

Lab book

Project: Size and Number/ Pex11 and Pex34

Team: Kate and Pauline

2017/07/13

- preparation of Pex11 IDT syntheses
- Golden Gate: ligation of Pex11 3b Insert in pYTK001

2017/07/14

- transformation of Golden Gate product Pex11 level 0 in competent E. coli
- retransformation of pYTK001

2017/07/17

- o/n culture Pex11 level 0

2017/07/19

- miniprep Pex11 level 0
- test digestion of Pex11 level 0
- gel of test digest
- Golden Gate: ligation of Pex11, ScTDH3, pYTK00ScSSA1, Venus in cassette plasmid level 1

2017/07/20

- Transformation of Pex11 level 1 in competent E. coli

2017/07/21

- o/n culture Pex11 level 1

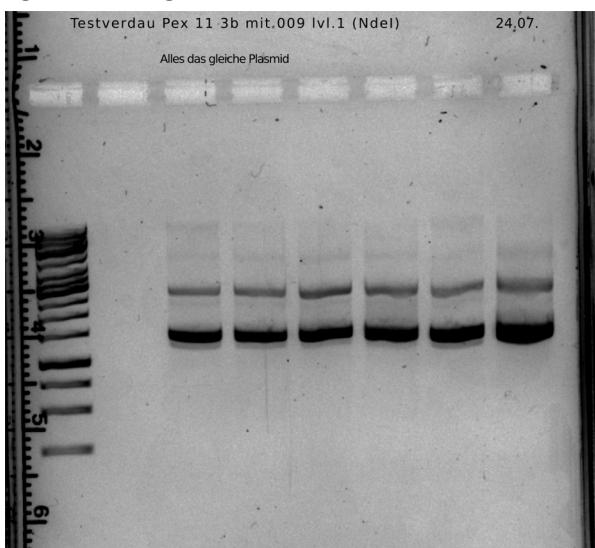
2017/07/22

-miniprep Pex11 level 1

2017/07/24

-test digest Pex11 level 1

-gel test digest: Pex11 level 1



2017/07/25

-test digest Pex11 level 0 and level 1



2017/07/26

-Golden Gate Pex11 level 1

2017/07/27

-transformation Golden Gate in E. coli

-Golden Gate with inducible promoters

2017/07/28

-transformation Golden Gate with inducible promoters in E. coli

-o/n culture Pex11 with ScTDH3 promoter

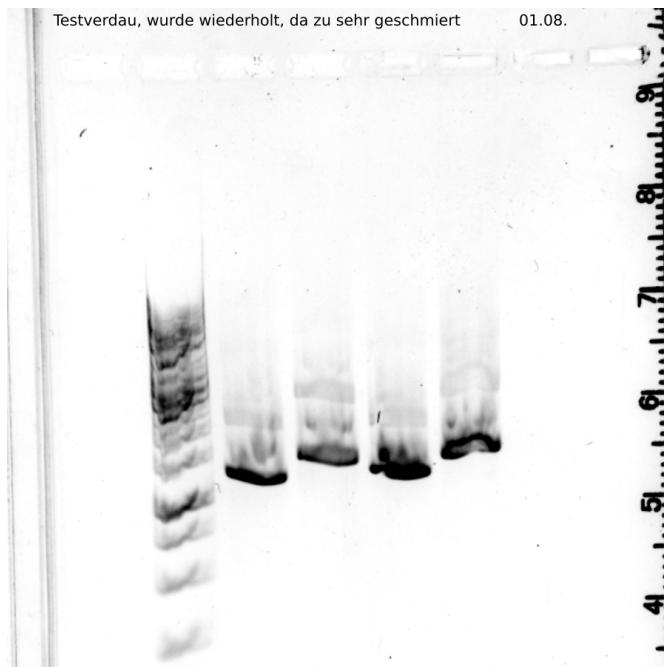
2017/07/31

-miniprep Pex11 with ScTDH3 promoter

-test digest Pex11 with ScTDH3 promoter

2017/08/01

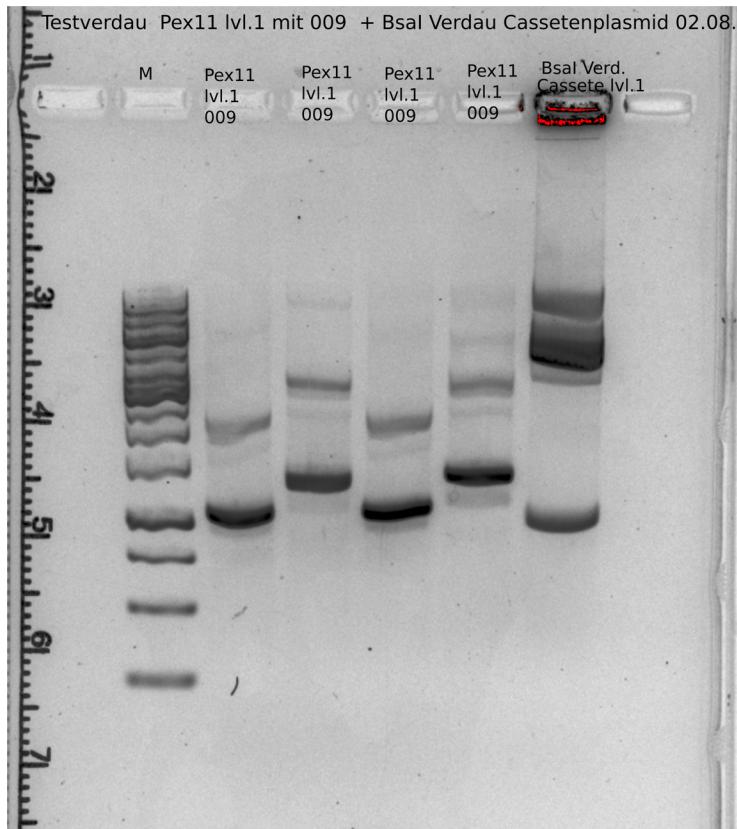
-gel test digest Pex11 with ScTDH3 promoter



-Bsal digest cassettenplasmid

2017/08/02

- repetition gel test digest Pex11 with ScTDH3 promoter
- gel Bsal digest cassettenplasmid



-gel clean up

-Go gate Pex11 with ScTDH3 ScScGAL1 and ScCUP1

2017/08/03

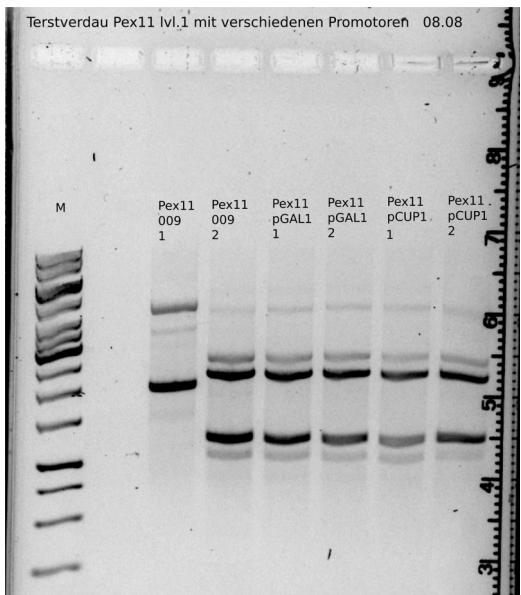
- transformation Golden Gate Pex11 with ScTDH3 ScGAL1 and ScCUP1 in E.coli
- retransformation of pYTK001, ScTDH3,Venus , ScSSA1

2017/08/07

-o/n cultures transformation and retransformation

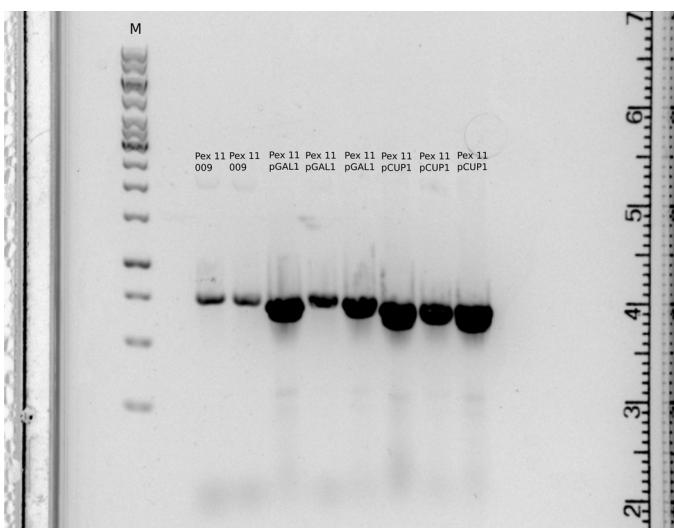
2017/08/08

- miniprep transformation and retransformation
- o/n culture yeast strain BY4747 pex11 Δ
- test digest Pex11 with ScTDH3, ScGAL1, ScCUP1
- new o/n cultures retransformations ScTDH3, Venus , ScSSA1
- gel test digest Pex11 with ScTDH3, ScGAL1 and ScCUP1



2017/08/09

- colony PCR Pex11 with ScTDH3 ScScGAL1 and ScCUP1
- gel colony PCR



- main culture pex11 Δ yeast strain
- glycerol stocks pex11 Δ yeast
- preparation of competent yeast cells pex11 Δ

