1	Group	Init
2	Command	"O2SSO5,0" wait
3	Set Variable	SealNumber "By how many seal layers should the plates be closed? (0 to 2)" "By how many seal layers should the plates be closed? (0 to 2)", 0 - 2
4	Set Variable	SamplingTime "How frequent should be sampled (Every 15 to 240 min)?" "How frequent should be sampled (Every 15 to 240 min)?", 15 - 240
5	Set Variable	SamplingRepetitions = 4
6	Set Variable	SamplingEvents = 96/SamplingRepetitions
7	User Prompt	"Turn on the heater of the shaking incubator and set the desired incubation temperature manually! Sampling in four repetitions; 300 rpm for shaking" sound : no
8	Set Variable	PauseNachSchütteln = 10
9	Set Variable	CurrentPlatePos = 4
10	Set Variable	InactivationTime = 5
11	Set Variable	InactivationVolume = 1200
12	Set Variable	MixingTime = 15
13	Set Variable	CurrentSampleNumber = 0
14	Group End	Init
15	Group	Init Pipetting
16	Wash Tips	 20 + 15 ml
17	Wash Tips	 15 + 10 ml
18	Group End	Init Pipetting
19	Group	Inactivation
20	Wash Tips	 6 + 8 ml

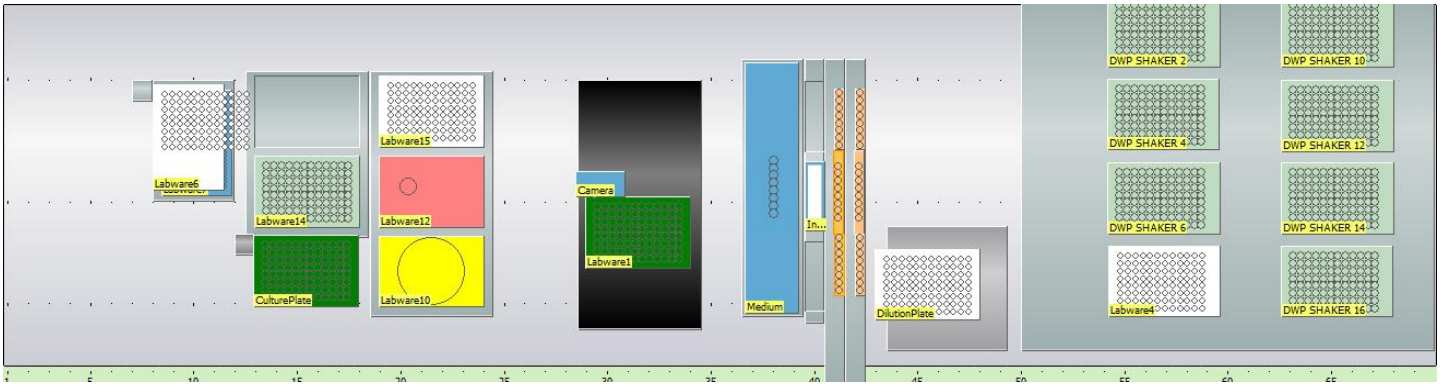
21	Aspirate		InactivationVolume µl >> DM_Water free dispense << "Inactivation" (Col. 1, Rows 1-8)
22	Start Timer		2
23	Wait for Timer		Timer 2 : InactivationTime sec
24	Dispense		InactivationVolume µl >> DM_Water free dispense << "Inactivation" (Col. 1, Rows 1-8)
25	Wash Tips		6 + 8 ml
26	Group End		Inactivation
27	Comment		Liquid-Liquid inoculation
28	Comment		Sealing
29	Begin Loop		SamplingRepetitions times "DilutionPlate"
30	Begin Loop		2 times "SwitchingHalfs"
31	Begin Loop		3 times "Sampling"
32	Group		Cultivation and Sampling
33	COVER		Open()
34	KUEHNER		stop()
35	Start Timer		1
36	Wait for Timer		Timer 1 : PauseNachSchütteln sec
37	Transfer Labware		Source: Grid '50,' Site 'CurrentPlatePos'; Destination: Grid '29', Site '1'; Narrow (ROMA 2)
38	KUEHNER		start(300)
39	COVER		Close()
40	Transfer Labware		Source: Grid '29,' Site '1'; Destination: Grid '12', Site '1'; Narrow (ROMA 1)
41	Set Variable		SampleOffset = 4*(LOOP_Sampling-1)+12*(LOOP_SwitchingHalfs-1)
42	Comment		TO DO: Enable random selection

