

Week	Date	Jobs
30	2017-07-24	
	2017-07-25	
	2017-07-26	<ul style="list-style-type: none"> <li>Golden Gate reaction for level 0 (see paper “A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly, Dueber <i>et al.</i>, 2015) for PEX5 variants (R8 and R15) and PTS1* variants (-YTNQE, -YTNWD, -YYNWL)</li> </ul>
	2017-07-27	<ul style="list-style-type: none"> <li>Heat shock transformation of golden gate products with DH5<math>\alpha</math></li> <li>Pre-digestion of entry vector</li> <li>Golden Gate level 2 cassette</li> </ul>
	2017-07-28	<ul style="list-style-type: none"> <li>Heat shock transformation of level 2 cassette plasmid</li> </ul>

Week	Date	Jobs
31	2017-07-31	<ul style="list-style-type: none"> <li>• Inoculation of R8, R15, PTS1* variant and lvl2 cassette transformants</li> <li>• Golden Gate reaction: Venus with PTS1 (wildtype – obtained from other subteam)</li> <li>• Heat shock transformation -&gt; no colonies on next day</li> </ul>
	2017-08-01	<ul style="list-style-type: none"> <li>• Miniprep of inoculated cultures</li> <li>• Test digestion of purified plasmids</li> <li>• Golden Gate reaction: <ul style="list-style-type: none"> <li>◦ R8, R15 with appropriate promotor, terminator</li> <li>◦ PTS1* variants with mVenus and promotor/terminator</li> <li>◦ Venus with PTS1 repetition</li> </ul> </li> <li>• Sequencing of level 0 constructs</li> </ul>
	2017-08-02	<ul style="list-style-type: none"> <li>• Heat shock transformation with golden gate products</li> </ul>
	2017-08-03	<ul style="list-style-type: none"> <li>• Inoculation</li> </ul>
	2017-08-04	<ul style="list-style-type: none"> <li>• Miniprep and test digestion</li> </ul>

Week	Date	Jobs
32	2017-08-07	<ul style="list-style-type: none"> <li>Digestion of Spectinomycin/Kanamycin level0 plasmids and subsequent gel purification to increase golden gate efficiency</li> </ul>
	2017-08-08	<ul style="list-style-type: none"> <li>Golden Gate reaction: GFP dropout for level 1</li> <li>Heat shock transformation</li> </ul>
	2017-08-09	<ul style="list-style-type: none"> <li>Inoculation of GFP dropout transformants</li> </ul>
	2017-08-10	<ul style="list-style-type: none"> <li>Minprep and test digestion</li> </ul>
	2017-08-11	

Week	Date	Jobs
33	2017-08-15	<ul style="list-style-type: none"> <li>• Transformation lvl2 DropOut (Leucine)</li> <li>• Miniprep receptor 8 and 15 and Venus+PTS-variants</li> <li>• Test digest receptor 8/15, peptide 1/2/3 and Venus+PTS-variants</li> </ul>
	2017-08-16	<ul style="list-style-type: none"> <li>• Miniprep Pex11 and Part E1</li> <li>• Test digest receptor 15 and Venus+wild-type PTS</li> <li>• Inoculate parts G10, G2, lvl1 and lvl2 leucine Dropout</li> <li>• Transformation of lvl1 leucine Dropout</li> <li>• Preparation of competent DH5alpha cells</li> <li>• Golden Gate lvl1 Dropouts and Venus+wild-type PTS1</li> </ul>
	2017-08-17	<ul style="list-style-type: none"> <li>• Miniprep and test digest parts G2, G10, lvl1 leucine Dropout</li> <li>• Test digest lvl1 P1, P2, P3, receptor 8 and receptor 15</li> <li>• Golden Gate lvl2 receptor + peptide combinations, lvl1 Pex11+mRuby</li> <li>• Inoculation of lvl1 uracil Dropout and Venus+wild-type PTS1</li> </ul>
	2017-08-18	<ul style="list-style-type: none"> <li>• Inoculation receptor8+P1-3, receptor15+P1-3, lvl1 uracil Dropout, Venus+wild-type PTS1, part D4 and lvl2 uracil and leucine Dropouts</li> <li>• Transformation of Pex11_mRuby Golden Gate product</li> <li>• Preparation of competent yeast cells</li> </ul>