2017/08/10

- miniprep retransformation pYTK001, ScTDH3, Venus , ScSSA1
- Golden Gate Pex11 with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)
- transformation Pex11 with ScTDH3 in yeast, in wild type strain and in pex11 Δ strain
- Bsmbl digest pYTK001

2017/08/11

- Transformation Pex11with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)

2017/08/14

- Colony PCR Pex11with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)
- gel colony PCR

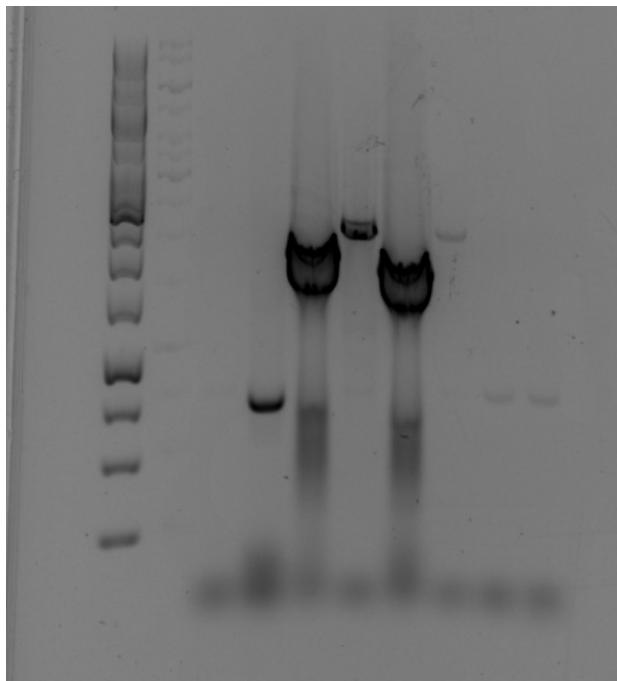


2017/08/16

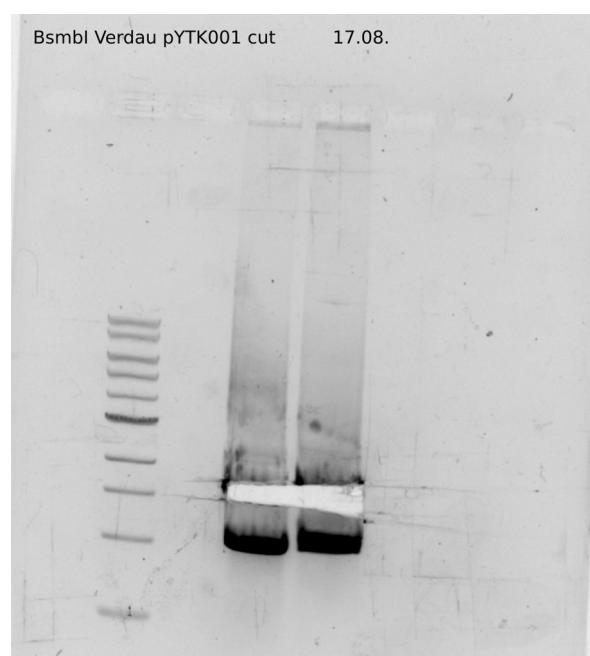
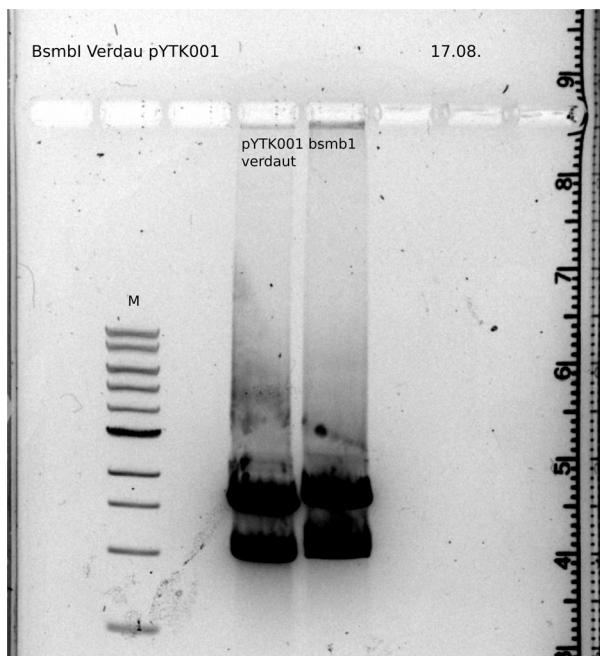
- Golden Gate Pex11with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)
- o/n culture pYTK001/ Pex11 with ScALD6/ Pex11 with ScGAL1

2017/08/17

- miniprep pYTK001/ Pex11 with ScALD6/ Pex11 with ScGAL1
- Bsmb1 digest pYTK001
- test PCR Pex11 with ScALD6/ Pex11 with ScGAL1
- gel colony PCR



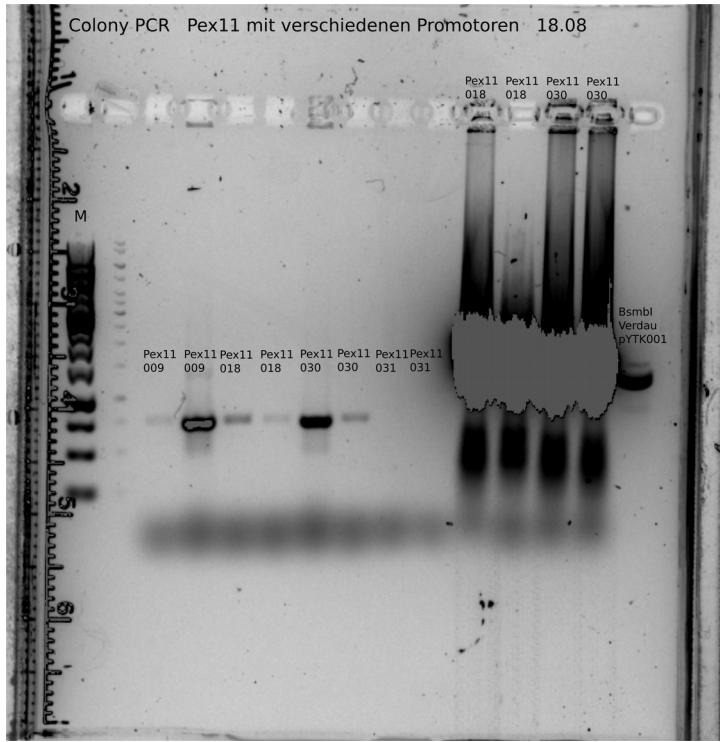
-gel Bsmb1 digest pytk001



-transformation Pex11 with four different promoters
(ScTDH3,0ScALD6,ScScGAL1,0ScCUP1)

2017/08/18

- colony PCR Pex11 with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)
- gel colony PCR Pex11 with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)



-gel clean up BsmbI digested pYTK001

-transformation Pex11 with ScALD6, ScGAL1 in competent yeast cells wild type and pex11 Δ

-Golden Gate Pex11 with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)

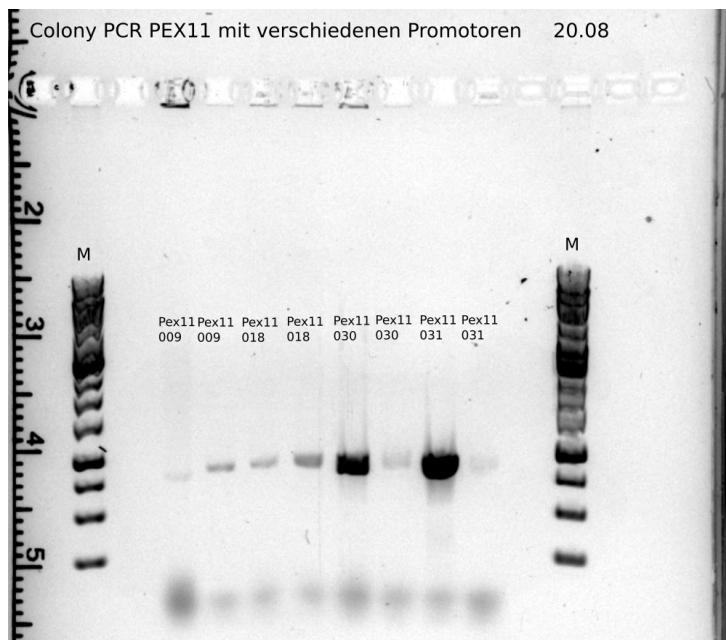
2017/08/19

-transformation Pex11 with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1) done by marvin

2017/08/20

-colony PCR

-gel colony PCR

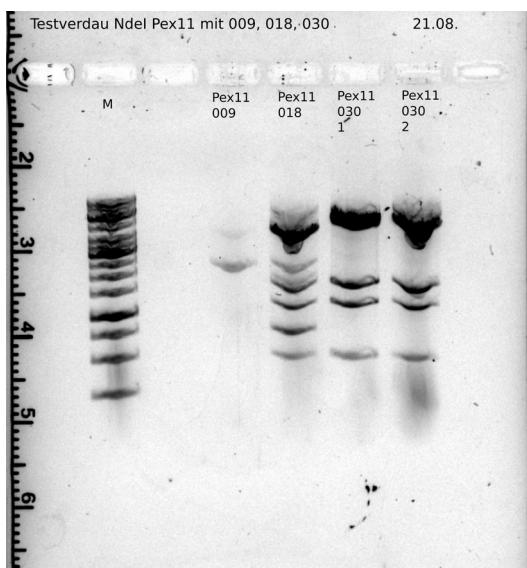


2017/08/21

-Golden Gate Pex11 with ScCUP1

-test digest Pex11 with ScTDH3/ Pex11 with ScALD6/ Pex11 with ScGAL1/ Pex11 with ScGAL1

