells at 3500G for 5 minutes, and discard the supernatant
7. Gently resuspend cells in 8mL Master Mix (of 100 mM LiAc, 10 mM DTT, 0.6 M sorbitol, 6 mM Tris HCl pH 8.5), then transfer to new 50mL falcon tubes, and incubate at room temperature for 30 minutes.

   Make sure cells do not precipitate, gently swirl every 10 minutes.
8. Pellet cells at 3500G for 5 minutes, and discard supernatant.
9. Work on ice: Resuspended cells in 1.5mL 1M ice-cold sorbitol and transfer to sterile 2mL eppendorff tubes.
10. Work on ice: Wash cells 3 times by centrifugation at 13000G for 30 seconds by resuspending in 1ml 1M ice-cold sorbitol, vortex between washes. After 3$^{rd}$ time add 1ml 1M ice-cold sorbitol.
11. Work on ice: Distribute cells in 5 sterile 1.5mL cryo tubes (200µL in each)
12. Immediately put the cryo tubes in the -80°C freezer

    Keep on ice until putting them in the freezer
    Note: It is a good idea to also have pre-cooled the cryo tubes before adding the washed cells
    1 tube = 1 electroporation reaction