Week	Date	Jobs
34	2017-08-21	<ul style="list-style-type: none"> <li>• Miniprep and test digest of the Dropouts</li> </ul>
	2017-08-22	<ul style="list-style-type: none"> <li>• Golden Gate lvl 1 receptor8/15, P1-3 and lvl2 uracil Dropouts</li> <li>• Miniprep and test digest of Pex11_mRuby and part D4 retransformation</li> </ul>
	2017-08-23	<ul style="list-style-type: none"> <li>• Miniprep and test digest of Venus+wild-type PTS1 and lvl1 Dropouts</li> <li>• Preparation of competent yeast cells</li> <li>• Golden Gate</li> <li>• Transformation of Golden Gate products from 2017-08-22</li> </ul>
	2017-08-24	<ul style="list-style-type: none"> <li>• Golden Gate Dropout</li> <li>• Test digest lvl2 uracil Dropout</li> <li>• Transformation of lvl2 Dropout</li> <li>• Inoculation of receptor8/15 and peptide1-3 (2017-08-22)</li> <li>• Golden Gate cytosolic fluorescence protein with lvl1 uracil Dropout</li> <li>• Pre-Digest of lvl1 receptor8/15 and peptide1-3</li> </ul>
	2017-08-25	<ul style="list-style-type: none"> <li>• Gel clean-up of Pre-Digest 24-08-2017</li> <li>• Preparation of Pex5-knockout yeast cells</li> <li>• Golden Gate lvl 1 receptor8/15, P1-3 and lvl2 uracil Dropouts</li> <li>• Miniprep and test digest of receptor8/15 and peptide1-3 (2017-08-22)</li> </ul>

Week	Date	Jobs
35	2017-08-28	<ul style="list-style-type: none"> <li>• Golden Gate of pre-digested lvl1 receptor8/15, lvl1 peptide1-3 and undigested lvl2 uracil Dropouts</li> <li>• Transformation of lvl1 constructs</li> </ul>
	2017-08-29	<ul style="list-style-type: none"> <li>• Colony PCR and test digest receptor8/15, wild-type PTS1 and peptide1-3 and inoculation afterwards</li> <li>• Transformation of lvl2 plasmids</li> </ul>
	2017-08-30	<ul style="list-style-type: none"> <li>• Golden Gate lvl1 Pex13 with mRuby</li> <li>• Miniprep and test digest of receptor8/15, wild-type PTS1 and peptide1-3 (2017-08-29)</li> <li>• Colony-PCR of R8/15+ peptide1-3</li> </ul>
	2017-08-31	<ul style="list-style-type: none"> <li>• Run Colony-PCR from 2017-08-30 on gel and inoculation of positive samples</li> <li>• Transformation Golden Gate lvl1 Pex13 with mRuby</li> <li>• Test digest Pex11 with mRuby</li> <li>• Repeat of Golden Gate lvl2 constructs followed by transformation</li> <li>• Yeast Transformation Pex11 with mRuby</li> </ul>
	2017-09-01	<ul style="list-style-type: none"> <li>• Inoculation, miniprep and test digest of lvl2 constructs</li> </ul>

