Hivebench Electronic Laboratory Notebook

Protocol

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qPCR Protocol

https://www.hivebench.com/protocols/20600

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General

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Abstract

This is a standardize qPCR protocol for *Megachilae rotundata* developed by Alex Torson (A.S. Torson et al., 2015).

Protocol **#1**: RNA Extraction

Materials

- 1.5 mL microcentrifuge tubes
- Blue pestils or microcentrifuge tubes with Droconum oxide beads
- TriZol
- BCP(1-Bromo-3-Chloropentane)
- Isopropyl Alcohol
- 75% ethanol
- 100% ethanol

Equipment

- Centrifuge (temperature regulated)
- Vortex

Procedure

Step #1: Homogenization

1. **Homogenize samples in 500µl TriZol within 1.5 mL microcentrifuge tubes**
    - **Note: Samples can be homogenized with either a blue pestil or a bullet blender using Droconium oxide beads**
2. **Add additional 500µl TriZol to homogenized sample**
    - **Additional Step- To clean up sample**
        - **Centrifuge samples at 12,000g for 10 minutes at 4°C to remove particulate**
        - **Decant supernatant (~850µl) to new RNase free 1.5mL tubes**
            - **Note: do not touch particulate**
3. **Samples can noe be kept at -80°C for up to one month before processing**

Step #2: Phase Seperation

1. Add 100µl (100µl/ml TriZol) of BCP(1-Bromo-3-Chloropentane) to the samples in the tri-reagant
2. Shake vigourously for 15 seconds
3. Incubate for 5-10 minutes at room temperature
4. Centrifuge at 12,000g for 8 minutes at 4°C
    - Following centrifuging, the mixture separates into a lower red phenol phase, interphase, and colorless aqueous phase(RNA). RNA remains exclusively in the aqueous phase, while DNA and proteins are in the interphase and organic phase

Step #3: RNA Precipitation

1. Transfer aqueous phase (~375µl) into RNase free 1.5mL microcentrifuge tubes
    - Be very **careful** to not touch the cloudy layer(Be very conservative because 375µl is not needed)
    - If layer is disturbed, repeat previous centrifugation step
2. Add 500µl of isopropyl alcohol to the aqueous phase
    - This allows the RNA to be precipitated from the aqueous phase
3. **Invert samples to mix**
4. Incubate at room temperature for 2-10 minutes
5. Centrifuge at 12,000g for 8 minutes at 4°C
6. White/clear pellet should now be visible

Step #4: RNA Wash

1. Remove supernatant
2. Wash RNA with 1000µl of 75% ethanol and vortex lightly
    - Vortex just enough to dislodge pellet from side of tube
3. Centrifuge at 7,500 RPM for 5 minutes at 4°C
    - If RNA pellet accumulates on the side of tge tube, or floats, sediment the pellet by spinning at 12,000g for 5 minutes at 4°C

Step #5: RNA Storing

1. Remove the 75% ethanol wash by using p1000 pipet
    - Collect by centrifugation at remove remainder of EtOH with p200 pipet
    - Be careful not to disturb pellet
2. Added 1000µl of 100% ethanol
3. Stored in -80°C freezer until ready for cDNA

Protocol #2: RNA Pellet Resuspension

Materials

- 1.5 mL microcentrifuge tubes
- DEPC- treated water (Buy or make)
- Ice

Equipment

- Centrifuge (temperature regulated)
- Nano Drop 1000

Procedure

1. Remove 100% ethanol from RNA tubes by using p1000 pipette to remove the large volume
2. Centrifuge tubes for 15 seconds to collect remaining ethanol at bottom of tube
3. Use p200 to collect and discard any remaining ethanol
4. Dry RNA tubes and pellet by inverting cetrifuge tubes
5. Allow the samples to complete dry
    - Note: Sides of pellet should start curling up and no clear halo of ethanol should be seen
6. Re-suspend RNA pellet in 30µl of DEPC- treated water
    - If pellet does not dissolve on its own, flick the tube to agitate (Spin down, do not vortex because it can destroy and ruin RNA)
7. Mix by gently pipetting
8. Chilled on ice
9. Use Nano Drop 1000 and software to measure concentration of RNA in water
    - Use same DEPC-treated water for initialization and reading blank on program as you used for re-suspending the RNA samples
10. Recorded concentration values, 260/280 values and 230/260 values
    - A 260/280 of 2.0 is generally considered pure for RNA
    - 230/260 should be 2.0-2.2, but this is less critical
    - Sample Table