-gel test digest



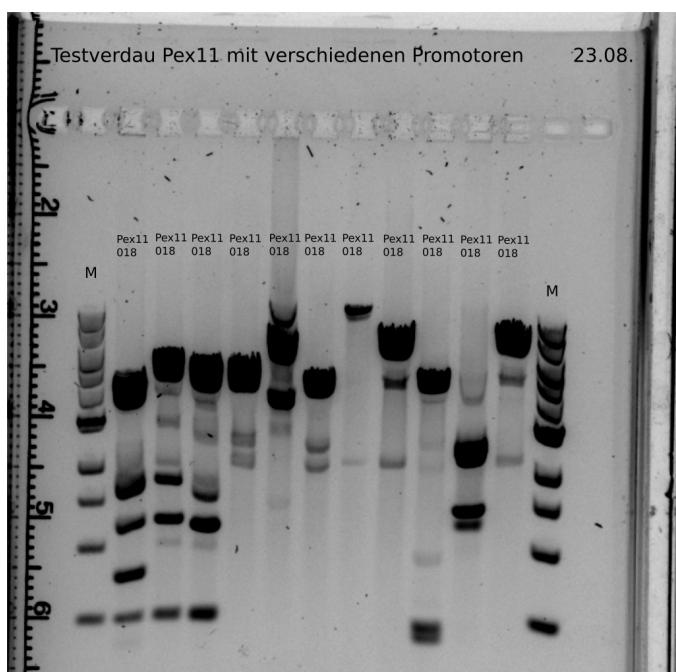
-o/n cultures Pex11 with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)

2017/08/22

- repetition test digest Pex11 with ScALD6/Pex11 with ScGAL1 1/ Pex11 with ScGAL1 2
- miniprep Pex11 ScTDH3,ScALD6, ScGAL1, ScCUP1
- test digest Pex11 with four different promoters ScTDH3, ScALD6, ScGAL1, ScCUP1
- retransformation pYTK083 and Pex11 with ScTDH3
- transformation Pex11 with ScCUP1 in competent E.coli

2017/08/23

- gel repetition test digest Pex11 with ScALD6/ Pex11 with ScGAL1 1/ Pex11 with ScGAL1 2
- test digest Pex11 with four different promoters ScTDH3, ScALD6, ScGAL1, ScCUP1

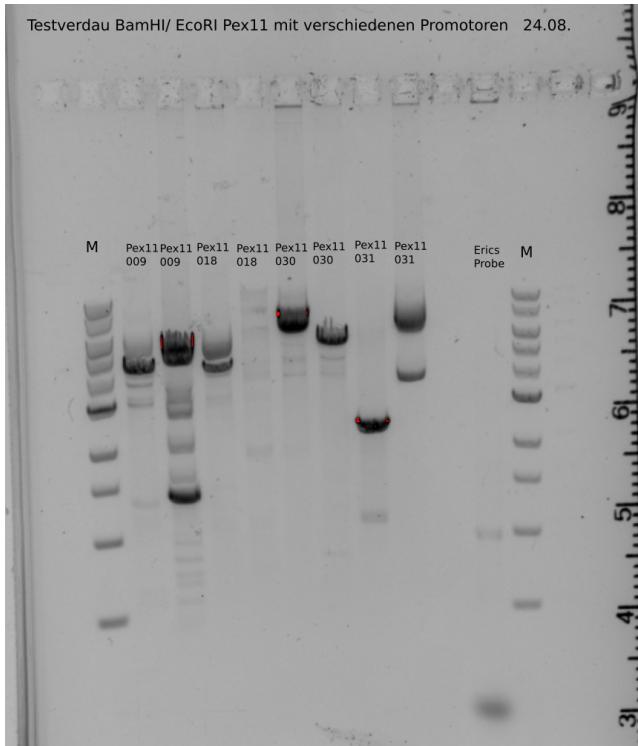


- Bsal digest cassettenplasmid level1
- colony PCR Pex11 with ScCUP1/ Pex11 with ScTDH3/ Pex11 level 0

2017/08/24

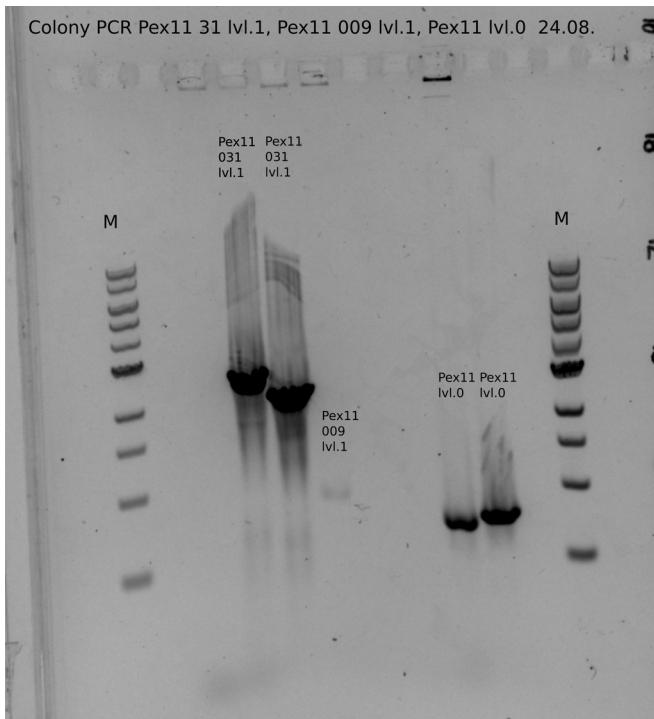
-test digest Pex11 with four different promoters (ScTDH3, ScALD6, ScGAL1, ScCUP1)

-gel test digest



-gel clean up BsmBI digested cassettenplasmid level 1

-gel colony PCR

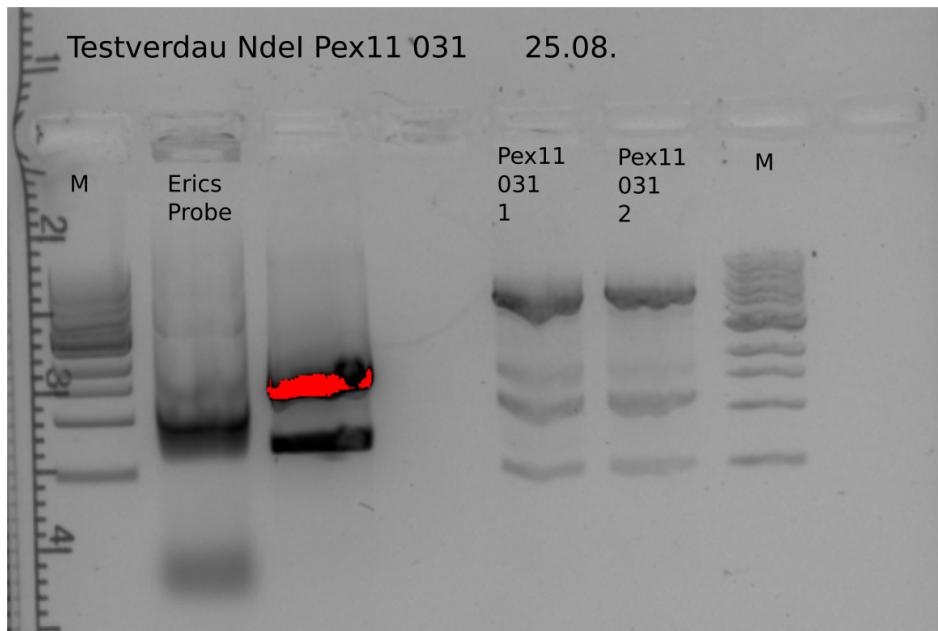


2017/08/25

-miniprep o/n culture Pex11 ScCUP1

-test digest Pex11 with ScCUP1

-gel test digest



2017/08/28

-Golden Gate Pex11 ScTDH3, ScALD6, ScGAL1

2017/08/28 ScTDH3

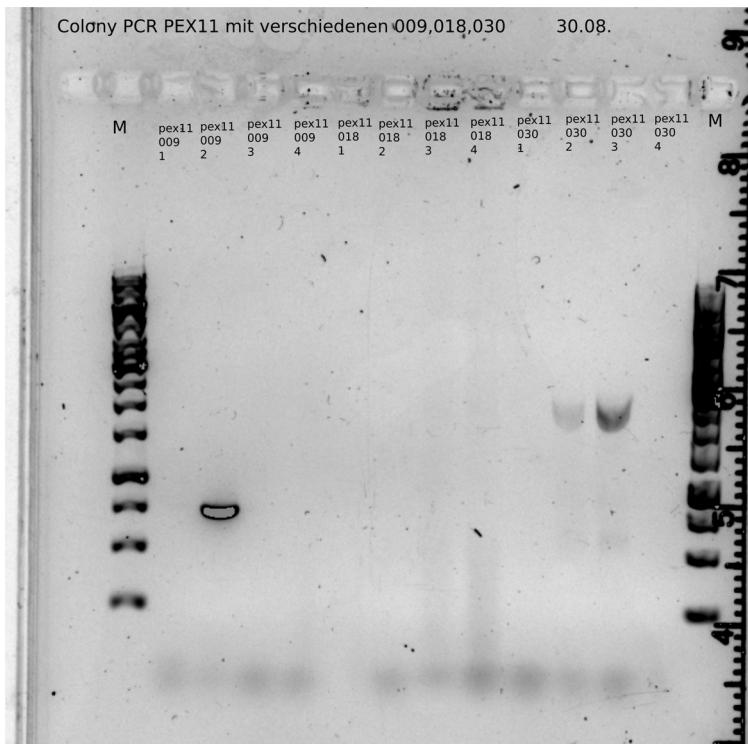
-transformation Pex11 ScTDH3, ScALD6, ScGAL1 in competent E. coli

