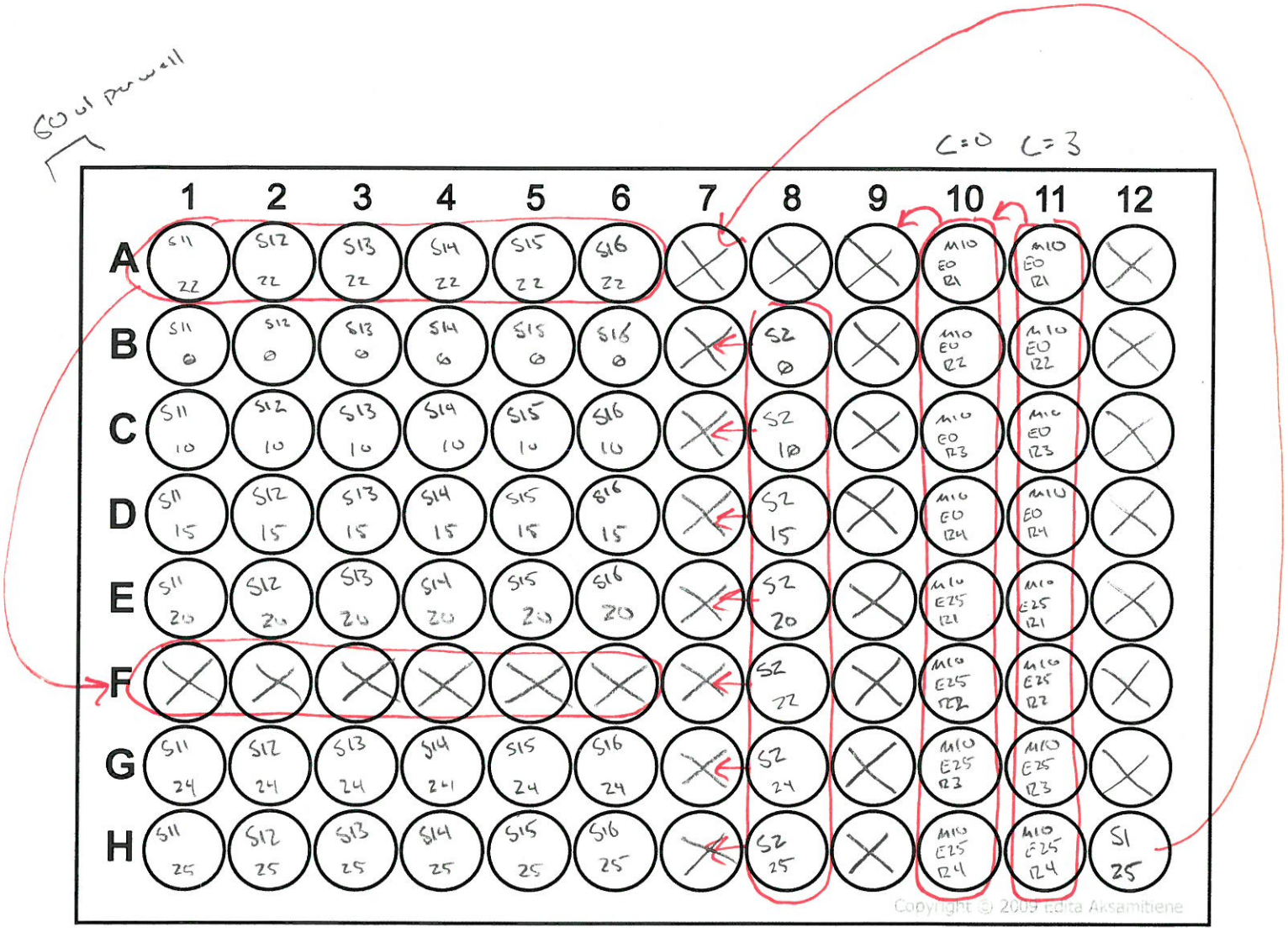
Phage + Virus

YEE_0002 Samples to reprocess

Date: March 10, 2017



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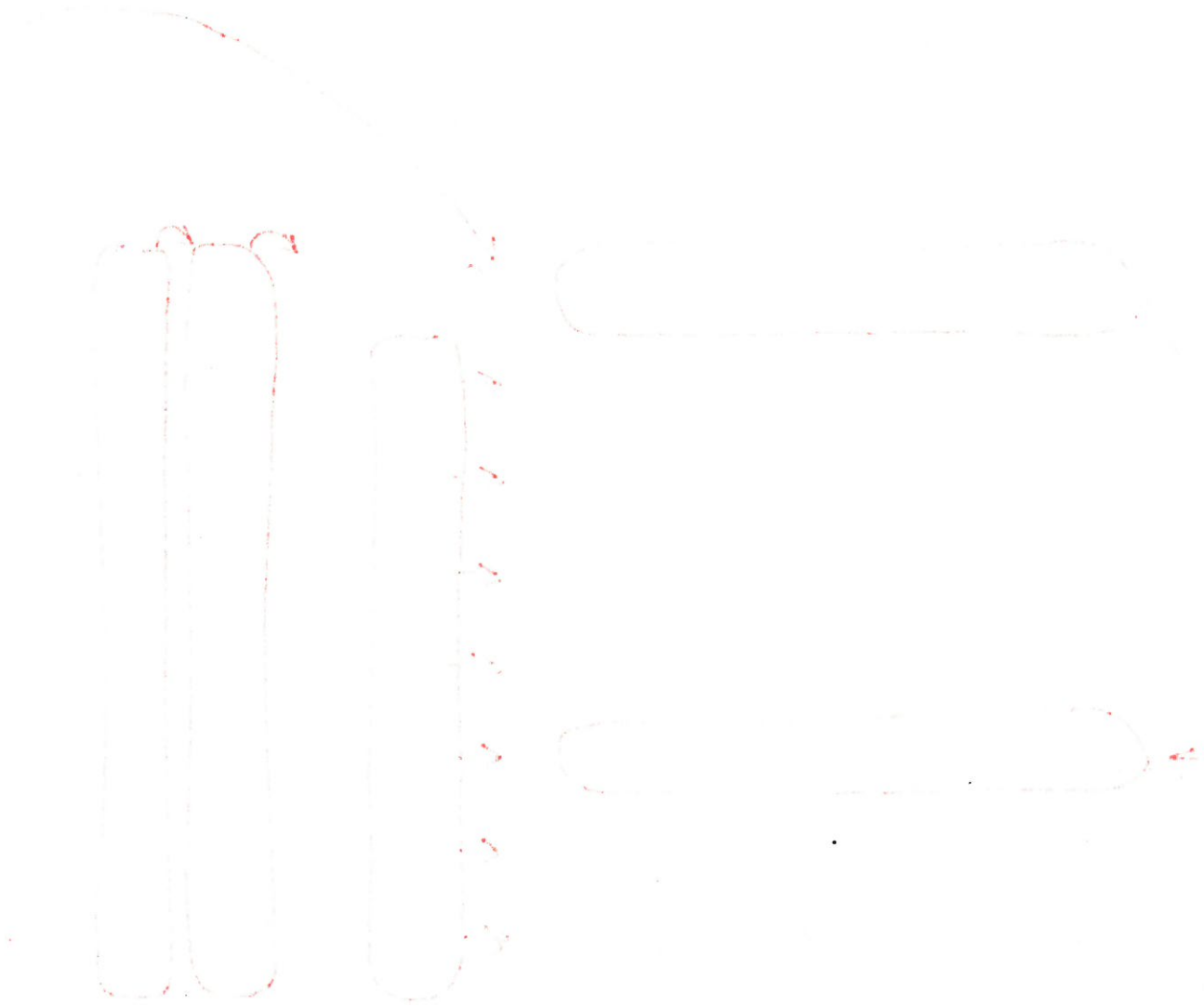

pooling design for each plate of samples													
		1	2	3	4	5	6	7	8	9	10	11	12
	A	S1						S2					
	B	S3			S4	S5	S6	S7			S8	S9	S10
	C	S11											
	D	S12											
	E	S13											
	F	S14											
	G	S15											
	H	S16											

Sample volume to use for each well for each of the 16 pools at each timepoint													
		1	2	3	4	5	6	7	8	9	10	11	12
	A	10ul	10ul	10ul	10ul	10ul	10ul	10ul	10ul	10ul	10ul	10ul	10ul
	B	20ul	20ul	20ul	60ul	60ul	60ul	20ul	20ul	20ul	60ul	60ul	60ul
	C	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul
	D	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul
	E	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul
	F	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul
	G	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul
	H	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul	5ul

