

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>

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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	Transformation lv12 DropOut (Leucine) Miniprep receptor 8 and 15 and Venus+PTS-variants Test digest receptor 8/15, peptide 1/2/3 and Venus+PTS-variants  ##
	2017-08-16	Miniprep Pex11 and Part E1 Test digest receptor 15 and Venus+wild-type PTS Inoculate parts G10, G2, lv1 and lv2 leucine Dropout, ## Transformation of lv1 leucine Dropout Preperation of competent DH5alpha cells Golden Gate lv1 Dropouts and Venus+wild-type PTS1
	2017-08-17	Miniprep and test digest parts G2, G10, lv1 leucine Dropout, ## Test digest lv1 P1, P2, P3, receptor 8 and receptor 15 Golden Gate lv2 receptor + peptide combinations, lv1 Pex11+mRuby Inoculation of lv1 uracil Dropout and Venus+wild-type PTS1 #
	2017-08-18	Inoculation receptor8+P1-3, receptor15+P1-3, lv1 uracil Dropout, Venus+wild-type PTS1, part D4 and lv2 uracil and leucine Dropouts Transformation of Pex11_mRuby Golden Gate product Peperation of competent yeast cells

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34	2017-08-21	Miniprep and test digest of the Dropouts
	2017-08-22	Golden Gate lvl 1 receptor8/15, P1-3 and lvl2 uracil Dropouts Miniprep and test digest of Pex11_mRuby and part D4 retransformation
	2017-08-23	Miniprep and test digest of Venus+wild-type PTS1 and lvl1 Dropouts Preperation of competent yeast cells ##Golden Gate Transformation of Golden Gate products from 2017-08-22
	2017-08-24	##Golden Gate Dropout Test digest lvl2 uracil Dropout Transformation of lvl2 Dropout Inoculation of receptor8/15 and peptide1-3 (2017-08-22) Golden Gate cytosolic flourescence protein with lvl1 uracil Dropout Pre-Digest of lvl1 receptor8/15 and peptide1-3
	2017-08-25	Gel clean-up of Pre-Digest 24-08-2017 Preperation of Pex5-knockout yeast cells Golden Gate lvl 1 receptor8/15, P1-3 and lvl2 uracil Dropouts Miniprep and test digest of receptor8/15 and peptide1-3 (2017-08-22)

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35	2017-08-28	Golden Gate of pre-digested lv1 receptor8/15, lv1 peptide1-3 and undigested lv2 uracil Dropouts Transformation of lv1 constructs
	2017-08-29	Colony PCR and test digest receptor8/15, wild-type PTS1 and peptide1-3 and inoculation afterwards Transformation of lv2 plasmids
	2017-08-30	Golden Gate lv1 Pex13 with mRuby Miniprep and test digest of receptor8/15, wild-type PTS1 and peptide1-3 (2017-08-29) Colony-PCR of R8/15+ peptide1-3
	2017-08-31	Run Colony-PCR from 2017-08-30 on gel and inoculation of positive samples Transformation Golden Gate lv1 Pex13 with mRuby Test digest Pex11 with mRuby Repeat of Golden Gate lv2 constructs followed by transformation Yeast Transformation Pex11 with mRuby
	2017-09-01	Inoculation, miniprep and test digest of lv2 constructs

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36	2017-09-04	Inoculation of tranformed yeast from KW 35 and lv2 constructs

		Colony PCR of Pex5-transformants and test digest of part K3/FPs
	2017-09-05	Yeast transformation of Pex11_mRuby with wild-type and Pex5-Ko Golden Gate of mTurquoise+receptor8/15 and peptide1-3+part 22 (weak promotor) Miniprep of lv2 constructs (2017-09-04)
	2017-09-06	Test digest of lv1 Pex13_mRuby Transformation of lv2 constructs ## Yeast transformation of lv1 uracil Pex13+mRuby
	2017-09-07	Inoculation of transformations from 2017-09-06 Extraction of yeast-genome Repeat of Golden Gate lv2 constructs and inoculation afterwards

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37	2017-09-11	<p>Miniprep and test digest of lv2 constructs from 2017-09-11</p> <p>Golden Gate of lv2 3-part cassette and transformation afterwards</p> <p>PCR extraction of wild-type Pex5-gene from yeast genome</p> <p>##</p> <p>Golden Gate peptide1-3 with part 22 (weak Promotor)</p> <p>Inoculation of receptor8/15+mTurquoise and receptor15+peptide3</p>
	2017-09-12	<p>Miniprep and test digest lv1 uracil Pex13_mRuby</p> <p>Repeat of PCR extraction of Pex5-gene from yeast genome</p> <p>Yeast transformation of receptor8/15+mTurquoise and receptor15+peptide3 and ###K3-Drop</p> <p>Golden Gate reaction lv1 constructs wild-type PTS, peptide1-3 and cytosolic mTurquoise</p>
	2017-09-13	<p>Golden Gate lv1 VioA, VioB, VioE, Pex13 (leucine) and lv2 3-part cassette</p> <p>Colony PCR of lv1 peptides built with part 24 and</p> <p>Inoculation of positive colonies</p> <p>Inoculation of wild-type and Pex5-Ko yeasts</p>
	2017-09-14	<p>Colony PCR of VioA,VioB,VioE,peptides and part K3</p> <p>Golden Gate lv1 VioE with part 17 and transformation afterwards</p> <p>Inoculation of yeasts for microscopy</p> <p>Repeat of Golden Gate VioB from 2017-09-13</p>



	2017-09-15	Transformation of VioB and lvl1 constructs with part24 Colony PCR of VioE and VioE+PTS Miniprep of VioA, K3, part11 and peptides with part 22 Colony PCR of VioE/VioE* Retransformation of VioE/VioE* Golden Gate lvl2 receptors + peptides with part22
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38	2017-09-18	Colony PCR VioB/E/E* Transformation of Golden Gate products from 2017-09-15 and transformation Golden Gate lvl2 receptors + peptides with part22, VioB/E/E*, receptor8/15+ His-tags Microscopy of transformed yeasts
	2017-09-19	Miniprep of lvl1 VioB/E/E* and lvl2receptor15+mturquoise Colony PCR from transformed Golden Gate products (2017-09-18) Yeast transformation with lvl1 (uracil) Pex13_mRuby Golden Gate lvl1 leucine Pex11/13+mRuby and lvl2 VioAB cassette followed by transformation
	2017-09-20	Miniprep of lvl2 constructs Yeast transformation of receptor8/15+peptide1-3/wild-type PTS Colony PCR of K3, VioAB, VioB, VioE/E*, Pex11/13, receptor8/15+His-tag followed by inoculation Test digest of K3
	2017-09-21	Miniprep of inoculated samples from 2017-09-20 Yeast transformation of lvl2 constructs (part24) Golden Gate lvl1 receptor8/15+His-tag yeast transformation of VioAB, Pex13_mRuby (leucine) in wild-type/Pex5-Ko yeast strain
	2017-09-22	Miniprep of VioE Transformation of Golden Gate product from 2017-09-21 Retransformation of receptor8/15 Repeat of Golden Gate VioE/E* from 2017-09-18 yeast co-transformation of VioAB+VioE in wild-type/Pex5-Ko yeast strain Transformation Golden Gate product VioE/E*

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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>

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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>