Plant defense against environmental pathogens:

Arabidopsis thaliana immune response to infection with isolates of *Hyaloperonospora arabidopsidis (HpA)**

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**********PLEASE BRING YOUR COMPUTER TO CLASS**********
**********INFORM US IF YOU DO NOT HAVE ONE**********

Meeting Time:

Group A:	Monday, July15, 09:00
Group B:	Monday, July15, 13:30
Group C:	Monday, July 22, 09:00
Group D:	Monday, July 22, 13:30

Meeting point:

MPI for Developmental Biology, Main Entrance

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*this handout is based on a previous version by Karasov *et al.* (2018)

BACKGROUND

ARABIDOPSIS AS A MODEL ORGANISM FOR PLANT-PATHOGEN INTERACTIONS

Plants growing in natural conditions are colonized by thousands of different species of microbes. Many of these microbes have no impact on plant growth, though thousands of strains and species have been identified as pathogens—as microbes that cause disease.

When plants become infected with pathogens, a common defense strategy is to confine the infection to the affected organ and prevent its spread throughout the rest of the plant by up-regulating the defense response. Proper recognition and defeat of pathogen infections is essential for the ability of plants to succeed in their natural environments. Plants have a set of receptors that recognize foreign organisms generally, and another set of receptors that can detect specific strains of pathogens. Pathogen attack recognized by these receptors will activate the expression of *PATHOGENESIS-RELATED 1* (*PR1*), a gene that is downstream of early defense response activation. The level of *PR1* gene expression is generally positively correlated with defense response and therefore it can be used as a molecular marker to assess the magnitude of plant defense response.

Hyaloperonospera arabidopsidis (*HpA*) is an oomycete pathogen species specific to *A.thaliana*. *A.thaliana-HpA* pathosystem shows a range of infection specificity, in that different *A. thaliana* natural populations show various level of resistance to specific *HpA* strains.

Previously, we have identified individual *A.thaliana* natural populations that are either resistant or susceptible to a specific strain of *HpA*. The common phenotype for the infected plants is visible *HpA* spores on the leaf surface and reduced growth of the plant. The mechanism of the growth inhibition, and how it relates to the defense response is unknown. In our experiment today, we will test the expression of the defense marker *PR1* in plants of different genotypes that have been treated with either *HpA* or water control. Treated tissue was collected at 5 days-post-inoculation (dpi) stage. The data will indicate whether different plants respond differently in their defense to treatment with the *HpA* isolate.

OBJECTIVES

As part of this lab course we will analyze *PR1* expression in various *A. thaliana* genotypes collected around Europe, and F1 heterozygotes from these genotypes. These plants are either infected by an isolate of *HpA* from Limburg, Germany or treated with water control. In particular we will perform reverse transcription on extracted RNA, perform qRT-PCR (quantitative real-time PCR) on the defense gene *PR1* and a plant housekeeping gene *UBC21*. We will then analyze each of our respective datasets to determine the level of *PR1* expression in our respective plants.

Lab Practical time-line:

***Prior to the course we will collect leaf RNA from plants infected the *HpA* isolate.**

I. In order to measure the plant immune response, you will first convert the RNA to complimentary DNA (cDNA) by performing a 'Reverse Transcription' reaction.

II. While the reaction is proceeding (about one hour), we will present an interactive lecture on quantitative PCR (qPCR) and data analysis, which you will use to measure the up- or down regulation of plant immune genes in response to pathogens infection.

III. When the Reverse Transcription reactions are done; you will perform qPCR on the cDNA for

i. A plant immune gene = Pathogenesis related protein 1 *PR1*
ii. A Plant 'housekeeping gene' for normalization = Ubiquitin conjugating enzyme *UBC21*

For each gene, 2 technical replicates of qPCR will be performed

During the qPCR reaction you will have about 30 minutes for a break.

IV. You will then proceed through your own qPCR data to determine a potential induction of the plant immune response. We will be there to help you if you have questions or need assistance.

DAY-OF EXPERIMENTAL PROCEDURE

I. Reverse Transcription

We will use the Fermentas 'RevertAid^{TM} First-Strand cDNA Synthesis Kit' in this course. cDNA will be synthesized from 1 µg total RNA using an oligo (dT)18 as a primer.

Experimental procedure:

1. Mix:
   - _ 1 µg total RNA (variable volume)
   - _ 1 µl oligo (dt)18
   - _ add nuclease-free H_2O to 12 µl final volume

2. Incubate at 70°C for 5 min on the heating block. Chill RNA on ice.

3. Add:
   - _4 µl 5x buffer (mix well before use)
   - _2 µl dNTPs
   - _1 µl RiboLock (RNAse inhibitor)
   - _1 µl AMV reverse transcriptase

4. Incubate at 42°C for 30 min
5. Stop reaction by heating to 70°C for 5 min
6. chill on ice
7. Add 80 µl H_2O to dilute the 1st strand cDNA; final volume: 100 µl

II. Lecture on quantitative PCR (qPCR) methodology and data analysis.

The basic steps of analysis include:

I. Importing data into Excel or R.
II. Averaging of technical replicates.
III. Assessment of technical variation.
IV. Normalization of *PRI* amount by *UBC21*.
V. Comparison of different strain infections and statistical tests (students t-test).

Notes:

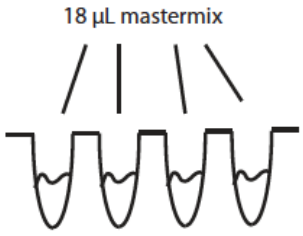
III. qPCR Experimental Procedure:

DNA oligonucleotides used in this course:
UBC21 Forward (F) and Reverse (R)
PR1 Forward (F) and Reverse (R)

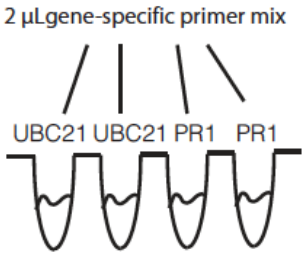
Step 1. prepare a master mix for 5 reactions (4 replicates + 1 extra to account for pipetting error):

	1 rxn	5 rxns
First strand cDNA template	4 µl	20 µl
2x SYBR green master mix	10 µl	50 µl
Water	4 µl	20 µl
Total	18 µl	90 µl

Step 2. add 18 µl of the master mix to each well of a 4-well PCR strip:



Step 3. add 2 µl of the gene-specific primer mix to the appropriate wells and cap the tubes:



Step 4:

Place your completed reactions in the appropriate space in the qPCR plate (positions will be indicated on the board). Make sure to change tips every time as this is a very sensitive procedure. Once all samples are pipetted, we will walk over to the machine and start the qPCR run. Your instructor will give details on parameters such as annealing temperature, elongation time, and number of cycles during the course and explain how one analyzes the amplification traces and the melt curve.

IV. Analysis of qPCR data

11:15-12:30 am