

Team Violacein

	shortcut
heat-shock transformation	HST
heat-shock retransformation	HSRT
Dropout	do
Dephosphorylation	dephos.
level	lvl
GoldenGate cloning	GG
Transformation	trafo
combination	#
BioBrick	BB
Overnight culture	ONC

Week	Date	Jobs
32	8/7/2017	heat-shock Retransformation (HSRT) from VioABCE (BBaK274004)
	8/8/2017	repetition of HSRT VioABCE predigest part pYTK083, Gel Clean-Up GoldenGate GFPdropouts for level1
	8/9/2017	HST GFPdo
	8/10/2017	overnight culture (ONC) GFPdo GoldenGate GFPdo for level2 and HST
	8/11/2017	MiniPreps GFPdo for level repetition of HSRT VioABCE test digest GFPdo level1
33	8/14/2017	test digest GFPdo level1 ONC from GFPdo level2 and VioABCE ColonyPCR VioABCE
	8/15/2017	MiniPreps GFPdo for level2 test digest GFPdo level 2
	8/18/2017	predigest + dephosphorylation GFPdo for level1 with Bsa1, Gel Clean-up
34	8/21/2017	repetition predigest and dephos. GFPdo for level1 with Bsa1, Gel Clean-up, HSRT GFPdo for level1
	8/22/2017	HSRT BBaK274004, ONC from GFPdo lvl1 HSRT
	8/23/2017	ColonyPCR VioABCE MiniPrep GFPdo lvl1 HSRT

34	8/24/2017	repetition predigest + dephos. GFPdo for lvl1 for VioA+ VioE, Gel Clean-up ONC from VioABCE (BBa_K274004)
	8/25/2017	MiniPrep VioABCE repetition predigest + dephos. GFPdo VioA, Gel Clean-Up Q5 PCR for VioA, B, C, E, Digest with Dph1, PCR purification
35	8/28/2017	repetition Q5 PCR VioC GoldenGate VioA, VioB, VioE for level0 and level1 (first try), HST GG1.1 test digest VioABCE
	8/29/2017	repetition Q5 PCR VioC with temperature gradient and touchdown HST GG level0 GG level1.2, ONC from GG1.1
	8/30/2017	MiniPreps from GG1.1, test digest GG1.1 -> VioE lvl1 is correct ONC from GG level0 HST GG 1.2
	8/31/2017	MiniPreps and ColonyPCR GG level0, 2nd test digest VioE lvl1 overnight culture from GG 1.2 HSRT GFPdo level 2
	9/1/2017	Test digest GG level0 -> VioA, VioB, VioE are correct MiniPrep GG1.2, test digest
36	9/4/2017	GG 1.3 VioA, VioA pts1, VioB, VioB pts1, VioE pts1 overnight culture from HSRT GFPdo level 2 predigest + dephos. GFPdo VioB, VioC and GFPdo lvl2 Q5 PCR VioC with new reverse Primer GG lvl 0 VioC VioA, VioB and VioE level0 sent for Sequencing -> correct
	9/5/2017	HST GG 1.3 MiniPrep GFPdo lvl2 Gel Clean-Up GFPdo VioB, VioC and GFPdo lvl2 two repeats of VioC Q5 PCR with both old and new reverse Primer with different temperature gradients

36	9/6/2017	Gel analysis Colony PCR ONC level1
	9/7/2017	predigest + Gel Clean-Up of VioABCE then Q5 PCR with temperature gradient, ordered VioC Synthese (Genescript), picked more colonies from GG1.2
	9/8/2017	MiniPrep GG1.2 + test digest GG 1.4 for VioA, VioB and VioA,B,E with PTS1, HST yeast transformation from VioE
37	9/11/2017	yeast ColonyPCR GFPdo lvl2 predigest + dephos. ONC from yeast VioE + <i>E. coli</i> GG 1.4
	9/12/2017	MiniPrep GG 1.4 + test digest > VioB pts ONC from yeast WT and <i>E. coli</i> VioA, VioB Gel Clean-Up GFPdo lvl2 Media and Gel preparation for WesternBlots 1.0
	9/13/2017	MiniPrep GG 1.4 + test digest > VioA pts WesternBlot 1.1: protein extraction, gel run, Membrane blocking and 1. AB overnight
	9/14/2017	testdigest of frequently used Toolbox parts and VioA pts yeast trafo VioA pts, VioB pts WesternBlot 1.2: 2.AB, membrane photo, ponceau staining
	9/15/2017	GG 1.5 VioE pts, VioB + HST yeast trafo VioA
38	9/18/2017	HSRT all level0 plasmids for glycerol stock ONC from yeast VioA, A pts, B pts, E and WT
	9/19/2017	ONC from HSRT and again yeast colonies for VioB pts and VioE WesternBlot 2.1 for VioA, VioA pts 2. AB overnight
	9/20/2017	prepared Glycerol stocks WesternBlot 2.2 and Ponceau staining ColonyPCR yeast VioA
	9/21/2017	WesternBlot 3.0 VioB pts, VioE and VioA yeast trafo VioB, test digest VioE pts
	9/22/2017	GG 1.7 VioE pts yeast cell harvest for WB