Sample	µg/µL (Concentration)	V1 (µL)	30µL - V1 = Vwater (µL)
#000	3000.0	2.00	2.00

        - Target Concentration: 0.333 µg/µL (this may vary depending on downstream application)
        - Final Volume = 30µL
11. Convert ng/µL by moving decimal place over to the left 3 times
    - Examples: (3000 ng/µL = 3.00 µg/µL)
12. Calculated dilution samples by using equation C1V1 = C2V2
    - Examples: (3.00 µg/µL)(V1) = (0.33 µg/µL)(30µL) =3.33 µL
    - Table Sample for Dilution

Sample	µg/µL (Concentration)	V1 (µL)	30 µL - V1 = Vwater (µL)
#000	3.000	3.33	26.67

13. Diluted samples per calculated table
14. Labelled samples and stored in fridge for DNase treatment

Keep RNA always on ice while conducting this protocol. RNA is time and temperature sensitive

Protocol #3: DNase Treatment

Materials

- 1.5 mL microcentrifuge tubes
- Invitrogen DNase Treatment. Catalog # 18068-0.15
- DEPC-treated water

- Ice

Equipment

- Heat block
- Vortex

Procedure

1. Use µg of RNA per DNase reaction (Can be scaled up if RNA concentrations allow)
    1. Starting concentration: 0.333µg/µL (May vary depending on initial concentrations and downstream applications)
    2. 0.333 µg/µL x 3 µL per reaction = 1 µg of RNA
2. Three DNase reactions are done for each sample (Triplicate)
    - Reaction components are from Invitrogen DNase Treatment kit

Component	Per Reaction
1 µg of RNA	3 µL x 3 = 9µL
10x DNase I Rxn Buffer	1 µL x 3 = 3µL
DNase I amp grade	1 µL x 3 = 3µL
DEPC-treated water	5 µL x 3 = 15µL
Total	**3 µL x 10 = 30µL**

    - Create Master Mix(MM) of reaction buffer, DNase I amp grade and DEPC and mixed by vortex
    - Add 21 µL of MM to 9µL to RNA in clean 1.5 microcentrifuge tubes
3. Incubate reaction at room temperature for 15 minutes
4. Inactivate DNase by adding 3 µL (1µL in single reaction) of EDTA (Invitrogen kit) to reaction
5. Gently mix by pipetting
6. Incubate reactions at 65°C for 10 minutes on heat block
7. Reactions are now ready for cDNA synthesis
    - Product can be stored at 4°C on ice overnight if proceeding to first-strand synthesis the following day. This will limit freeze/thaw cycle on the RNA samples
8. Residual volumes can be stored at -80°C freezer afer cDNA synthesis

Keep RNA always on ice while conducting this protocol. RNA is time and temperature sensitive

Protocol #4: First-strand cDNA Synthesis

Materials

- 1.5 mL microcentrifuge tue
- PCR tubes
- Invitrogen superscript III First-strand synthesis system for RT-PCR. Catalog # 18080-51
- Ice

Equipment

- Thermocycler
- Centrifuge

Protocol

1. Combine following in strip cap 0.2 mL tubes
    - Components from invitrogen superscript III First-strand synthesis system
    -
        -

Component	Per Reaction(Rxn)	x68 ((22 x 3) + 2)
Up to µg RNA	8 µL (0.8 µg)	-
50 mM oligo (dt) 20 primer	1 µL	68
10 mM dNTP mix	1 µL	68
Total	**10 µL**	**136**