Evolution Samples (N= 50; contains all pcr2 samples and all of sample set S2)									
	Sample Set	Replicate	Evolutionary	Competition	a.primer	p1.primer	bc.mean.con	bc.median.co	pcr2
	S1	NA	25	NA	Ion_17	Ion_R7	14.1152696	3.38029571	yes
	S2	NA	0	NA	Ion_18	Ion_R1	0.08827349	0.06183735	no
	S2	NA	10	NA	Ion_18	Ion_R2	1.36412557	0.84897371	no
	S2	NA	15	NA	Ion_18	Ion_R3	24.8615259	14.1323601	no
	S2	NA	20	NA	Ion_18	Ion_R4	11.4260136	3.65388631	no
	S2	NA	22	NA	Ion_18	Ion_R5	20.6938972	20.6820262	yes
	S2	NA	24	NA	Ion_18	Ion_R6	22.022152	18.8304087	yes
	S2	NA	25	NA	Ion_18	Ion_R7	14.5133095	5.39488153	no
	S11	NA	0	NA	Ion_27	Ion_R1	14.1512697	10.6392496	yes
	S11	NA	10	NA	Ion_27	Ion_R2	31.4590158	27.8609729	yes
	S11	NA	15	NA	Ion_27	Ion_R3	34.1867453	30.8105911	yes
	S11	NA	20	NA	Ion_27	Ion_R4	34.5891969	31.6449058	yes
	S11	NA	22	NA	Ion_27	Ion_R5	35.1246987	28.4033952	yes
	S11	NA	24	NA	Ion_27	Ion_R6	28.7353292	23.6471234	yes
	S11	NA	25	NA	Ion_27	Ion_R7	37.8129457	32.8645298	yes
	S12	NA	0	NA	Ion_28	Ion_R1	23.3023631	22.1392882	yes
	S12	NA	10	NA	Ion_28	Ion_R2	34.8426347	36.4293855	yes
	S12	NA	15	NA	Ion_28	Ion_R3	41.5139216	42.1978351	yes
	S12	NA	20	NA	Ion_28	Ion_R4	46.1019639	46.3949564	yes
	S12	NA	22	NA	Ion_28	Ion_R5	50.1842168	53.161934	yes
	S12	NA	24	NA	Ion_28	Ion_R6	23.0165099	21.1650822	yes
	S12	NA	25	NA	Ion_28	Ion_R7	44.9864086	44.1823343	yes
	S13	NA	0	NA	Ion_29	Ion_R1	29.3383551	29.9844505	yes
	S13	NA	10	NA	Ion_29	Ion_R2	42.9601739	36.4727831	yes
	S13	NA	15	NA	Ion_29	Ion_R3	36.7913415	31.9589828	yes
	S13	NA	20	NA	Ion_29	Ion_R4	44.591743	42.0532527	yes
	S13	NA	22	NA	Ion_29	Ion_R5	41.4167466	37.727021	yes
	S13	NA	24	NA	Ion_29	Ion_R6	39.6471882	35.4367726	yes
	S13	NA	25	NA	Ion_29	Ion_R7	42.6642723	43.0782999	yes
	S14	NA	0	NA	Ion_30	Ion_R1	29.9710825	30.4303945	yes
	S14	NA	10	NA	Ion_30	Ion_R2	28.7479618	24.261063	yes
	S14	NA	15	NA	Ion_30	Ion_R3	34.3190193	26.873492	yes
	S14	NA	20	NA	Ion_30	Ion_R4	43.0559351	36.6878952	yes
	S14	NA	22	NA	Ion_30	Ion_R5	39.7980915	37.9217795	yes
	S14	NA	24	NA	Ion_30	Ion_R6	36.2222689	31.2068756	yes
	S14	NA	25	NA	Ion_30	Ion_R7	39.3524257	34.8629139	yes
	S15	NA	0	NA	Ion_31	Ion_R1	52.8268995	57.9990636	yes
	S15	NA	10	NA	Ion_31	Ion_R2	44.6068871	44.750351	yes
	S15	NA	15	NA	Ion_31	Ion_R3	21.0522351	16.9405569	yes
	S15	NA	20	NA	Ion_31	Ion_R4	48.167732	52.6620179	yes
	S15	NA	22	NA	Ion_31	Ion_R5	47.9421064	47.3551564	yes
	S15	NA	24	NA	Ion_31	Ion_R6	48.1970821	50.4575971	yes
	S15	NA	25	NA	Ion_31	Ion_R7	35.3397788	31.7390849	yes
	S16	NA	0	NA	Ion_32	Ion_R1	26.9911755	19.7845528	yes
	S16	NA	10	NA	Ion_32	Ion_R2	58.755851	63.2331152	yes
	S16	NA	15	NA	Ion_32	Ion_R3	21.8175747	17.2786558	yes
	S16	NA	20	NA	Ion_32	Ion_R4	43.9097998	39.8296687	yes
	S16	NA	22	NA	Ion_32	Ion_R5	20.6203919	15.0336288	yes
	S16	NA	24	NA	Ion_32	Ion_R6	56.5033875	49.824418	yes
	S16	NA	25	NA	Ion_32	Ion_R7	41.9039173	33.3038751	yes