-sequencing Pex11 with ScCUP1 1 and 2

-retransformation Pex11 with ScCUP1

2017/08/ScScGAL1

- colony PCR Pex11 with ScTDH3, ScALD6, ScGAL1
- gel colony PCR



2017/08/31

- o/n culture Pex11 with ScGAL1 2 and 3
- repetition retransformation Pex11 and ScCUP1
- Golden Gate Pex11 with ScSSw12, ScTDH3

2017/09/01

- transformation Pex11 ScSSw12 01ScTDH3 in competent e.colis
- o/n culture Pex11 0ScCUP1 retransformation

2017/09/04

- transformation Pex11 ScCUP1, ScGAL1 in competent E. coli
- miniprep
- retransformation Pex11 with ScGAL1, pYTK082, pYTK055

2017/09/05

-o/n cultures Golden Gate Pex11 with ScSSW12, ScTDH3

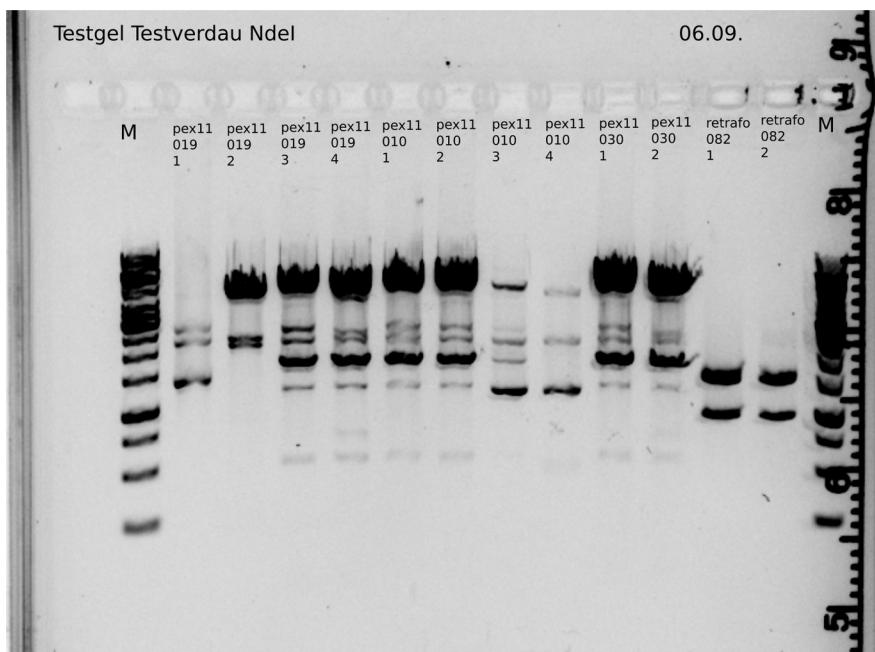
-retransformation Pex11 with ScScGAL1, pYTK082

2017/09/06

-Miniprep o/n culture

-test digest

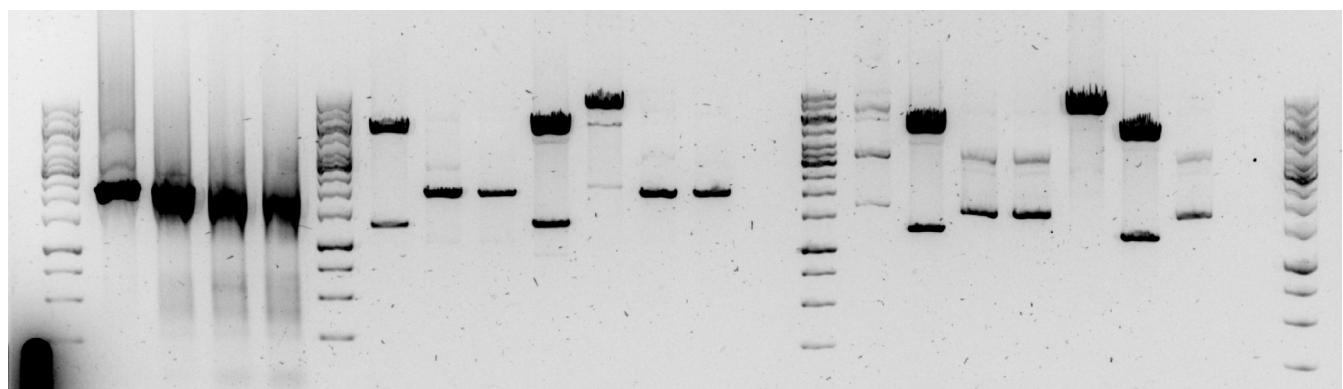
-gel test digest



2017/09/07

-test PCR Pex11 with ScPAB1 4, Pex11 with ScSSW12 1, Pex11 with ScSSW12 2

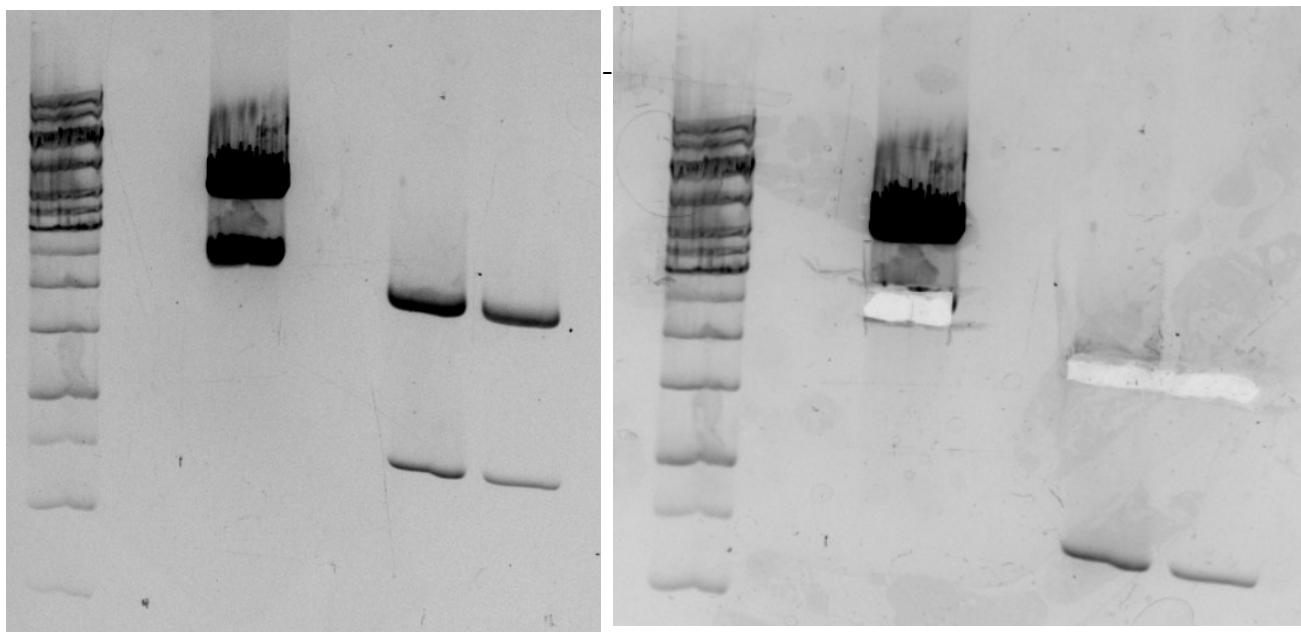
-gel test PCR



2017/09/10

-Biobrick digest EcoRI and PstI

-gel Biobrick digest



-o/n culture yeast

-gel clean up Biobrick insert

2017/09/11

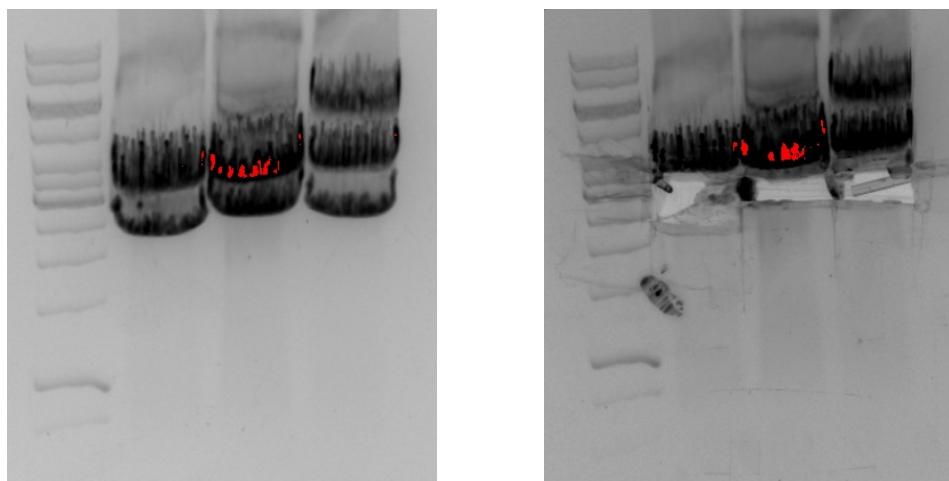
-Biobrick digest

-o/n culture yeast

-glycerin stocks yeast strain Pex11 with ScPAB1 Pex11Δ, Pex11 with ScGAL1 in WT ,
Pex11 with ScScGAL1 in pex11Δ