        - Note: We only had 21 samples, however we added 1 to get 22 in order to have residual. After we multiplied 22 by 3, we also added 2 to produce more residual
        - Multiplied the amount needed for total volume of all reactions (Scale up by 10%)
        - Add the 50mM oligo and 10mM dNTP in a clean 1.5 mL tube and gently mixed by pipetting
        - Place 2µL of the mixed oligo and dNTP in each tube followed by the 8µL of RNA
2. Incubate the tubes at 65°C for 5 minuters in thermocycler
3. Place on ice for at least one minute
4. Prepare the following cDNA synthesis Rxn Master Mix(MM) in the indicated order
    - Components from invitrogen superscript III First-strand synthesis system

Component	Per Rxn	x65 ((21 samples x 3) + 2 = 65)
10x RT Rxn Buffer	2 µL	130 µL
25mM MgCl2	4 µL	260 µL
0.1M DTT	2 µL	130 µL
RNase OUT (40U/µL)	1 µL	65 µL
Superscript RT III	1 µL	65 µL
Total	**10 µL**	**650 µL**

5. Add 10µL of cDNA MM to each reaction
6. Mix gently and collect by brief centrifugation
7. Incubate in thermocycler as follows:
    - 50°C for 50 minutes
    - 85°C for 5 minutes
8. Chill tubes on ice
9. Collect reactions by brief centrifugation
10. Add 1µL on RNase H (From invitrogen superscript kit) to each reaction
11. Incubate at 37°C for 20 minutes in thermocycler
12. cDNA synthesis reactions can now be stored at -20°C or used for qPCR immediatly

Protocol #5: Dilution of cDNA

Materials

- 2 mL microcentrifuge tube
- PCR tubes
- PCR water

Equipment

- None

Protocol

1. Combine each sample replicates from first-strand synthesis into a clean and labeled 1.5mL microcentrifuge tube to a volume of 60µL (20µL from each replicate)
    - If 20µL from each sample is not possible, then a smaller amount that is similiar across replicates needs to be taken
2. Dilute each 60µL sample to 1:10
    - This may vary depending on concentration and downstream application
    - Added 540µL of PCR water
3. Gently mixed by pipetting
    - A completely homogenous sample is paramount at this step
4. Pooled samples(RT +) were produced
    - 3x as much RT + was needed to compare to samples
        - 75µL(from each samples) x 21 samples = 1,575µL into clean 2mL centrifuge tube
    - Pooled samples were used on dissociation plate to make sure primer sets worked
5. The RT+ positive was split into 175µL aliquots in seperate PCR tubes. RT+ positive used immediatly was kept in the fridge, whereas the samples not used immediately were stored into -20°C freezer
    - Note: this is done to limit freeze-thaw
6. Each sample was(approximately 520µl remaining) was split into 175µL aliquots in seperate PCR tubes. cDNA samples used immediatly was kept in the fridge, whereas the samples not used immediately were stored into -20°C freezer
    - Note: this is done to limit freeze-thaw

Keep cDNA always on ice while conducting this protocol. cDNA is time and temperature sensitive

Protocol #6: Primer Dilution and Worker stock

Materials

- 1.5 mL microcentrifuge tue
- Primers, which were designed in Integrated DNA Technology (IDT). Two Primers sets were needed per experimental gene, including atleast 10 reference genes.
    - Both Forward and reverse primers are needed
    - For the two primers sets for experimental genes, the primers need to be found on different parts of the DNA
- PCR water

Equipment

- None

Protocol

1. We made 100µm stock solutions for the primers by multiplying nmoles of primer(this number is on primer label) by 10. Then, we added that volume in µl of PCR water
    -

ID	nmoles	nmoles x 10
#000	34	340
#001	26	260

    - Dilutions were made for both Forward and Reverse primer seperately
2. A working stock was produced by combining 40µl of both the Forward and Reverse primer with 720 µl of PCR water

Protocol #7: Dissociation Plate (Used for reference genes)

Documents

- Both templates(Primer template and cDNA template), loading plate, and reaction conditions

  Garett-Dissociation_Plate_Feb_3_2017.docx

- Light layout for LightOne Pro

  Garett_Dissociation_Plate_Feb_3_2017.csv

Materials

- 96 well plate
- PCR water
- SYBR Green Real-Time PCR Master mixes
- 384 well PCR microplate
- Pooled samples (RT+) from Protocol #5
- Primer working stocks from Protocol #6