Week	Date	Jobs
36	2017-09-04	<ul style="list-style-type: none"> <li>• Inoculation of transformed yeast from KW 35 and lvl2 constructs</li> <li>• Colony PCR of Pex5-transformants and test digest of part K3/FPs</li> </ul>
	2017-09-05	<ul style="list-style-type: none"> <li>• Yeast transformation of Pex11_mRuby with wild-type and Pex5-Ko</li> <li>• Golden Gate of mTurquoise+receptor8/15 and peptide1-3+part 22 (weak promotor)</li> <li>• Miniprep of lvl2 constructs (2017-09-04)</li> </ul>
	2017-09-06	<ul style="list-style-type: none"> <li>• Test digest of lvl1 Pex13_mRuby</li> <li>• Transformation of lvl2 constructs</li> <li>• Yeast transformation of lvl1 uracil Pex13+mRuby</li> </ul>
	2017-09-07	<ul style="list-style-type: none"> <li>• Inoculation of transformations from 2017-09-06</li> <li>• Extraction of yeast-genome</li> <li>• Repeat of Golden Gate lvl2 constructs and inoculation afterwards</li> </ul>

Week	Date	Jobs
37	2017-09-11	<ul style="list-style-type: none"> <li>• Miniprep and test digest of lvl2 constructs from 2017-09-11</li> <li>• Golden Gate of lvl2 3-part cassette and transformation afterwards</li> <li>• PCR extraction of wild-type Pex5-gene from yeast genome</li> <li>• Golden Gate peptide1-3 with part 22 (weak Promotor)</li> <li>• Inoculation of receptor8/15+mTurquoise and receptor15+peptide3</li> </ul>
	2017-09-12	<ul style="list-style-type: none"> <li>• Miniprep and test digest lvl1 uracil Pex13_mRuby</li> <li>• Repeat of PCR extraction of Pex5-gene from yeast genome</li> <li>• Yeast transformation of receptor8/15+mTurquoise and receptor15+peptide3 and K3-Drop</li> <li>• Golden Gate reaction lvl1 constructs wild-type PTS, peptide1-3 and cytosolic mTurquoise</li> </ul>
	2017-09-13	<ul style="list-style-type: none"> <li>• Golden Gate lvl1 VioA, VioB, VioE, Pex13 (leucine) and lvl2 3-part cassette</li> <li>• Colony PCR of lvl1 peptides built with part 24 and Inoculation of positive colonies</li> <li>• Inoculation of wild-type and Pex5-Ko yeasts</li> </ul>
	2017-09-14	<ul style="list-style-type: none"> <li>• Colony PCR of VioA, VioB, VioE, peptides and part K3</li> <li>• Golden Gate lvl1 VioE with part 17 and transformation afterwards</li> <li>• Inoculation of yeasts for microscopy</li> <li>• Repeat of Golden Gate VioB from 2017-09-13</li> </ul>
	2017-09-15	<ul style="list-style-type: none"> <li>• Transformation of VioB and lvl1 constructs with part24</li> <li>• Colony PCR of VioE and VioE+PTS</li> <li>• Miniprep of VioA, K3, part11 and peptides with part 22</li> <li>• Colony PCR of VioE/VioE*</li> <li>• Retransformation of VioE/VioE*</li> </ul>



		<ul style="list-style-type: none"><li>• Golden Gate lvl2 receptors + peptides with part22</li></ul>
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38	2017-09-18	<ul style="list-style-type: none"> <li>• Colony PCR VioB/E/E*</li> <li>• Transformation of Golden Gate products from 2017-09-15 and transformation</li> <li>• Golden Gate lvl2 receptors + peptides with part22, VioB/E/E*, receptor8/15+ His-tags</li> <li>• Microscopy of transformed yeasts</li> </ul>
	2017-09-19	<ul style="list-style-type: none"> <li>• Miniprep of lvl1 VioB/E/E* and lvl2receptor15+mturquoise</li> <li>• Colony PCR from transformed Golden Gate products (2017-09-18)</li> <li>• Yeast transformation with lvl1 (uracil) Pex13_mRuby</li> <li>• Golden Gate lvl1 leucine Pex11/13+mRuby and lvl2 VioAB cassette followed by transformation</li> </ul>
	2017-09-20	<ul style="list-style-type: none"> <li>• Miniprep of lvl2 constructs</li> <li>• Yeast transformation of receptor8/15+peptide1-3/wild-type PTS</li> <li>• Colony PCR of K3, VioAB, VioB, VioE/E*, Pex11/13, receptor8/15+His-tag followed by inoculation</li> <li>• Test digest of K3</li> </ul>
	2017-09-21	<ul style="list-style-type: none"> <li>• Miniprep of inoculated samples from 2017-09-20</li> <li>• Yeast transformation of lvl2 constructs (part24)</li> <li>• Golden Gate lvl1 receptor8/15+His-tag</li> <li>• yeast transformation of VioAB, Pex13_mRuby (leucine) in wild-type/Pex5-Ko yeast strain</li> </ul>
	2017-09-22	<ul style="list-style-type: none"> <li>• Miniprep of VioE</li> <li>• Transformation of Golden Gate product from 2017-09-21</li> <li>• Retransformation of receptor8/15</li> <li>• Repeat of Golden Gate VioE/E* from 2017-09-18</li> <li>• yeast co-transformation of VioAB+VioE in wild-type/Pex5-Ko yeast strain</li> <li>• Transformation Golden Gate product VioE/E*</li> </ul>