Competition Samples (N= 16; contains all of mix M10)									
1	M10	R1	0	0	Ion_33	Ion_R11	19.905329	19.158917	no
2	M10	R2	0	0	Ion_34	Ion_R11	8.818273	7.671685	no
3	M10	R3	0	0	Ion_35	Ion_R11	2.357958	2.128046	no
4	M10	R4	0	0	Ion_36	Ion_R11	2.597363	2.184959	no
5	M10	R1	25	0	Ion_37	Ion_R11	18.183827	11.227402	no
6	M10	R2	25	0	Ion_38	Ion_R11	50.161297	48.882898	no
7	M10	R3	25	0	Ion_39	Ion_R11	23.629128	11.153746	no
8	M10	R4	25	0	Ion_40	Ion_R11	33.003047	27.801369	no
9	M10	R1	0	3	Ion_25	Ion_R12	35.046062	29.249595	no
10	M10	R2	0	3	Ion_26	Ion_R12	52.713971	56.093657	no
11	M10	R3	0	3	Ion_27	Ion_R12	30.290724	28.500221	no
12	M10	R4	0	3	Ion_28	Ion_R12	10.544308	10.073059	no
13	M10	R1	25	3	Ion_29	Ion_R12	41.028604	36.21226	no
14	M10	R2	25	3	Ion_30	Ion_R12	3.550894	1.673805	no
15	M10	R3	25	3	Ion_31	Ion_R12	32.16729	26.177167	no
16	M10	R4	25	3	Ion_32	Ion_R12	4.870915	2.431687	no

Layout for DNA extraction

Plate T=0

	1	2	3	4	5	6	7	8	9	10	11	12
A	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
B	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
C	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
D	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
E	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11
F	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11
G	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11
H	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11

Plate T=3

	1	2	3	4	5	6	7	8	9	10	11	12
A	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
B	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
C	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
D	T0.M1	T0.M2	T0.M3	T0.M4	T0.M5	T0.M6	T0.M7	T0.M8	T0.M9a	T0.M9b	T0.M10	T0.M11
E	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11
F	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11
G	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11
H	T25.M1	T25.M2	T25.M3	T25.M4	T25.M5	T25.M6	T25.M7	T25.M8	T25.M9a	T25.M9b	T25.M10	T25.M11

Layout for Primer addition

Plate T=0

	Ion_R1	Ion_R2	Ion_R3	Ion_R4	Ion_R5	Ion_R6	Ion_R7	Ion_R8	Ion_R9	Ion_R10	Ion_R11	Ion_R12
Ion_33	1	9	17	25	33	41	49	57	65	73	81	89
Ion_34	2	10	18	26	34	42	50	58	66	74	82	90
Ion_35	3	11	19	27	35	43	51	59	67	75	83	91
Ion_36	4	12	20	28	36	44	52	60	68	76	84	92
Ion_37	5	13	21	29	37	45	53	61	69	77	85	93
Ion_38	6	14	22	30	38	46	54	62	70	78	86	94
Ion_39	7	15	23	31	39	47	55	63	71	79	87	95
Ion_40	8	16	24	32	40	48	56	64	72	80	88	96