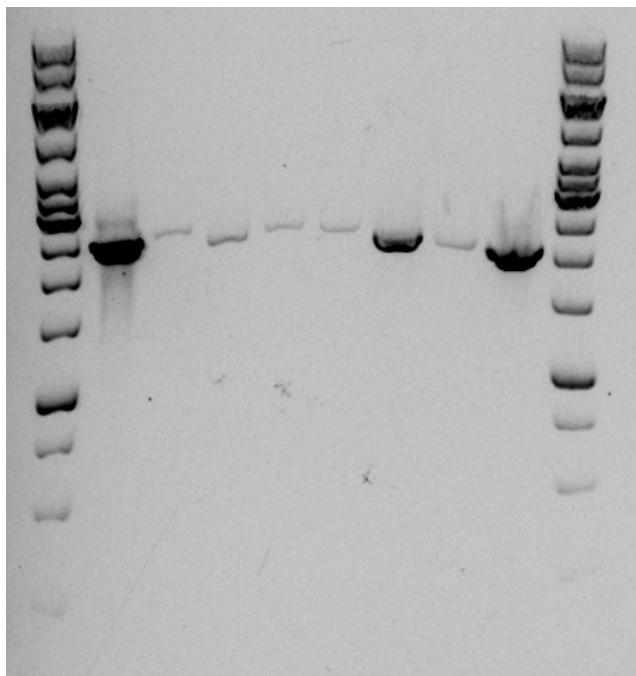
-cell fixitation for fluorescence microscopie

-gel Biobrick digest Pex11 ScSSw12 , ScScGAL1, 01ScPAB1

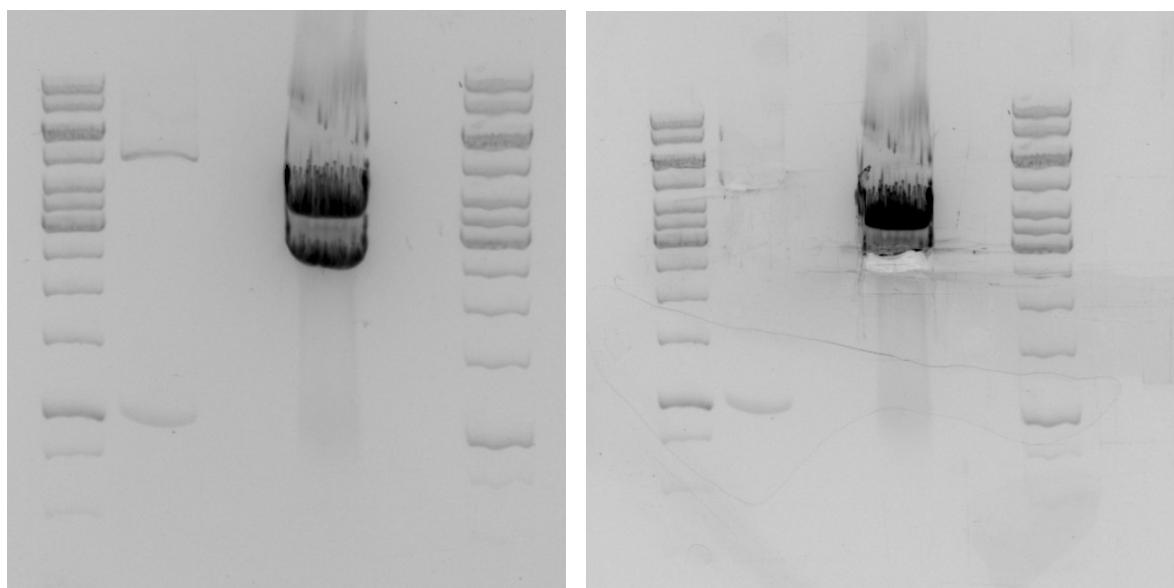


2017/09/12

- gel clean up Pex11 ScSSw12 , ScScGAL1, ScPAB1
- Biobrick digest Pex11 ScSSw12
- Bsal digest cassette URA
- colony PCR yeast Pex11 ScSSw12 , ScPAB1, ScGAL1, ScCUP1
- gel colony PCR



- gel digest cassette URA Pex11 ScSSw12



- retransformation cassette URA, TEV protease
- gel clean up Biobrick insert Pex11 ScSSw12
- o/n culture yeast

2017/ 09/13

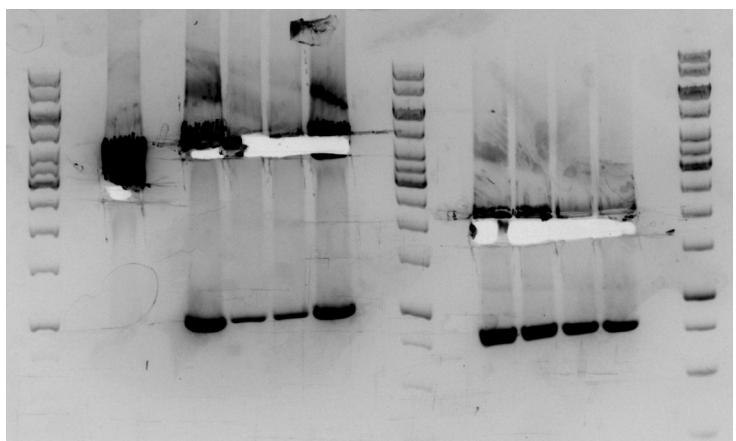
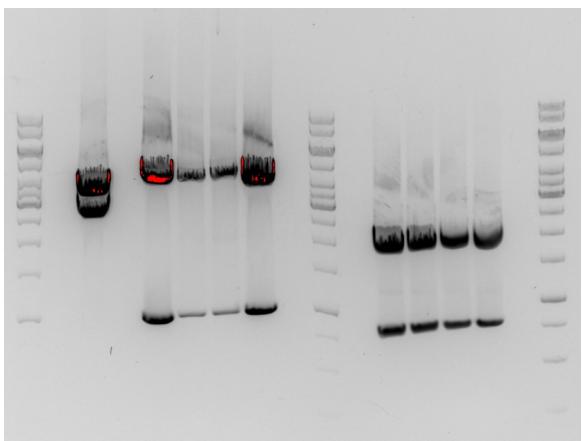
- main culture yeast
- test gel purified Biobrick inserts Pex11 ScSSw12 , ScPAB1, ScGAL1, ScCUP1
- Biobrick digest Pex11 ScGAL1
- Glycerin stocks yeast cultures
- fixation yeast cells

2017/09/14

- miniprep retransformation cassette URA, TEV(Biobrick backbone)
- sequencing
- BsmBI digest cassette URA
- Biobrick digest TEV
- o/n culture yeast

2017/09/15

- gel digestion backbones



- main culture yeast
- gel clean up
- fixation yeast culture

2017/09/18

- ligation Biobricks Pex11 ScSSw12 , ScPAB1, ScGAL1, ScCUP1
- transformation Biobrick ligation in comp e.coli
- transformation Pex11 ScSSw12 in wt yeast
- fixation yeast cells on object slide

2017/09/20

- miniprep Biobricks Pex11 ScSSw12 , ScPAB1, ScGAL1, ScCUP1
- retransformation Pex11 ScSSw12 ScTDH3
- yeast o/n culture

2017/09/21

- o/n culture Pex11 ScSSw12 01ScPAB1
- yeast main culture

2017/09/22

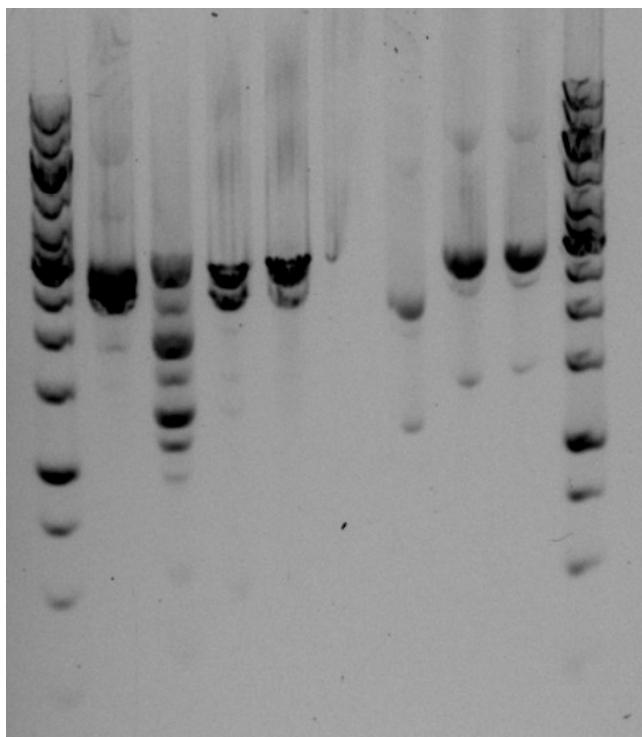
- miniprep retransformations Pex11 ScSSw12 , ScPAB1
- yeast main culture Pex11 ScSSw12 in WT