Week	Date	Jobs
39	2017-09-25	<ul style="list-style-type: none"> <li>• Colony PCRs</li> <li>• Golden Gate VioA</li> <li>• Transformation of golden gate product</li> </ul>
	2017-09-26	<ul style="list-style-type: none"> <li>• Miniprep VioE-PTS</li> <li>• Golden Gate VioE</li> <li>• Transformation VioE</li> <li>• Microscopy PEX13-mRuby</li> <li>• Inoculation VioA</li> </ul>
	2017-09-27	<ul style="list-style-type: none"> <li>• Miniprep VioA</li> <li>• Test digestion</li> <li>• Inoculation VioE</li> </ul>
	2017-09-28	<ul style="list-style-type: none"> <li>• Miniprep &amp; test digestion of VioE → negative</li> <li>• Repetition of VioE</li> <li>• Golden gate Vio A and VioB level2 construct Transformation</li> </ul>
	2017-09-29	<ul style="list-style-type: none"> <li>• Colony PCR</li> <li>• Inoculation</li> <li>• Transformation VioE</li> </ul>

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40	2017-10-02	<ul style="list-style-type: none"> <li>• Synthesized genes arrived in Duesseldorf on Friday, the 29<sup>th</sup> of September <ul style="list-style-type: none"> <li>◦ Golden Gate reactions for level 0 were done there and plasmids arrived in Cologne on Monday, the 2<sup>nd</sup> of October (PTS variant, WT PEX5, R19, nPTS)</li> <li>◦ Level 1 for the new receptor variant R19 and WT receptors were also done there</li> </ul> </li> <li>• Test digest of level 1 plasmids <ul style="list-style-type: none"> <li>◦ Positive for R19 constructs, negative for WT</li> </ul> </li> <li>• Golden Gate reactions for PTS variant P*, WT Pex5 and nPTS (level 1)</li> <li>• Transformation of golden gate products</li> </ul>
	2017-10-03	<i>Lab closed due to German holiday</i>
	2017-10-04	<ul style="list-style-type: none"> <li>• Repetition of VioE golden gate reaction due to previous failures and later transformation</li> <li>• Golden Gate reactions (lvl2) for R19 and FP-PTS variants</li> <li>• Microscopy of co-transformed yeasts with PEX13, mTurquoise+PTS and PEX5 variant</li> <li>• Inoculation level 1 constructs</li> </ul>
	2017-10-05	<i>Out of lab due to SynBio day</i>
	2017-10-06	<ul style="list-style-type: none"> <li>• Miniprep of level 1 constructs</li> <li>• Colony PCR of VioE</li> <li>• Testdigest level 1 constructs</li> <li>• PCR to remove stop codon from R8, R15 and WT PEX5</li> <li>• Gel purification of PCR product</li> <li>• Golden gate reaction for level0 plasmids with purified PCR product</li> </ul>

