FACs with the microscopy/bulk conditions for the paper

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Title: FACs experiment with the same conditions as the microscopy done to quantify the cell sizes for the pAl1-Cdc42-sfGFP strains (III).

Date

04022020-06022020

Objective

To be able to compare the results of the FACs with the microscopy conditions. And also to see if we get a difference in expression when increasing the incubation temperature to 36C.

Method

Insights from the Gal1 promoter

- The location in the genome at which the gal promoter was inserted can have a strong effect on the expression pattern of the gal promoter (*Ramon feedback*). Hence we should not compare different studies of the Gal1 promoter with ours if the integration in the genome is in a different location and also if it is a plasmid or not.
- We should compare systematically the Gal1p expression pattern of the strains that has the sfGFP (Werner strains) and the mneonGreen ones (Ramon/Miranda strains), because they have the same type of genomic integration of the Gal1 promoter.
  - **Ask Reza for his data with WT+mneongreen to compare with mine**
    - Look for the postprocessing results, in this folder
- Follow the same protocol as I followed for the microscopy measurements

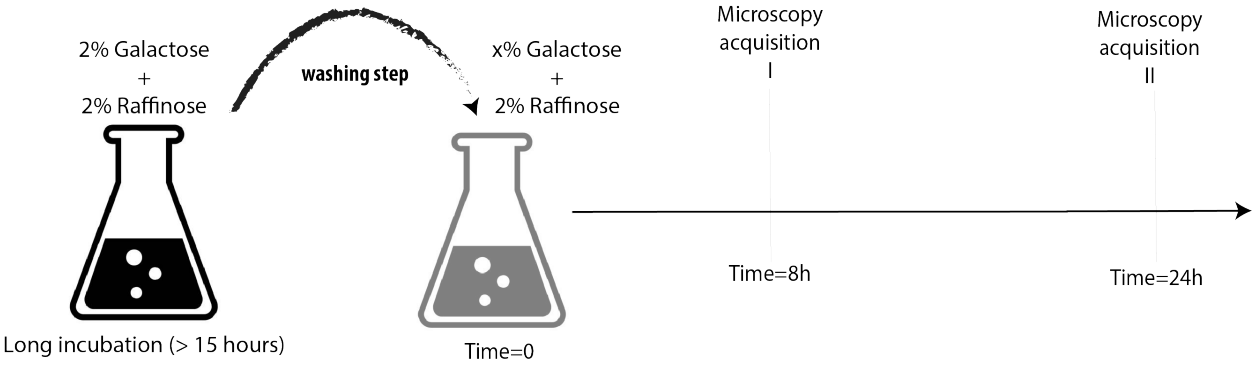

Figure 1: Experimental Design for the microscopy measurements

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Planned procedure

- 15 hours of incubation in 2% Gal +2% Raff (i.e. overnight incubation from 17:00 to 08:00)
- Washing step with CSM+2% Raff+0%Gal to the respective Galactose (at 08:00-09:00) concentrations. Incubate
- Measure FACs after 24 hours of incubation . (next day of the washing step)
  - Use the references cdc42-GFP ywkd038 and ywkd001 in 2% dextrose +2% Raff, in CSM-met and CSM respectively.

- In order to have the same conditions as the microscopy done in December 2018 , where we quantify the cell sizes after 24 hours of incubation in X% galactose (after a washing step from a firsto overnight incubation in 2% galactose), the media has to have 4x the normal amount of aminoacids. This maybe has an impact on the regulation of the promoter.
- The new plate design for this is shown in @fig:plate-design

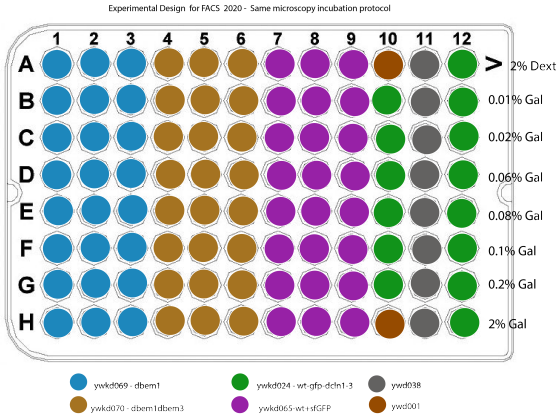

Figure 2: Plate design for this FACS experiment

- The strain ywkd024 will be measured to still compared with previous measurements done by Marit, in 2017.