Plate T=3

	Ion_R8	Ion_R9	Ion_R10	Ion_R11	Ion_R12	Ion_R13
Ion_17	97	105	113	121	129	137
Ion_18	98	106	114	122	130	138
Ion_19	99	107	115	123	131	139
Ion_20	100	108	116	124	132	140
Ion_21	101	109	117	125	133	141
Ion_22	102	110	118	126	134	142
Ion_23	103	111	119	127	135	143
Ion_24	104	112	120	128	136	144

Plate T=3 (cor

	Ion_R8	Ion_R9	Ion_R10	Ion_R11	Ion_R12	Ion_R13
Ion_25	145	153	161	169	177	185
Ion_26	146	154	162	170	178	186
Ion_27	147	155	163	171	179	187
Ion_28	148	156	164	172	180	188
Ion_29	149	157	165	173	181	189
Ion_30	150	158	166	174	182	190
Ion_31	151	159	167	175	183	191
Ion_32	152	160	168	176	184	192

2	Do PCR using A and P1 primers for DNA plate 1 and 2	PCR plate label: FIT PCR 4-1, 4-2	
		ul	
a	DNA (lyticase+beads)	5	
	primer A+P1	0.25+0.25	
	Taq plus mix (Lamda Cat#D124R)	12.5	
	water	7	
	total volume	25	
b	program: UPTAG		
	94C	5min	
	94C	10sec	35 cycles
	50C	10sec	
	72C	10sec	
	72C	5min	
c	run 2% gel to check PCR result	clean single band of 131bp	

	Ion_17	Ion_18	Ion_19	Ion_20	Ion_21	Ion_22	Ion_23	Ion_24	Ion_25	x	x	x
Ion_R1	x	x	x	x	x	x	S1_25	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R2	S11_0	S12_0	S13_0	S14_0	S15_0	S16_0	S2_0	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R3	S11_10	S12_10	S13_10	S14_10	S15_10	S16_10	S2_10	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R4	S11_15	S12_15	S13_15	S14_15	S15_15	S16_15	S2_15	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R5	S11_20	S12_20	S13_20	S14_20	S15_20	S16_20	S2_20	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R6	S11_22	S12_22	S13_22	S14_22	S15_22	S16_22	S2_22	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R7	S11_24	S12_24	S13_24	S14_24	S15_24	S16_24	S2_24	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R8	S11_25	S12_25	S13_25	S14_25	S15_25	S16_25	S2_25	M10_E25_C0	M10_E25_C3	x	x	x
	1	2	3	4	5	6	7	8	9			

Protocol: Lyticase + beads DNA extraction
Date Edited: April 17, 2017

1. Prepare the samples
    a. Add 60ul of yeast sample in each appropriate well of a 96-well pcr microplate
    b. Centrifuge at 3000 rcf for 5 minutes to pellet
    c. Pull the supernatant one well at a time, don't need to get all of it.
    d. Store covered in freezer (-20 C) if the rest of the protocol will not be run immediately

2. Prepare the lyticase and beads mixture
    a. Fill a single microcentrifuge tube about 1/3 of the way with lyticase solution (100 units / ml).
    b. Add glass beads (0.5 mm diameter) to the tube for a total volume of approximately 2/3 of the microcentrifuge tube's capacity.
    c. Refill the tube with beads or lyticase solution as needed throughout the protocol. Keep the lyticase stock tube on ice and the lyticase + beads mixture on cooled plate holder when not in use.

3. Add the lyticase and beads mixture to each well
    a. Cut the tip off of a large pipette tip (p1000 size) at a 45 degree angle to allow both lyticase and beads to be drawn into the tip.
    b. Transfer 100ul of lyticase + beads to each well (do not touch the well or sample, change pipette tips if you do) – aim for ~75% lyticase and ~25% beads per well.