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41	2017-10-09	<ul style="list-style-type: none"> <li>• Miniprep of VioE cultures, that were inoculated on the 6<sup>th</sup> of October (they were put in the fridge on the 7<sup>th</sup> of October)</li> <li>• Transformation of R8, R15, WT level 0 plasmids without stop codon</li> <li>• PCR for basic Biobricks with Q5-Polymerase <ul style="list-style-type: none"> <li>◦ R8, R15</li> <li>◦ Pex13</li> <li>◦ PTS variants P1, P2, P3, P*</li> </ul> </li> <li>• Sequencing of level 1 plasmids (nPTS, P* and WT PEX5) → positive</li> <li>• Golden Gate reactions with PEX5 and mTurquoise-PTS variants (R8, R15 and R19)</li> <li>• Later Transformation</li> <li>• Test Digest of VioE → negative</li> <li>• Repetition Golden Gate reaction for VioE with predigested backbone</li> <li>• Digestion of PCR product with EcoRI, PstI and DpnI for biobricks and later Ligation with backbone</li> <li>• Transformation of biobricks and VioE</li> <li>• Yeast co-transformation of R19 with P* and nPTS</li> </ul>
	2017-10-10	<ul style="list-style-type: none"> <li>• Inoculation of VioE and biobricks</li> <li>• Repetition of PCR for Biobricks due to missing colonies <ul style="list-style-type: none"> <li>◦ R8, R19, P1 and P3</li> </ul> </li> <li>• Gel purification of PCR products</li> <li>• Digestion with EcoRI and PstI</li> <li>• Golden gate (level 2) reactions with WT receptor and mTurquoise-PTS variants and VioAB</li> <li>• Transformation of Golden gate products</li> <li>• PCR to remove stop codon in R19 for later tagging with his-flag tag and later golden gate reaction for level 0</li> <li>• Colony PCR of level 2 constructs and inoculation of positive ones</li> </ul>
	2017-10-11	<ul style="list-style-type: none"> <li>• Miniprep of inoculated cultures</li> <li>• Transformation of R19* (w/o stop codon) and level 2 constructs</li> <li>• Test digestion of VioE and Biobricks <ul style="list-style-type: none"> <li>◦ everything turned out to be negative</li> </ul> </li> <li>• Colony PCR VioAB and level 2 wildtype constructs and inoculation of positive ones</li> </ul>
	2017-10-12	<ul style="list-style-type: none"> <li>• Inoculation of co transformed yeast containing R19 and P* or nPTS for microscopy</li> </ul>

		<ul style="list-style-type: none"> <li>• Sequencing of VioE → turned out to be negative</li> <li>• Colony PCR of level 2 constructs → inoculation of positive ones</li> <li>• PCR to attach random PTS variants to VioE</li> <li>• Later Purification of PCR product and golden gate reaction with terminator (part 54)</li> <li>• Miniprep of inoculated cultures</li> <li>• Test digestion of those</li> <li>• Transformation of VioE-PTS constructs</li> <li>• Yeast co-transformation R19 with wildtype PTS</li> </ul>
	2017-10-13	<ul style="list-style-type: none"> <li>• Repetition of VioE PCR to attach PTS due to the use of a wrong plasmid the day before <ul style="list-style-type: none"> <li>◦ PCR was unsuccessful</li> </ul> </li> <li>• Miniprep and test digestion</li> <li>• Transformation of biobrick backbone with RFP from distribution</li> <li>• Yeast co-transformation of WT receptor with P*</li> <li>• Digestion of an other team members Biobrick to purify backbone for further ligations with EcoRI and PstI</li> <li>• Yeast co-transformation with VioABR19 construct that we got from another subteam from Dusseldorf and VioE-PTS</li> </ul>