2017/09/25

- test digest Biobricks Pex11 ScSSw12 , ScPAB1, ScGAL1, ScCUP1
- o/n culture yeast

2017/09/26

-gel test digest Biobrick



-main culture yeast

-fixation yeast cells

-plate reader measurement Pex11 ScGAL1 Venus

-ligation Biobricks

2017/09/27

-transformation ligation Biobricks

-fixation yeast cells on object slide

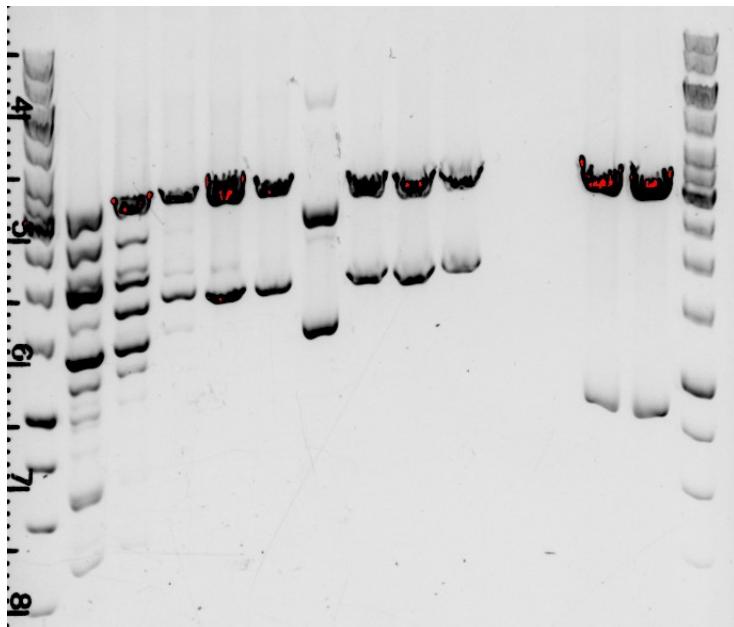
2017/09/2ScTDH3

-miniprep Biobrick Pex11 ScSSw12 , ScPAB1, ScGAL1

-Pex34 level 0 Golden Gate

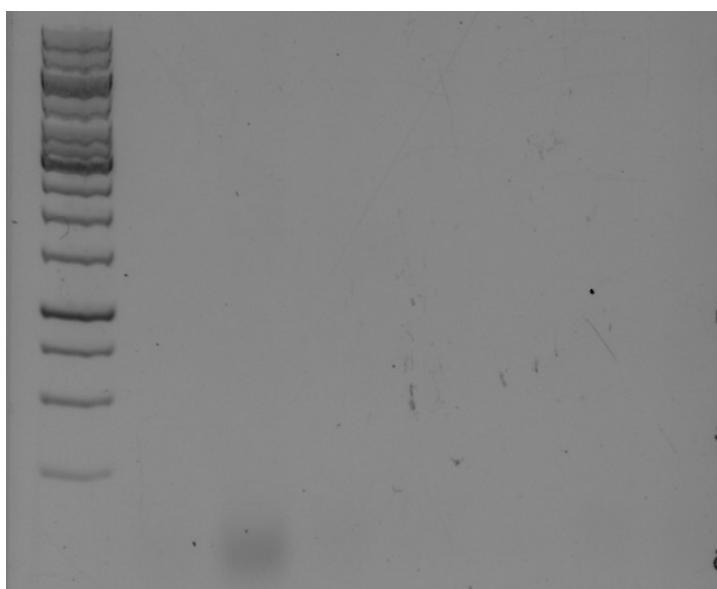
2017/09/ScScGAL1

- Golden Gate Pex34 level 1 (8 constructs)
- test digest Biobrick
- transformation Golden Gate level 1 Pex34
- colony PCR Pex34 lvl 0
- gel test digest Biobrick colony PCR Pex34 level 0



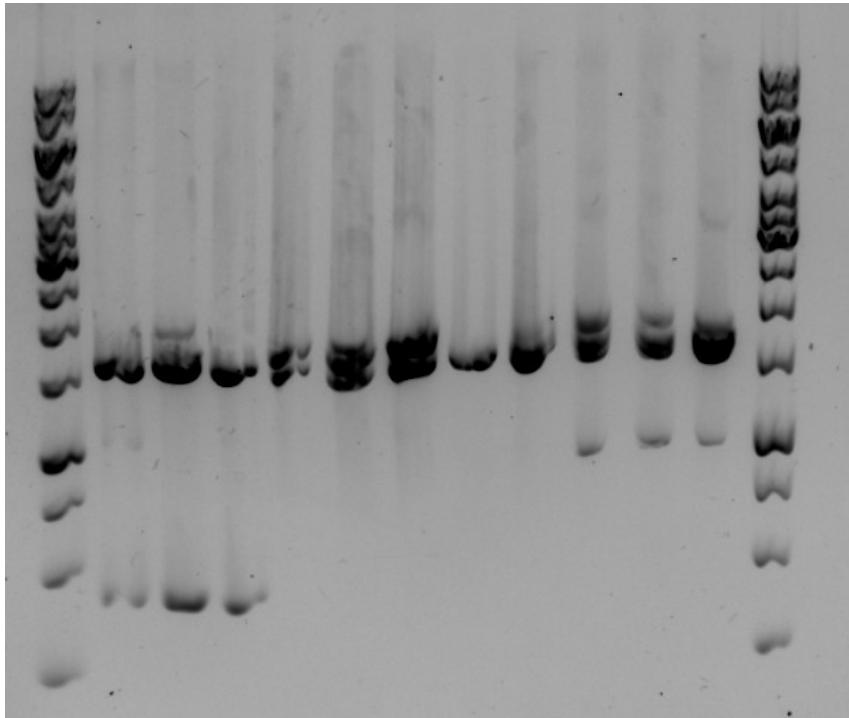
2017/10 /01

- colony PCR Pex34 level 0
- gel colony PCR



2017/10 /02

- test digest Pex34 level 0, baker ptsl, non ptsl
- gel test digest



-Golden Gate Pex34 level 1

- transformation Golden Gate Pex34
- retransformation ScSSw12 , ScPAB1, pYTK055, pYTK060

2017/10 /03

- fixation yeast cultures
- Golden Gate Pex34 lvl1
- fixation on object slide
- o/n culture retrtransformations ScSSw12 , ScPAB1, pYTK055, pYTK060
- miniprep Pex11 ScSSw12 , pYTK074

2017/10 /04

- transformation Golden Gate Pex34 level 1
- sequencing Pex34 level 0

2017/10 /05

-miniprep retransformations ScSSw12 , ScPAB1, pYTK055, pYTK060

-Golden Gate Pex34 lvl. 1

2017/10 / 06

o/n culture Pex34 level 1

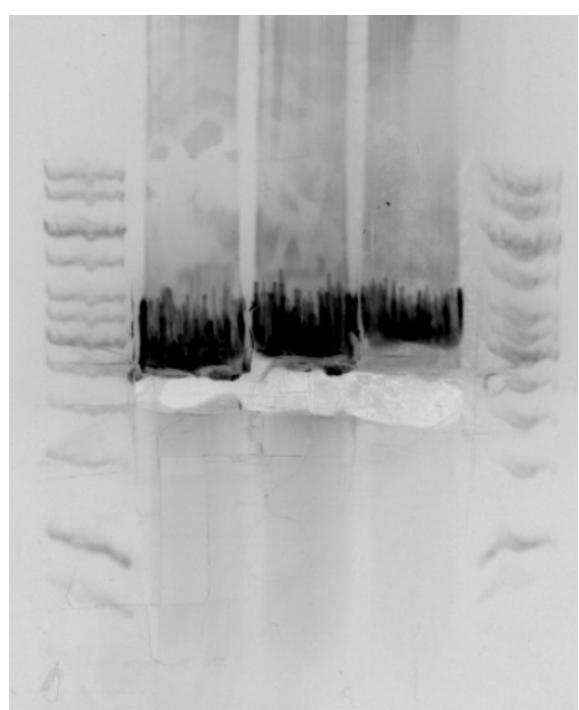
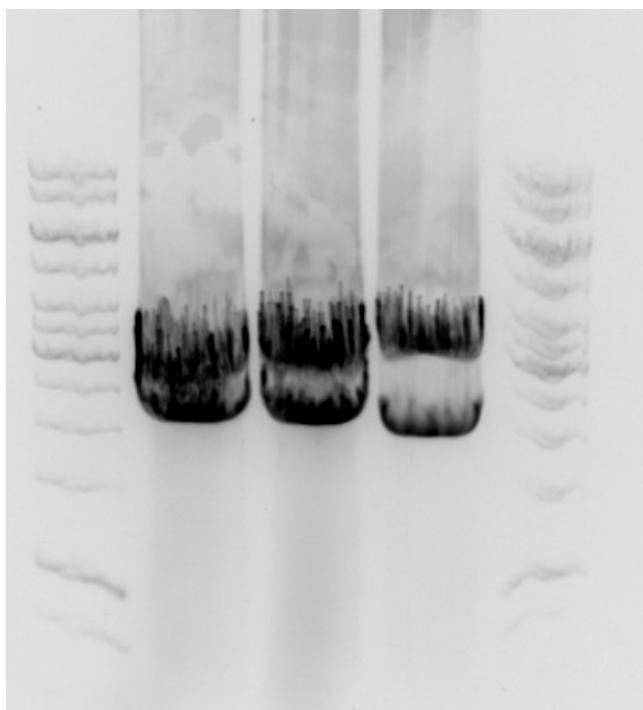
2017/10 /08