4. Resuspend / bead-beat the samples before PCR
    a. Cover the plate with a microseal 'B' seal cover (brown backing) completely, ensuring that each individual well is completely sealed so that there is no contamination between wells.
    b. Vortex the plate at the highest setting
        i. Push down on each pair of columns for approximately 2 seconds of vortexing to ensure that all wells are mixed evenly and completely
        ii. Repeat as needed until all wells are resuspended

5. Incubate the PCR plate in the thermocycler
    a. Load the plate into the pcr machine ensuring that it is firmly in place
    b. Navigate to the program named "37C_30" on the thermocycler and double check that the program specifications match those outlined in appendix 1.
    c. close the thermocycler lid and press run.
        i. Do not use the rubberish compression pad that is stored near the vortexer.
    d. Pause the PCR program after 15 minutes and vortex / bead-beat all samples again as specified in the "resuspend" step above.
        i. Double check before vortexing that all wells are still fully sealed by the pcr cover to avoid contamination across wells.
    e. Place the plate back in the thermocycler and resume the PCR program. Allow the program to run to completion.
        i. *If tubes are allowed to transition fully from the highest temperature at the end of the program to the 4 degrees C holding temperature they will be highly deformed. Pulling the tubes out as soon as the program has finished running will prevent this deformation.

6. Spin down the plate (Hermle Z 300)
    a. Spin at 3000 rcf for 5 mminutes
    b. Remove supernatant (50ul per well) and add to a new pcr-plate ("DNA storage plate")
        i. Will only need 5ul for each round of pcr, so there should be a lot of sample in reserve for future use
        ii. OK to use multichannel pipette for this step
    c. If you are stopping here, the plate can be stored at -20 C in the freezer until you are ready to do the PCR protocol.

Appendix 1: Thermocycler program specifications

1. Parameters
    a. Volume = 100 *u*l
    b. Lid = 105 C
2. Protocol
    a. 37 C for 30 minutes
    b. 95 C for 10 minutes
    c. 4 C for infinite hold

	Ion_17	Ion_18	Ion_19	Ion_20	Ion_21	Ion_22	Ion_23	Ion_24	Ion_25	x	x	x
Ion_R1	x	x	x	x	x	x	S1_25	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R2	S11_0	S12_0	S13_0	S14_0	S15_0	S16_0	S2_0	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R3	S11_10	S12_10	S13_10	S14_10	S15_10	S16_10	S2_10	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R4	S11_15	S12_15	S13_15	S14_15	S15_15	S16_15	S2_15	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R5	S11_20	S12_20	S13_20	S14_20	S15_20	S16_20	S2_20	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R6	S11_22	S12_22	S13_22	S14_22	S15_22	S16_22	S2_22	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R7	S11_24	S12_24	S13_24	S14_24	S15_24	S16_24	S2_24	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R8	S11_25	S12_25	S13_25	S14_25	S15_25	S16_25	S2_25	M10_E25_C0	M10_E25_C3	x	x	x

moved to group 2

	1 Ion_17	2 Ion_18	3 Ion_19	4 Ion_20	5 Ion_21	6 Ion_22	7 Ion_23	8 Ion_24	9 Ion_25	10 x	11 x	12 x
Ion_R1												
Ion_R2	4.37	5.61	36.67	6.40	6.36	6.83	3.80	0.71	2.38			
Ion_R3	5.67	5.39	45.83	5.24	8.40	7.24	3.07	1.37	1.72			
Ion_R4	6.32	5.73	5.09	41.04	16.27	9.40	17.24	0.89	0.60			
Ion_R5	8.33	7.33	5.29	15.45	6.71	6.32	2.81	0.72	0.81			
Ion_R6	5.29	33.13	7.53	6.88	6.55	6.25	4.17	0.89	2.60			
Ion_R7	6.15	33.74	6.08	8.46	9.02	9.09	3.68	2.67	0.59			
Ion_R8	6.36	25.70	7.28	7.05	7.19	10.38	15.72	0.67	0.68			
							3.14	0.74	1.28			

Group 1	Group 2

in group 2 →

	ng/ul	vol to add
S12_22	7.86	13.99
S12_24	9.16	12.01
S12_25	8.02	13.72
S13_0	5.70	19.30
S13_10	9.58	11.48
S14_15	7.76	14.18

Group 1

Group 2

Total volume

154.34 ul (775)

266.60 ul (1,333)

– re-run PCR on these samples

↳ increase sample volume relative to water volume in PCR protocol to improve DNA yields