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42	2017-10-16	<ul style="list-style-type: none"> <li>• Repetition: Ligation of our parts with biobrick backbone and later transformation</li> <li>• Microscopy of R19-wt-PTS and WTPEX5-P* yeasts → both showed no import</li> <li>• Repetition of random PTS-attachment to VioE via PCR</li> </ul>
	2017-10-17	<ul style="list-style-type: none"> <li>• Gel purification of PCR products</li> <li>• Golden gate reaction with purified PCR product and terminator (part 54)</li> <li>• Digestion of level 2 cassette (Uracil auxotrophy) with BsmBI and gel purification</li> <li>• Inoculation of transformed biobricks</li> </ul>
	2017-10-18	<ul style="list-style-type: none"> <li>• Repetition of VioE golden gate reaction</li> <li>• Miniprep of biobrick cultures</li> <li>• Transformation of VioE-PTS constructs – plasmid existed already</li> <li>• Repetition of Transformation with RFP-Biobrick</li> </ul>
	2017-10-19	<ul style="list-style-type: none"> <li>• Miniprep of VioE-PTS</li> <li>• PCR for basic biobricks: P2, Pex13* and WT-PTS → negative</li> <li>• Test digestion of biobricks → negative</li> <li>• Transformation VioE product from previous day</li> <li>• Sequencing of the two VioE-PTS plasmids → negative</li> <li>• Digestion of level 1 plasmids for composite biobricks <ul style="list-style-type: none"> <li>◦ Pex13-mRuby, R8, R15 and R19</li> </ul> </li> <li>• Repetition of PCR for basic biobricks: <ul style="list-style-type: none"> <li>◦ P1, P2, P3, P*, wtPTS</li> <li>◦ R8, R15, R19</li> <li>◦ Pex13*</li> </ul> </li> <li>• Digestion of PCR products with DpnI, EcoRI and PstI</li> <li>• PCR cleanup</li> <li>• Ligation with biobrick backbone and transformation</li> </ul>
	2017-10-20	<ul style="list-style-type: none"> <li>• Inoculation of Biobricks (somebody put them in the freezer on Saturday, the 21<sup>st</sup> of October)</li> <li>• Yeast co-transformation of several PEX5 – mTurquoise-PTS combinations</li> <li>• Golden Gate reaction of R19* with His-Flag tag and later transformations</li> </ul>

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43	2017-10-23	<ul style="list-style-type: none"> <li>• Miniprep and test digestion of biobricks → all negative</li> <li>• Repetition: Ligation of purified inserts with newly purified backbone</li> <li>• Heat shock transformation of biobrick plasmids</li> <li>• Mini prep and test digestion of PEX5 variant R19 with His/Flag tag for western blot → positive</li> <li>• Yeast transformation with R19 level 1 plasmid for western blot</li> <li>• Inoculation of yeast for microscopy</li> </ul>
	2017-10-24	<ul style="list-style-type: none"> <li>• Yeast co-transformation of various level 2 constructs with the PEX13 mRuby marker</li> <li>• Golden gate reaction to integrate the receptor R19 into the genome of the PEX5 knock out strain and later heat shock transformation</li> <li>• Inoculation of biobrick plasmids</li> </ul>
	2017-10-25	<ul style="list-style-type: none"> <li>• Inoculation of colonies from the previous day</li> <li>• Miniprep of biobrick plasmids and test digest</li> <li>• Sequencing of purified plasmids</li> <li>• Yeast co-transformation of lvl2 constructs (R19 PTS1* P*, R19 PTS1)</li> </ul>
	2017-10-26	<ul style="list-style-type: none"> <li>• Miniprep and test digest of inoculated cultures → positive</li> </ul>
	2017-10-27	<ul style="list-style-type: none"> <li>• Yeast transformation with plasmid for genome integration</li> <li>• Inoculation of yeast colonies for microscopy</li> </ul>



Week	Date	Jobs
44	2017-10-30	<ul style="list-style-type: none"> <li>• Microscopy of yeast cultures with several constructs</li> </ul>
	2017-10-31	Regional holiday
	2017-11-01	Regional holiday
	2017-11-02	<ul style="list-style-type: none"> <li>•</li> </ul>
	2017-11-03	<ul style="list-style-type: none"> <li>•</li> </ul>