-test PCR Pex34 lvl1

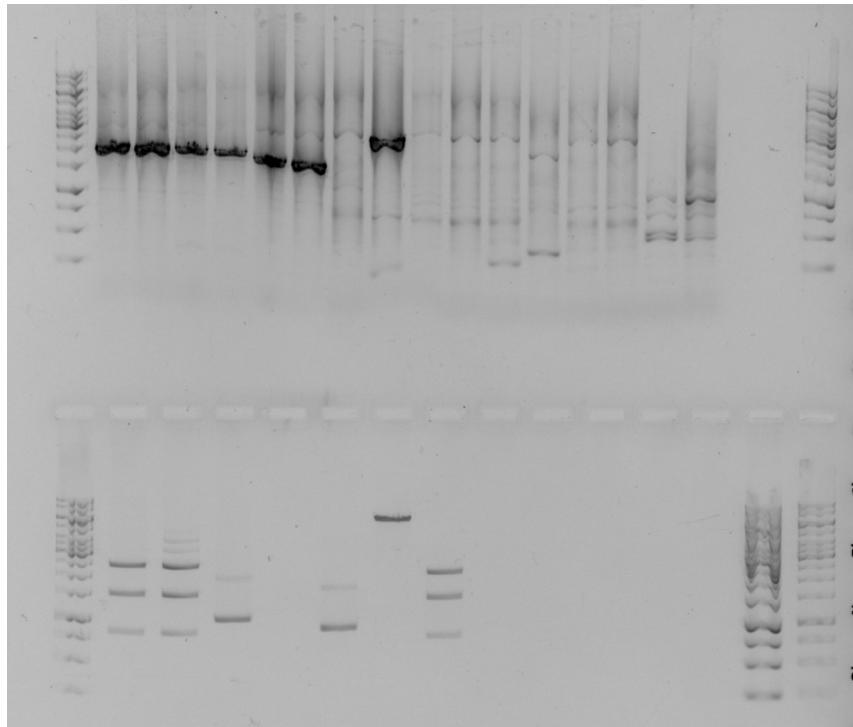
-test digest Biobricks Pex11 ScSSw12 ScScGAL1

-Biobrick digest inserts Pex11 ScSSw12 , ScPAB1, ScCUP1

-gel Biobrick digest



-gel testPCR Pex34 lvl1+ testverdau Biobricks



-sequencing Pex34 lvl0

2017/10 /ScTDH3

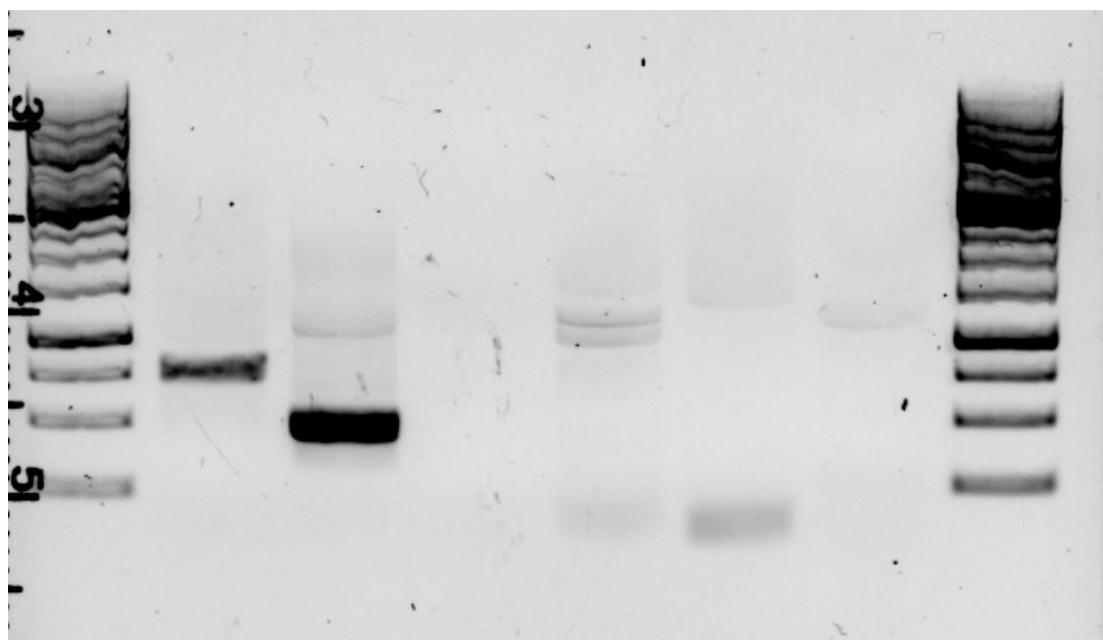
-PCR Biobrickx Pex34 and Pex11

-gel clean up (zymo clean gel dna recovery kit)

-PCR clean up (machery-Nagel)

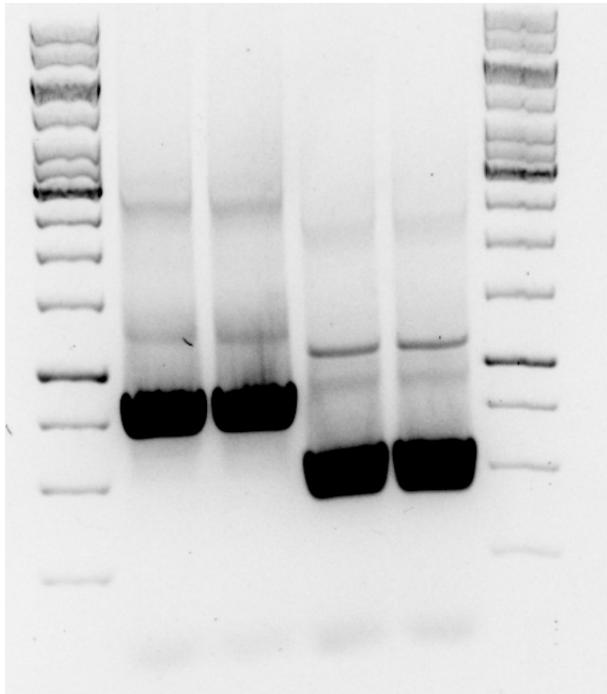
-gel PCR-clean up

-o/n culture Yeast Pex11 four constructs in wt and Pex11Δ



2017/10 /10

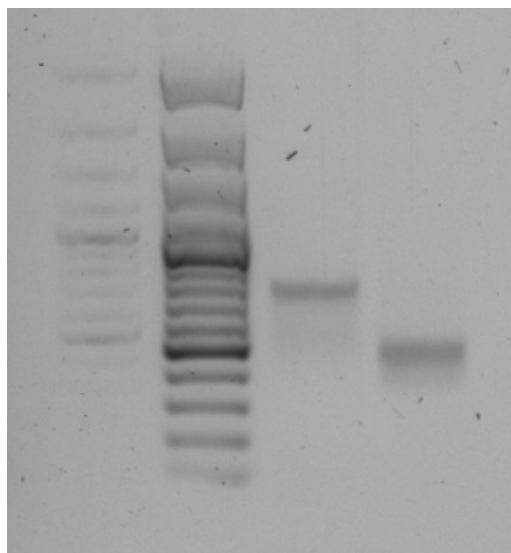
- main culture yeast Pex11 four constructs in wt and Pex11 Δ
- EcoRi PstI digest PCR products Pex34 and Pex11
- gel digest



- Golden Gate Pex34 lvl1
- fixation yeast cells

2017/10 /11

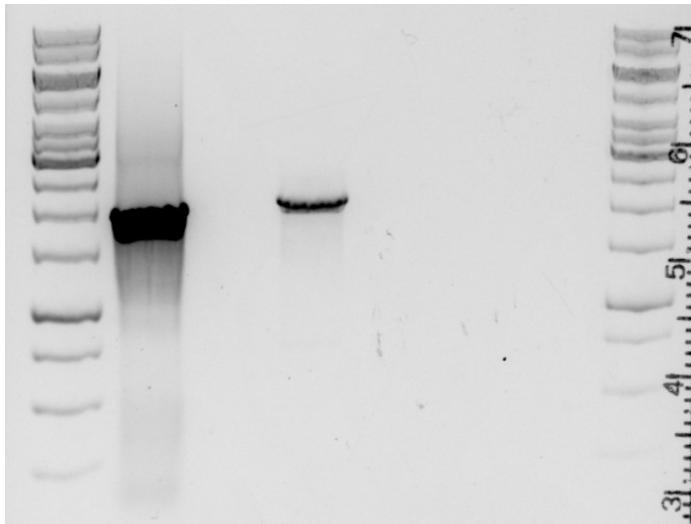
- Transformation Pex34 lvl1 in competent e.coli
- gel clean up Pex11 an dPex34 Biobrick inserts
- testgel gel clean up