- Strains :

  - ywkd024 : RWS119 Wedlich-Söldner Lab collection a W303 *can 1 1-100 his3 11,15 Galpr-myc-**GFP**-CDC42 YipLac204-MET-CLN2 cln1Δ::HisG, cln2Δ, cln3Δ::HisG* (**strain to compare with ywkd065(sfGFP)**)
  - ywkd065a New YWKD055c W303 *URA-Gal1pr-**sfGFP**-Cdc42 sandwich (pWKD011 integrated) leu2 3,112 his 3 11,15*
  - ywkd069 : New YWKD055c a W303 *bem1Δ::KanmX URA-Gal1pr-sfGFP-Cdc42 sandwich (pWKD011 integrated) MFAprHIS3 3,112 11,15*
  - ywkd070 : YWKD070a,b,c New YWKD055c a W303 *bem1Δ::KanmX bem3Δ::clonNAT URA-Gal1pr-sfGFP-Cdc42 sandwich (pWKD011 integrated) MFAprHIS3 3,112 11,15*

- ywkd038: RWS1421 Wedlich-Söldner Lab collection a W303 *can1 1-100 his3 11,15 CDC42pr-myc-**GFP**-CDC42 YipLac204-MET-CLN2 cln1Δ::HisG, cln2Δ, cln3Δ::HisG* (**Reference for the native CDC42 expression**)

- Settings of the FACs experiment
  - **Equipment-Model**: BDFACSCelesta
  - **Lasers**: Alexa Fluor 488 at 495V
  - **Flow Rate**: 2ul/sec
  - **Sample volume**: 130ul
  - **Plate**: 96 well plate with flat bottom
  - **# of events per well**: 10000
  - **FSC threshold**:20000
  - **FSC voltage**: 407V
  - **SSC volatge**: 275V
  - **Mixing volume**: 65ul
  - **Mixing speed**: 200ul/sec
  - **Nr. of mixes**: 5

Actual procedure

- 1st Incubation , at 9:00 in 04022020 in 2% Gal.
  - With 10ul of thawed and vortexed glycerol stocks, because last experiment had some trouble with the incubation from this step, so perhaps I did not put many cells in the tubes from the frozen glycerol stock.
  - Remarks from 1st incubation:
    - ywkd070 grew very miserably
    - ywkd069 was denser than ywkd070
- 2nd incubation , at 11:45 in 05022020 in the respective galactose concentrations.
  - remarks from 2nd incubation:
    - I miscalculated the amount of ywkd024 cells to equally distribute among galactose concentrations, and I could not reach the 2% gal tube, so I took from the already prepared 0.2% gal tube into the 2%. So, I expect a small amount of cells from 2% gal, when measuring, because of this.
- Experiment : at 13:00
  - Before the experiment:
    - I had to dilute ywkd065 and ywkd024 20X because of their high density after 24h of incubation.
    - I did not dilute the 0% case of ywkd065 niether the 2% case for ywkd024.
    - I diluted them with CSM and CSM-met without any galactose, basically because it was not necessary taking into acount the time from the dilution (12:30) to the measuring (13:00).

      Even for the case of 2 hours difference, because they are not the first to be measured in the FACs machine due to the well position , no degradation should occur via degradation of Cdc42-GFP or CDC42-

sfGFP or via division.

