Experiment 26	**Eukaryotic microorganisms as research tools: Detecting protein-protein interactions with the yeast 2-hybrid system**
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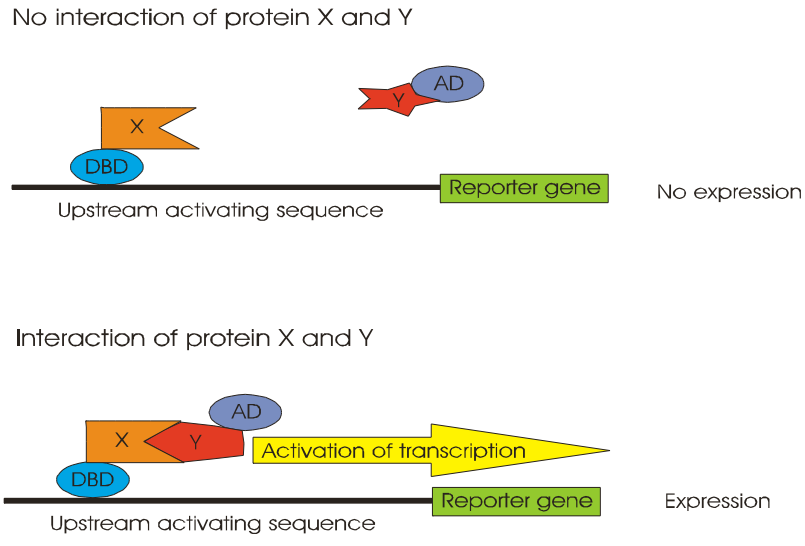
Reading	Chapters in BBOM 9th: 9.14, 10, 17.4 Chapters in BBOM 10th: 14.6, 15, 14.9 BBOM: Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9th Edition, 1999. 10th Edition, 2003. Prentice Hall For abbreviations and terms see Appendix
Objectives	To understand the yeast 2-hybrid system Including yeast transformation and growth on selective media
Background	The yeast 2-hybrid system is a genetic method for detecting interactions between proteins *in vivo* in the yeast *Saccharomyces cerevisiae*. The system can be used to screen a library for novel proteins that specifically interact with a known bait protein of interest or to test complex formation between two known proteins or protein domains for which there is a prior reason to expect an interaction. The yeast 2-hybrid system exploits the fact that transcription factors are comprised of two domains, a DNA binding domain (DBD) and an activation domain (AD). When these two domains are in close contact they switch on the transcription of their target genes. Two plasmids are constructed, one of them coding for the DBD fused to protein X also known as the bait protein, while the second plasmid codes for the AD fused to protein Y also known as the prey protein. Protein Y can be replaced by a cDNA library coding for the complete set of proteins of any organism to screen for novel interaction partners. The two hybrid proteins X and Y each on their separate plasmids are transformed into a yeast strain that contains two reporter genes, the *lacZ* gene and an auxotrophic marker. The regulatory regions for these two reporters contain the particular binding site for the transcription factor. If protein X interacts with protein Y in the nucleus, the activation domain comes into contact with the DNA-binding domain reconstituting the transcription factor. Thus the transcription of the target genes is activated, resulting in expression of the reporter genes. Positive interactions can be detected by selection on plates lacking the auxotrophic marker, followed by a second screen for ß-galactosidase expression. 

Literature	Current Protocols in Molecular Biology, Frederick M Ausubel et al., Wiley Interscience, 1999, Volume 2, Chapter 13
www.Links	http://www.bdbiosciences.com/clontech/techinfo/manuals/PDF/PT3024-1.pdf http://www.umanitoba.ca/faculties/medicine/biochem/gietz/Trafo.html
Practical work	The students will perform a transformation experiment by introducing respectively the bait or the prey plasmid into competent cells of *Saccharomyces cerevisiae*. General laboratory skills are required to do this experiment, such as working with fungi aseptically. It is important not to introduce contaminating fungi into the experiment. The students will transform two different yeast lines with different plasmids. For one condition the transformation efficiency will be correlated to different durations of the heat shock at 42°C. The plates will be incubated for 3-4 days at 30°C. On the second day the ß-galactosidase assay detecting protein-protein interaction will be performed. The students will observe and analyse the results.
Materials and Experimental Protocols	Material and equipment needed: 1. Competent yeast cells, carrying either the bait protein or the prey protein: line A carrying the LRX1 gene on the bait plasmid, line B carrying the RALF gene on the prey plasmid. 2. Plasmid DNA: a RALF gene on the prey plasmid, the empty bait plasmid, the empty prey plasmid 3. Solutions and reagents: carrier DNA, Polyethyleneglycol (PEG) 3500 50% w/v, Lithium Acetate 1.0 M, sterile H_2O, stock solution for the ß-galactosidase assay (0.5 M K-PO_4-buffer pH 7.0, 0.1% SDS (Sodium Dodecylsulfate), 6% DMF (Dimethylformamide * be careful this is toxic*), 0.5% low melting agarose), x-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside, an indicator substrate for beta-galactosidase) 10% in DMF, ß-mercaptoethanol (* be careful this is toxic*) 4. Plates: SC selection plates for yeast: 9 plates lacking tryptophane (TRP) and leucine (LEU), 1 plate lacking tryptophane, leucine and histidine (HIS). 5. Working items: micro pipettes, sterile yellow and blue tips, sterile Eppendorf tubes, racks, floating aid, forceps, tissue paper, water resistant pen, waste container Common work station: Centrifuge for Eppendorf tubes, vortex mixer, water bath at 42°C, microwave, 30°C incubator, autoclave **Day 1** – yeast transformation 1. Label 6 Eppendorf tubes: LRX+R10, LRX+R20, LRX+R30, LRX+RALF, LRX+bait and RALF+prey. 2. Pipette 100 µl competent cells into each tube, into the tubes marked with LRX+… pipette the LRX line, into the tube marked with RALF+… pipette the RALF line. Pellet cells at top speed in a microcentrifuge for 30 sec and discard the supernatant. 3. Add the following components of the Transformation Mix to the cell pellet in the order listed below. The correct order is very important! To