Group 1 : 20 samples

Group 2: 46 samples

Sample ID	N Wells	N Barcodes	A Primer	P1 Primer	PCR (ng/ul)	PCR (ng/ul) [RUN 2]	Vol to add	Vol to add rerun		Total Volume	Total volume for pool (rerun)
S11_0	11	22	Ion_17	Ion_R2	25.2		4.37		1		
S11_10	11	22	Ion_17	Ion_R3	19.4		5.67		1		
S11_15	11	22	Ion_17	Ion_R4	17.4		6.32		1		
S11_20	11	22	Ion_17	Ion_R5	13.2		8.33		1		
S11_22	11	22	Ion_17	Ion_R6	20.8		5.29		1		
S11_24	11	22	Ion_17	Ion_R7	17.9		6.15		1		
S11_25	11	22	Ion_17	Ion_R8	17.3		6.36		1		
S12_0	11	22	Ion_18	Ion_R2	19.6		5.61		1		
S12_10	11	22	Ion_18	Ion_R3	20.4		5.39		1		
S12_15	11	22	Ion_18	Ion_R4	19.2		5.73		1		
S12_20	11	22	Ion_18	Ion_R5	15		7.33		1		
S12_22	11	22	Ion_18	Ion_R6	3.32	7.86	33.13	13.99	1		
S12_24	11	22	Ion_18	Ion_R7	3.26	9.16	33.74	12.01	1		
S12_25	11	22	Ion_18	Ion_R8	4.28	8.02	25.70	13.72	1		
S13_0	11	22	Ion_19	Ion_R2	3	5.7	36.67	19.30	1		
S13_10	11	22	Ion_19	Ion_R3	2.4	9.58	45.83	11.48	1		
S13_15	11	22	Ion_19	Ion_R4	21.6		5.09		1		
S13_20	11	22	Ion_19	Ion_R5	20.8		5.29		1		
S13_22	11	22	Ion_19	Ion_R6	14.6		7.53		1		
S13_24	11	22	Ion_19	Ion_R7	18.1		6.08		1		
S13_25	11	22	Ion_19	Ion_R8	15.1		7.28		1	272.90	168.33
S14_0	11	22	Ion_20	Ion_R2	17.2		6.40		2		
S14_10	11	22	Ion_20	Ion_R3	21		5.24		2		
S14_15	11	22	Ion_20	Ion_R4	2.68	7.76	41.04	14.18	2		
S14_20	11	22	Ion_20	Ion_R5	7.12		15.45		2		
S14_22	11	22	Ion_20	Ion_R6	16		6.88		2		
S14_24	11	22	Ion_20	Ion_R7	13		8.46		2		
S14_25	11	22	Ion_20	Ion_R8	15.6		7.05		2		
S15_0	11	22	Ion_21	Ion_R2	17.3		6.36		2		
S15_10	11	22	Ion_21	Ion_R3	13.1		8.40		2		
S15_15	11	22	Ion_21	Ion_R4	6.76		16.27		2		
S15_20	11	22	Ion_21	Ion_R5	16.4		6.71		2		
S15_22	11	22	Ion_21	Ion_R6	16.8		6.55		2		
S15_24	11	22	Ion_21	Ion_R7	12.2		9.02		2		
S15_25	11	22	Ion_21	Ion_R8	15.3		7.19		2		
S16_0	11	22	Ion_22	Ion_R2	16.1		6.83		2		
S16_10	11	22	Ion_22	Ion_R3	15.2		7.24		2		
S16_15	11	22	Ion_22	Ion_R4	11.7		9.40		2		
S16_20	11	22	Ion_22	Ion_R5	17.4		6.32		2		
S16_22	11	22	Ion_22	Ion_R6	17.6		6.25		2		
S16_24	11	22	Ion_22	Ion_R7	12.1		9.09		2		
S16_25	11	22	Ion_22	Ion_R8	10.6		10.38		2		
S1_25	6	12	Ion_23	Ion_R1	15.8		3.80		2		
S2_0	5	10	Ion_23	Ion_R2	16.3		3.07		2		
S2_10	5	10	Ion_23	Ion_R3	2.9		17.24		2		
S2_15	5	10	Ion_23	Ion_R4	17.8		2.81		2		
S2_20	5	10	Ion_23	Ion_R5	12		4.17		2		
S2_22	5	10	Ion_23	Ion_R6	13.6		3.68		2		
S2_24	5	10	Ion_23	Ion_R7	3.18		15.72		2		
S2_25	5	10	Ion_23	Ion_R8	15.9		3.14		2		
M10_E0_C0_R1	1	12 + ref @50%	Ion_24	Ion_R1	14.1		0.71		2		
M10_E0_C0_R2	1	12 + ref @50%	Ion_24	Ion_R2	7.32		1.37		2		
M10_E0_C0_R3	1	12 + ref @50%	Ion_24	Ion_R3	11.2		0.89		2		
M10_E0_C0_R4	1	12 + ref @50%	Ion_24	Ion_R4	13.8		0.72		2		
M10_E25_C0_R1	1	12 + ref @50%	Ion_24	Ion_R5	11.2		0.89		2		
M10_E25_C0_R2	1	12 + ref @50%	Ion_24	Ion_R6	3.74		2.67		2		
M10_E25_C0_R3	1	12 + ref @50%	Ion_24	Ion_R7	14.9		0.67		2		
M10_E25_C0_R4	1	12 + ref @50%	Ion_24	Ion_R8	13.5		0.74		2		
M10_E0_C3_R1	1	12 + ref @50%	Ion_25	Ion_R1	4.2		2.38		2		
M10_E0_C3_R2	1	12 + ref @50%	Ion_25	Ion_R2	5.8		1.72		2		
M10_E0_C3_R3	1	12 + ref @50%	Ion_25	Ion_R3	16.7		0.60		2		
M10_E0_C3_R4	1	12 + ref @50%	Ion_25	Ion_R4	12.3		0.81		2		
M10_E25_C3_R1	1	12 + ref @50%	Ion_25	Ion_R5	3.84		2.60		2		
M10_E25_C3_R2	1	12 + ref @50%	Ion_25	Ion_R6	16.9		0.59		2		
M10_E25_C3_R3	1	12 + ref @50%	Ion_25	Ion_R7	14.8		0.68		2		
M10_E25_C3_R4	1	12 + ref @50%	Ion_25	Ion_R8	7.84		1.28		2	273.08	252.61
							552.38				