Results

Plate data

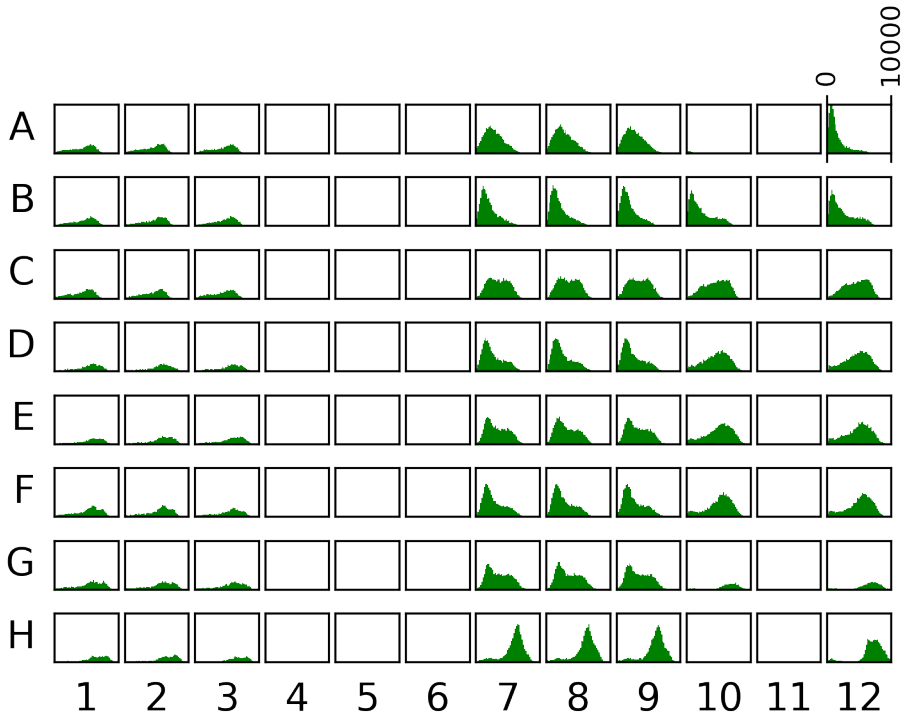

Figure 3: Whole plate for 02062020 measurement

Box plots

Measure per strain

Cdc42 relative expression

Correlation between the GFP and sfGFP fluorophuores

Conclusion

- In this experiment the WT+pGal behaves more as expected than in experiment 004.
- The ywkd070 behaves very weirdly, it did not grow form the first incubation. I dont know why. Also ywkd038 and ywk001 have very miserably growth, in 36C.
- The correlation between GFP and sfGFP, observed in @fig:correlation, shows that when we are dividing the pGal-sfGFP expresion with the native CC42-GFP we are underestimating the final result because the GFP intensity is slightly brighter than the sfGFP.