2017/10 /12

-colony PCR Pex34

-gel colony PCR



-Ligation Biobrick Pex11 and Pex34

-fixation yeast cell on mikroskopy slides

-transformation Golden Gate in competent e.coli

2017/10 /13

-yeast main culture sfGFP in Pex11 Δ

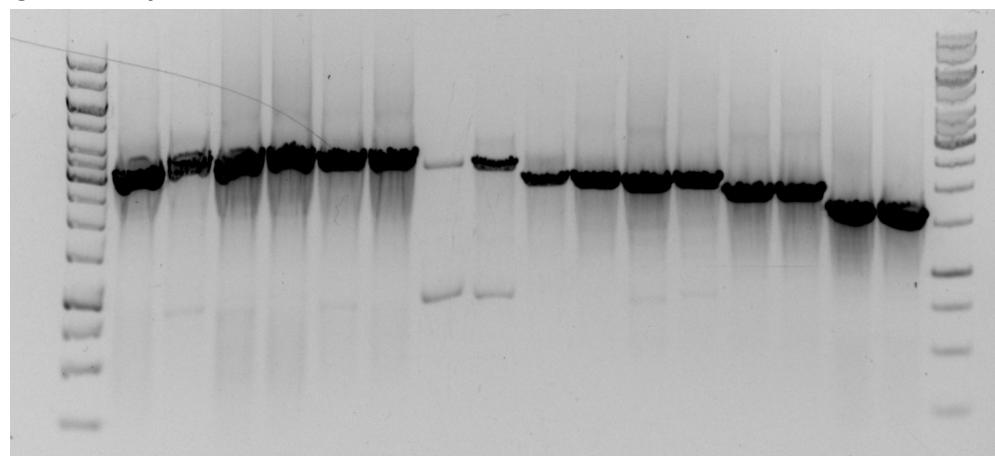
-fixation yeast cell in PBS

2017/10 /18

-Colony PCR Pex34 lvl1

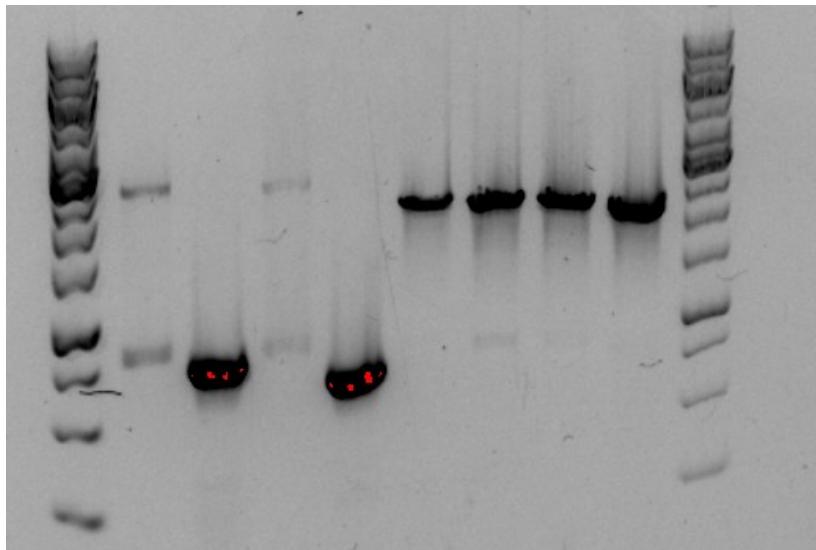
2017/10 /19

-gel colony PCR



-colony PCR Pex34 level 1

-gel colony PCR



-transformation ligation of Pex11 and Pex34 in competent E. coli

2017/10 /20

-miniprep Pex34 lvl1

-sequencing Pex34 lvl1

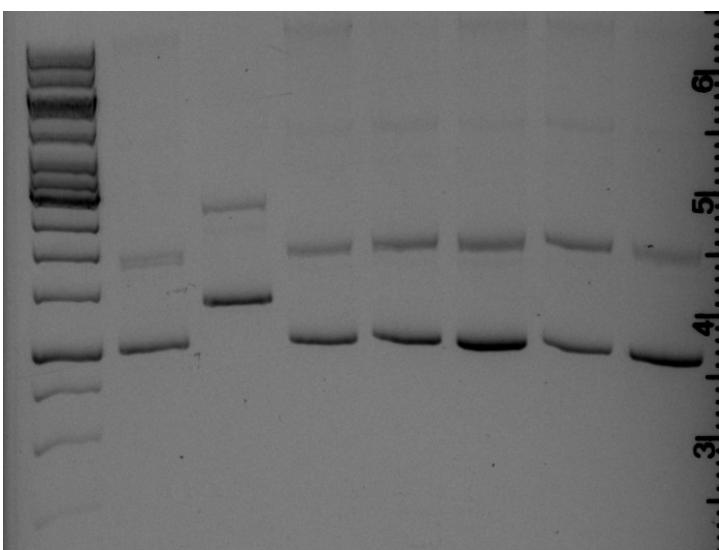
2017/10 /22

-retransformation Pex34 3, pYTK016, ScCUP1, ScGAL1,TEV, pYTK053

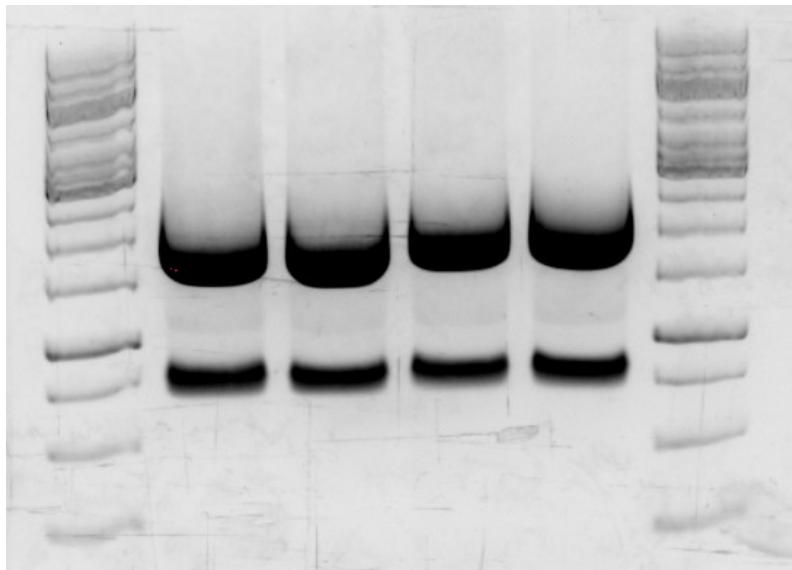
-miniprep Pex34 ScPAB1 no tag, Biobricks Pex11 and Pex34

-test digest Biobricks Pex11 and Pex34

-gel test digest

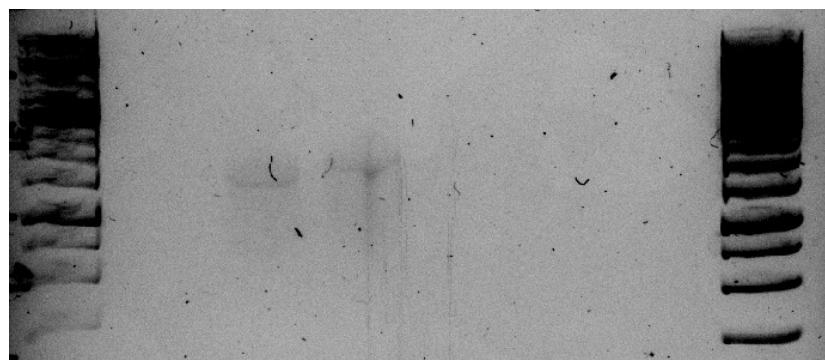


-Biobrick digest tev(Biobrick backbone)
-sequencing Pex34 SCPAB1 no tag, Biobricks Pex11 and Pex34
-gel digest tev



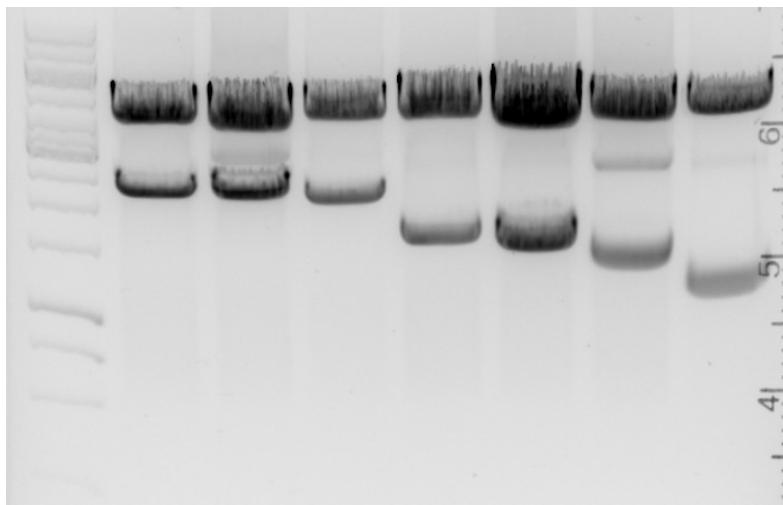
2017/10/23

-Gel clean up Tev cut
-test gel tev clean up



-Biobrick digest Pex34 six different constructs

-Gel Biobrick digest



-yeast b/n culture Pex34 six different constructs

-o/n culture retransformation

-yeast transformation Pex34 01ScTDH3 no tag

-ligationPex34 in Biobrick backbone

-transformation ligation

2017/10 /24

-yeast main culture

-fixation yeast cells

-sequencing

-copper induction constructs with copper inducible promoters

2017/10 /25

-fixation yeast cells with copper induced constructs