43	Set Variable		WELL_OFFSET = SampleOffset
44	Aspirate		250 µl >> DM_Water free dispense << "CulturePlate" (Col. 1, Row 1)
45	Aspirate		250 µl >> DM_Water free dispense << "CulturePlate" (Col. 1, Row 2)
46	Aspirate		250 µl >> DM_Water free dispense << "CulturePlate" (Col. 1, Row 3)
47	Aspirate		250 µl >> DM_Water free dispense << "CulturePlate" (Col. 1, Row 4)
48	Set Variable		WELL_OFFSET = 0
49	Dispense		250 µl >> DM_Water free dispense << "DilutionPlate" (Col. 1, Rows 1-4) , 2 options
50	Wash Tips		1 + 2 ml
51	Transfer Labware		Source: Grid '12,' Site '1'; Destination: Grid '29', Site '1'; Narrow (ROMA 1)
52	COVER		Open()
53	KUEHNER		stop()
54	Start Timer		1
55	Wait for Timer		Timer 1 : PauseNachSchütteln sec
56	Transfer Labware		Source: Grid '29,' Site '1'; Destination: Grid '50', Site '4'; Narrow (ROMA 2)
57	KUEHNER		start(300)
58	COVER		Close()
59	Group		Inactivation
60	Wash Tips		6 + 8 ml
61	Aspirate		InactivationVolume µl >> DM_Water free dispense << "Inactivation" (Col. 1, Rows 1-8)
62	Start Timer		2
63	Wait for Timer		Timer 2 : InactivationTime sec
64	Dispense		InactivationVolume µl >> DM_Water free dispense << "Inactivation" (Col. 1, Rows 1-8)

65	Wash Tips	6 + 8 ml
66	Group End	Inactivation
67	Group End	Cultivation and Sampling
68	Group	Dilution
69	Aspirate	600 µl >> DM_Water free dispense << "Medium" (Col. 1, Rows 1-4)
70	Begin Loop	3 times "MediumFill"
71	Dispense	200 µl >> DM_Water free dispense << "DilutionPlate" (Col. 2, Rows 1-4) , 3 options
72	End Loop	"MediumFill"
73	Begin Loop	3 times "Serial Dilution"
74	Aspirate	50 µl >> DM_Water free dispense << "DilutionPlate" (Col. 1, Rows 1-4) , 3 options
75	Dispense	50 µl >> DM_Water free dispense << "DilutionPlate" (Col. 2, Rows 1-4) , 3 options
76	Wash Tips	6 + 8 ml
77	Te-Shake Shaker	SetFrequency(1200)
78	Te-Shake Shaker	Start(1)
79	Start Timer	3
80	Wait for Timer	Timer 3 : MixingTime sec
81	Te-Shake Shaker	Stop()
82	End Loop	"Serial Dilution"
83	Aspirate	50 µl >> DM_Water free dispense << "DilutionPlate" (Col. 4, Rows 1-4) , 2 options
84	Group	Inactivation
85	Wash Tips	6 + 8 ml
86	Aspirate	InactivationVolume µl >> DM_Water free dispense << "Inactivation" (Col. 1, Rows 1-8)

87	Start Timer	2
88	Wait for Timer	Timer 2 : InactivationTime sec
89	Dispense	 InactivationVolume µl >> DM_Water free dispense << "Inactivation" (Col. 1, Rows 1-8)
90	Wash Tips	 6 + 8 ml
91	Group End	Inactivation
92	Group End	Dilution
93	Group	OD600nm determination
94	Transfer Labware	Source: Grid '44,' Site '1'; Destination: Grid '19', Site '1'; Narrow (ROMA 2)
95	Transfer Labware	Source: Grid '19,' Site '1'; Destination: Grid '8', Site '1'; Narrow (ROMA 1)
96	Infinite Reader	Open()
97	Transfer Labware	Source: Grid '9,' Site '1'; Destination: Grid '6', Site '1'; Wide (ROMA 1)
98	Infinite Reader	Close()
99	Set Variable	CurrentSampleNumber = CurrentSampleNumber+1
100	Set Variable	LAST_BARCODE = CurrentSampleNumber