GROUP	sample/grou	concentratio	volume to mi	vol to mix / 2	
G1	220	7.78	28.277635	14.14	
G2	299	10.2	29.3137255	14.66	
					Water to add
	519 H20:		57.5913605	28.80	
	add up 1ng /	519	129.75	64.875	36.08
F2-2	131bp PCR products	4 ng/ul			
		50nM			

≤20,000 counts =

Contamination ≥ 5% =

	Ion_17	Ion_18	Ion_19	Ion_20	Ion_21	Ion_22	Ion_23	Ion_24	Ion_25	x	x	x
Ion_R1	x	x	x	x	x	x	S1_25	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R2	S11_0	S12_0	S13_0	S14_0	S15_0	S16_0	S2_0	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R3	S11_10	S12_10	S13_10	S14_10	S15_10	S16_10	S2_10	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R4	S11_15	S12_15	S13_15	S14_15	S15_15	S16_15	S2_15	M10_E0_C0	M10_E0_C3	x	x	x
Ion_R5	S11_20	S12_20	S13_20	S14_20	S15_20	S16_20	S2_20	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R6	S11_22	S12_22	S13_22	S14_22	S15_22	S16_22	S2_22	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R7	S11_24	S12_24	S13_24	S14_24	S15_24	S16_24	S2_24	M10_E25_C0	M10_E25_C3	x	x	x
Ion_R8	S11_25	S12_25	S13_25	S14_25	S15_25	S16_25	S2_25	M10_E25_C0	M10_E25_C3	x	x	x

	Ion_17	Ion_18	Ion_19	Ion_20	Ion_21	Ion_22	Ion_23	Ion_24	Ion_25	x	x	x
Ion_R1							3.80	0.71	2.38			
Ion_R2	4.37	5.61	36.67	6.40	6.36	6.83	3.07	1.37	1.72			
Ion_R3	5.67	5.39	45.83	5.24	8.40	7.24	17.24	0.89	0.60			
Ion_R4	6.32	5.73	5.09	41.04	16.27	9.40	2.81	0.72	0.81			
Ion_R5	8.33	7.33	5.29	15.45	6.71	6.32	4.17	0.89	2.60			
Ion_R6	5.29	33.13	7.53	6.88	6.55	6.25	3.68	2.67	0.59			
Ion_R7	6.15	33.74	6.08	8.46	9.02	9.09	15.72	0.67	0.68			
Ion_R8	6.36	25.70	7.28	7.05	7.19	10.38	3.14	0.74	1.28			

Group 1	Group 2