Yeast Colony PCR

Introduction

Protocol from Tomas Strucko, adapted to fit our Taq polymerase Master Mix.

This protocol is used for quick and easy genetic validation of engineered yeast cells. The Taq method was optimized to obtain PCR bands up to ~ **3 kb** (**4.5 k**b bands have been obtained too, but with much lower success rate). Our largest fragment is ~4.7 kb.
Total PCR reaction volume is **50 µL** (but can be decreased if needed). In this case the Taq DNA Polymerase Master Mix RED. The PCR product (if column purified) is also suitable as a template for Sanger sequencing.
Different brand polymerases can also be used, however, reaction conditions should be modified to fit the manufacturers recommendations .

Materials

- Plates with fresh yeast colonies (preferably directly from incubator)
- Taq DNA Polymerase 2x Master Mix RED (AMPLIQON)
- Primers (10uM)
- Milli-Q or ddH_20
- PCR vials/strips
- Sterile tooth picks (pipette tips can be used instead)

Procedure

Disrupt yeast colonies

1. Pipette 6.5 uL of sterile ddH_2O into each PCR vial

2. CRITICAL! Using sterile toothpick or pipette tip pick **miniature** amount of cell material from an agar plate and dip it into PCR vial with water. **Too many cells or agar from plate will inhibit the PCR reaction!**

3. Run the following PCR program:

   98^oC 5 minutes
   4^oC 30 seconds
   98^oC 5 minutes
   80^oC 10 minutes

Prepare Master mix

4. While **Step 3** is running combine primers and Taq DNA Polymerase Master Mix RED.

   Use the reagent volumes shown in the **Table 1**. If more than two primers are used, then reduce the amount of water accordingly.
   Note! Always prepare sufficient volume for one extra reaction!

Master Mix preparation on PCR reaction number basis

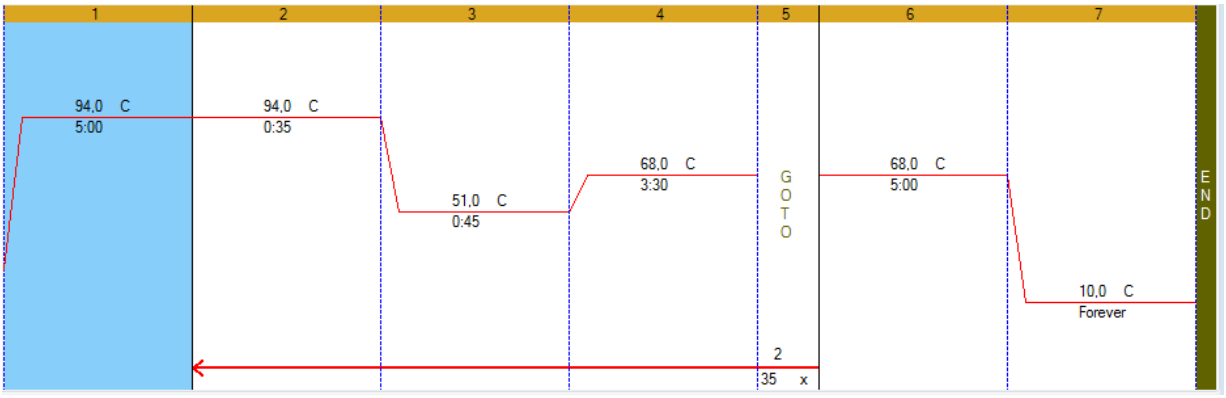
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1		Number of PCR reactions													
2	**Reagent**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**20**	**30**	**40**	**50**
3	Taq DNA Polymerase Master Mix RED	25	50	75	100	125	150	175	200	225	250	500	750	1000	1250
4	Primer A (10uM)	1	2	3	4	5	6	7	8	9	10	20	30	40	50
5	Primer B (10uM)	1	2	3	4	5	6	7	8	9	10	20	30	40	50
6	Water	16.5	33	49.5	66	82.5	99	115.5	132	148.5	165	330	495	660	825

Run PCR reaction

5. Centrifuge PCR tubes from the **Step 3** using table-top centrifuge, in order to get all the liquid to be at the bottom of the tubes.

6. Add 43.5 uL (or appropriate volume) of Master Mix from **Step 4** into each PCR tube with water and disrupted cell suspension.

7. Run the following PCR program:



Analyze

8. Load at least 6 uL of the PCR reaction mix into 1% agarose gel for analysis