101	Infinite Reader	Measure(C:\ProgramD ata\Tecan\EVOware\O utputFiles_Reader\G rowthcurve_<YYYY-MM -DD HH-mm-ss>.xls|< TecanFile xmlns:xsi equal; quote; htt p://www.w3.org/2001 /XMLSchema-instance quote; xsi:schema Locationequal; quote; tecan.at.schem a.documents Main.xs dquote; fileforma tequal; quote; Te can.At.Measurement quote; fileversion equal; quote; 2.0 quote; xmlnsequa l; quote; tecan.at .schema.documentsq uote; ><FileInfo ty peequal; quote; quote; instrument equal; quote; infi nite 200Proquote; versionequal; qu ote; quote; creat edFromequal; quot e; localadminquote ; createdAtequal; quote; 2015-07-22 T15:04:02.4813319Z quote; createdWith equal; quote; Tec an.At.XFluor.Reader Editor.XFluorReader Editorquote; desc riptionequal; quo te; quote; /><Tec anMeasurement ideq ual; quote; 1quot e; classequal; q uote; Measurementq uote; ><Measurement ManualCycle idequa l; quote; 2quote; numberequal; qu ote; 1quote; type equal; quote; Sta ndardquote; ><Cycl ePlate idequal; q uote; 3quote; fil eequal; quote; CO R96fc UV transparen tquote; plateWith Coverequal; quote ; Falsequote; ><Pl ateRange idequal; quote; 4quote; r angeequal; quote; A1:H12quote; aut oequal; quote; fa lsequote; ><Measur ementAbsorbance id equal; quote; 5qu ote; modeequal; quote; Normalquote ; typeequal; quo te; quote; namee qual; quote; ABSq uote; longnameequ al; quote; quote; descriptionequal ; quote; quote; > <Well idequal; qu ote; 6quote; auto equal; quote; tru equote; ><Measurem entReading idequal ; quote; 7quote; nameequal; quote ; quote; beamDiam eterequal; quote; 500quote; beamGr idTypeequal; quot e; Singlequote; b eamGridSizeequal; quote; 1quote; b eamEdgeDistanceequ al; quote; autoqu ote; ><ReadingLabel idequal; quote; 8quote; nameequa l; quote; Label1q uote; scanTypeequ al; quote; ScanFix edquote; refIDeq ual; quote; 0quot e; ><ReadingSetting s numberequal; qu ote; 25quote; rat eequal; quote; 25 000quote; /><Read ingTime integration Timeequal; quote; 0quote; lagTime equal; quote; 0qu ote; readDelayequ al; quote; 0quote ; flashequal; qu ote; 0quote; dark equal; quote; 0q uote; excitationTi meequal; quote; 0 quote; /><Reading Filter idequal; q uote; 0quote; typ eequal; quote; Ex quote; wavelength equal; quote; 600 0quote; bandwidth equal; quote; 90 quote; attenuation equal; quote; 0q uote; usageequal; quote; ABSquote; /></ReadingLabel> </MeasurementReadin g></Well><CustomDat a idequal; quote; 9quote; /></Meas urementAbsorbance>< /PlateRange></Cycle Plate></Measurement ManualCycle><Measur ementInfo idequal; quote; 0quote; descriptionequal; quote; quote; ><S criptTemplateSettin gs idequal; quote ; 0quote; ><Script TemplateGeneralSett ings idequal; quo te; 0quote; Title equal; quote; qu ote; Groupequal; quote; quote; In foequal; quote; quote; Imageequal ; quote; quote; /><ScriptTemplateDe scriptionSettings i dequal; quote; 0 quote; Internaleq ual; quote; quote ; Externalequal; quote; quote; Is Externalequal; qu ote; Falsequote; /></ScriptTemplateS ettings></Measureme ntInfo></TecanMeasu rement></TecanFile>)
102	Infinite Reader	Open()
103	Transfer Labware	Source: Grid '6,' Site '1'; Destination: Grid '9', Site '1'; Wide (ROMA 1)
104	Infinite Reader	Close()
105	Transfer Labware	Source: Grid '8,' Site '1'; Destination: Grid '19', Site '1'; Narrow (ROMA 1)
106	Transfer Labware	Source: Grid '19,' Site '1'; Destination: Grid '44', Site '1'; Narrow (ROMA 2)
107	Group End	OD600nm determination
108	End Loop	"Sampling"
109	End Loop	"SwitchingHalfs"
110	End Loop	"DilutionPlate"


Labware6
Labware14
CulturePlate
Labware15
Labware12
Labware10
Camera
Labware1
Medium
DilutionPlate
DWP SHAKER 2
DWP SHAKER 10
DWP SHAKER 4
DWP SHAKER 12
DWP SHAKER 6
DWP SHAKER 14
Labware4
DWP SHAKER 16