	the LRX+R… marked tubes add the RALF plasmid, to the LRX+bait marked tube add the empty bait plasmid and to the RALF+prey marked tube add the empty prey plasmid. 1. PEG 3500 50% w/v 240 µl 2. Lithium Acetate 1.0 M 36 µl 3. Carrier DNA (2 mg/ml) 50 µl Vortex mix the carrier DNA! 4. Plasmid DNA (1 µg) 34 µl 5. Incubate the tubes in a water bath at 42°C for 10 to 30 min respectively. The tubes marked with R10, R20 or R30 are to be incubated for this duration in minutes, all other tubes are to be incubated for 20 min. 6. Centrifuge at top speed for 30 sec and remove the Transformation Mix with a micropipettor. 7. Pipette 1.0 ml of sterile water into the tube and resuspend the cells by stirring with a micropipette tip and then vortex mixing vigorously. 8. Make a 1:10 dilution of the cell suspension of LRX+R10, 20 and 30. Pipette 10 µl of this dilution onto SC selection plates lacking TRP and LEU and pipette 10 µl of the undiluted cell suspension onto a second set of plates. Label your plates! Of all other cell suspensions pipette 100 µl undiluted samples onto plates of appropriate SC selection medium lacking TRP and LEU. The 10 µl samples should be pipetted into 100 µl puddles of sterile water. Incubate at 30°C for 3-4 days. For preparation of the ß-galactosidase assay streak transformants onto a selection plate lacking TRP, LEU and HIS (done by assistant). **Day 2** – ß-galactosidase assay and evaluation 1. Melt the stock solution for the ß-galactosidase assay with a microwave. Take 4 ml of this solution and add 10 µl X-gal solution and 1 µl ß-mercaptoethanol. 2. Pour this mixture onto the SC plate lacking TRP, HIS and LEU. 3. Incubate at 30°C over night. You will evaluate the plate of the previous student group. 4. Look at the plates with the different incubation period at 42°C. Count the colonies and calculate the transformation efficiency.
Laboratory Rules and Precautions	Handling *Saccharomyces cerevisiae* (yeast) requires the use of standard microbiological laboratory techniques. Collect all of the waste materials in a biohazard bag, and autoclave them. All contaminated liquid and solid wastes will have to be autoclaved before disposal as well. Please wash your hands after you have handled material containing recombinant DNA molecules and before leaving the laboratory. Eating, drinking, smoking and applying cosmetics are not permitted in the work area.
Goals and Experience gained	Familiarity with yeast transformation and selection of transformants and calculating the transformation efficiency; interpreting results of the yeast 2-hybrid system in terms of protein-protein interactions
Timing	90 min
Reporting	Keep proper notes on experimental steps and observations in your laboratory book.

Questions to be answered	Each lab session will start with 2 "open book " test questions related to topics of this experiment.
Appendix	**Abbreviations and Terms** DBD: binding domain AD: activation domain LRX1 gene / RALF gene: These genes are both genes of the plant *Arabidopsis thaliana* for whose proteins an interaction is anticipated. *lac*Z gene / ß-galactosidase: The *lac*Z gene is a reporter gene coding for the enzyme ß-galactosidase. This enzyme converts the colourless substance x-gal (5-bromo-4-chloro-3-indolyl-ß-D-galactosid) into the deep blue 5-bromo-4-chloro-indigo. auxotrophic marker: An auxotrophic marker is a reporter gene coding for a gene that complements specific nutritional requirements. It typically codes for a key enzyme in a metabolic pathways towards essential monomers like amino acids. SC selection plates: SC stands for synthetic complete drop out medium. SC selection plates lack one or more amino acids to select for yeast cells that have overcome the need for this essential monomer.