39	9/25/2017	HST GG 1.7 ONC for glycerol stocks and yeast VioB
	9/26/2017	ColonyPCR and overnight culture VioE test digest VioE_pts (GG1.6) VioA sent for Sequencing > neg.
	9/27/2017	MiniPrep VioE, test digest > VioE_pts correct yeast trafo VioE_pts WesternBlot 4.1: VioE, VioB sent for Sequencing: VioA> wrong VioB> correct
	9/28/2017	WesternBlot 4.2 yeast trafo VioA HST VioA GG level 2
	9/29/2017	HST GG 2.0
40	10/2/2017	picked yeast colonies from WT, VioB, VioB pts yeast trafo VioEpts
	10/3/2017	GG 1.8 VioA, VioC, VioC pts VioC lvl0 sent for sequencing ColonyPCR lvl 2.0 HST GG 1.8
	10/4/2017	GG 1.9 VioA, VioC, VioC pts WesternBlot 5.1: VioB pts, VioB, WT overnight culture GG 1.8
	10/5/2017	Syn bio day
	10/6/2017	MiniPrep GG 1.8 + test digest, WesternBlot 5.2 HST GG1.9 repetition yeast trafo VioE pts
41	10/9/2017	ONC from GG 1.9 test digest VioC, VioC pts, VioA BioBrick: predigest VioE lvl1 and BB Backbone, gel Clean-Up, ligation of VioE and BB repetition GG 2.0
	10/10/2017	picked new colonies from GG 1.9 HST BB VioE MiniPrep 1.9 + test digest > VioA correct meeting with mass spectrometry experts, MS try out GG 2.1: # 1, 3, 4, 7 and VioE_pts, VioC, VioC pts

41	10/11/2017	HST GG 2.1 MiniPrep GG 1.9 + test digest (twice) yeast trafo VioA ONC from BB VioE
	10/12/2017	VioA lvl1 sent for Sequencing ONC from GG 2.1 VioC, VioC_pts, level2 MiniPrep BB VioE+ test digest
	10/13/2017	MiniPrep GG 2.1 + test digest, yeast trafo VioE pts Overnight culture of VioA, VioB, VioB pts and WT Predigest of GFPdo for VioC and lvl2 + Gel Clean-Up
	10/14/2017	GG 2.2 #1, #2 HSRT VioB lvl1
	10/15/2017	HSRT VioB pts, VioE pts, VioB, VioA pts, VioC lvl0, VioA HST GG 2.2 Harvest of ONC VioA, VioB, VioB pts and WT
42	10/16/2017	BioBrick PCR of basic parts (VioE pts, VioA pts, VioB pts) + digest Overnight culture <i>E. coli</i> GG 2.2 and yeast VioB, VioB pts, VioA pts Sent for sequencing: VioE pts, VioB pts ColonyPCR VioE pts, all colonies WesternBlot 6: WT, VioB, VioB pts, VioA
	10/17/2017	Harvest of yeast VioB, VioB pts, VioA pts MiniPrep GG 2.2 + test digest GG 2.3 #3, #4, #7
	10/18/2017	HST GG 2.3, VioE pts Repetition BioBrick PCR of VioB pts GG 2.4 #1, VioC, VioC pts Predigest GFPdo VioC and lvl2
	10/19/2017	Gel Clean-Up GFPdo VioC and lvl2 PCR Clean-Up BioBrick VioA pts, VioB pts Test digest of VioC HST GG 2.4 Sent for Sequencing: BioBrick part VioE, VioC lvl0_2 WesternBlot 7: WT, VioA pts, VioB, VioB pts ColonyPCR lvl2 #4, #7 and overnight culture VioE pts, #4, #7, #1 ONC VioE pts, #4, #7, #1 new colonies GG 2.5 lvl2 #1, #3, VioC, VioC pts

42	10/20/2017	HST 2.5: VioC, VioC pts, #1, #3, BioBrick VioA pts, VioB pts MiniPrep VioE pts, #4, #7, #1 + test digest Yeast triple trafo #1 and #7
	10/21/2017	ONC GG 2.5 MiniPrep
	10/22/2017	Test digest Harvest of ONC GG 2.5
43	10/23/2017	Miniprep biobricks, VioC, VioC_pts, lv2; testdigest; sent for sequencing VioE biobrick, VioC, VioC_pts; colonyPCR triple trafo
	10/24/2017	Digestion of Level1 plasmids with biobrick enzymes + gel purification; biobrick PCR VioE_pts; ligation in biobrick backbone; GG 2.6 #1 #7 #3 #4 #5 #8 #10 #13; picked yeast colonies for <i>in vitro</i> assay
	10/25/2017	harvest yeast ONC , planning in vitro assay and sample preparation; biobrick hst
	10/26/2017	in vitro assay prodeoxyviolacein; picked biobrick colonies
	10/27/2017	miniprep biobricks + testdigest + sent to HQ and sent for sequencing
	10/28/2017	mass spectrometry analysis in vitro assay samples Prodeoxyviolacein