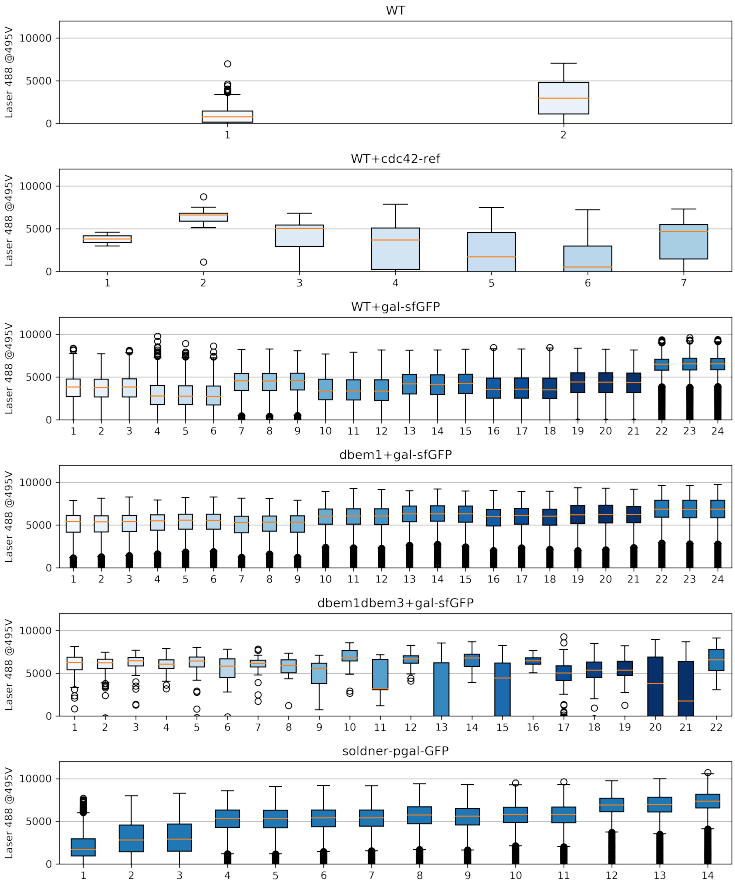

Figure 4: Boxplots of all the data

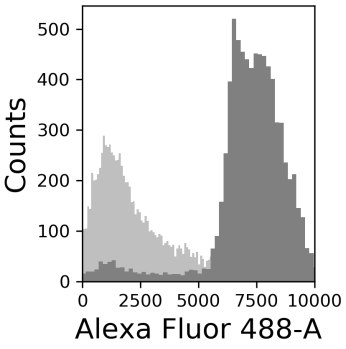

Figure 5: Non normalized histogram for ywkd024 fold change from 0% gal to 2% gal

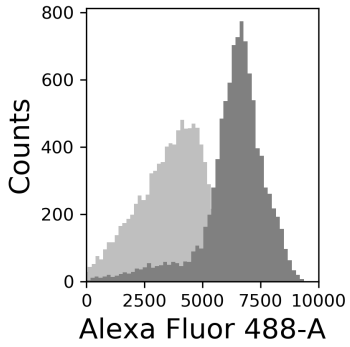

Figure 6: Non normalized histogram for ywkd065 fold change from 0% gal to 2% gal

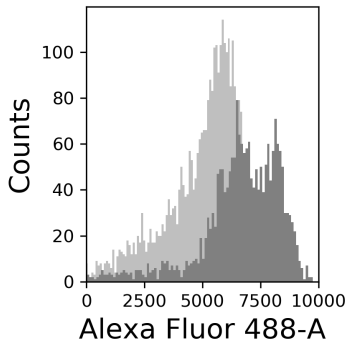

Figure 7: Non normalized histogram for ywkd069 fold change from 0% gal to 2% gal

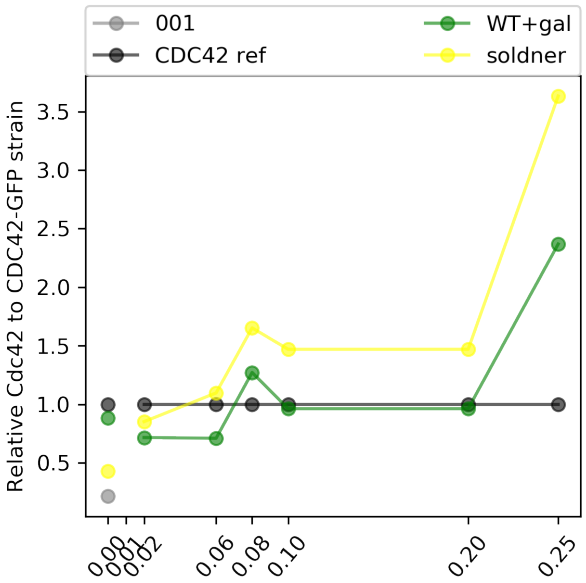

Figure 8: Relative cdc42 expression compared to ywkd038

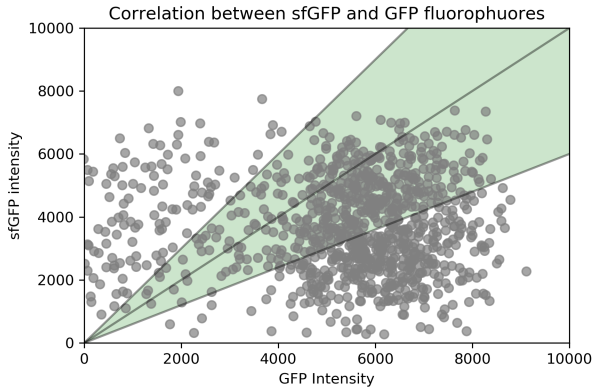

Figure 9: Correlation between sfGFP and GFP fluorophuores exp_005 at 0.1%-gal