Equipment

- Roche 480 LightCycler
- LightOne Pro

Protocol

1. Pooled samples (RT +) of cDNA was used for dissociation plate, along with all possible reference genes(9 in this case) and experimental genes(6 in this case)
2. Plate layout for the dissociation plate were produced. Two template layouts were produced: (1) Primer template and (2) cDNA template
   - **Primer template-The numbers represent the the different genes and primer sets. The color blue denoting the 9 reference genes and the green denoting the 6 experimental genes**

1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B	10_1	10_1	10_1		14_3	14_3	14_3		26_1	26_1	26_1		18_1	18_1	18_1									
C																								
D	10_3	10_3	10_3		14_5	14_5	14_5		26_5	26_5	26_5		18_2	18_2	18_2									
E																								
F	11_1	11_1	11_1		16_1	16_1	16_1		3_1	3_1	3_1		19_1	19_1	19_1									
G																								
H	11_2	11_2	11_2		16_5	16_5	16_5		3_2	3_2	3_2		19_2	19_2	19_2									
I																								
J	12_1	12_1	12_1		26_3	26_3	26_3		7_1	7_1	7_1		25_1	25_1	25_1									
K																								
L	12_2	12_2	12_2		26_4	26_4	26_4		7_2	7_2	7_2		25_3	25_3	25_3									
M																								
N	13_1	13_1	13_1		27_1	27_1	27_1		15_1	15_1	15_1													
O																								
P	13_3	13_3	13_3		27_2	27_2	27_2		15_5	15_5	15_5													

   - Note: This template is how the primers were tested for validity.
   - **cDNA Template-There was a total of three replicates: 2 RT+ and 1 non-template for each primer set. For non-template Master Mix(MM), 4µl water is used instead of RT +**

1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None		RT+	RT+	None									
C																								
D	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None		RT+	RT+	None									
E																								
F	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None		RT+	RT+	None									
G																								
H	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None		RT+	RT+	None									
I																								
J	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None		RT+	RT+	None									
K																								
L	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None		RT+	RT+	None									
M																								
N	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None													
O																								
P	RT+	RT+	None		RT+	RT+	None		RT+	RT+	None													

3. To produce the dissociation plate, a loading plate (96 well plate) was used for the transer
   - **Primer and Master Mix Loading plate (96 well)**

1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10_1	14_3	26_1	18_1						Template Master Mix		No Template Master Mix
B	10_3	14_5	26_1	18_2								
C	11_1	16_1	3_1	19_1								
D	11_2	16_5	3_2	19_2								
E	12_1	26_3	7_1	25_1								
F	12_2	26_4	7_2	25_3								
G	13_1	27_1	15_1									
H	13_3	27_2	15_5									

   - Volumes for each component in loading plate:
   - Primers-> 1µl/Rxn which equates to a total of 6µl (3µl + 3 for residual) per well (Columns 1-4)
   - **Template and Non-template master mix (column 10 & 12)**
   -

Template Master Mix		No Template Master Mix	
For 1	For 66((21 samples x3 replicates)+2)	For 1	For 33
4µ RT +	264µl	RT+	-
5µl SYBR Green Real-Time Master Mix	330µl	5µl Master Mix	165µl
PCR Water	-	4µl PCR Water	132µl
Total	**594µl**	**9µl/ Rxn**	**297µl**

- Template and non-template master mix were mixed via pipetting

4. **A 384 well PCR microplate was used for the dissociation plate, and primer and master mix were transferred by the folowing protocol:**
    1. **First**, 1µl of the primers from columns 1-4 in the master mix plate were transfered onto the dissociation plate according to the Primer template layout above
    2. **Second**, 9µl of either the template or the non-template master mix in columns 10 or 12 were transferred onto the dissociation plate according to cDNA template layout above
        - Note: Lights using LightOne Pro were used to transfer the primer and master mixes into the PCR microplate to make sure transfers occurred correctly. The light layout is shown below:

Plate	Wells							
Plate1	p1	n1	l1	j1	h1	f1	d1	b1
Plate1	p2	n2	l2	j2	h2	f2	d2	b2
Plate1	p3	n3	l3	j3	h3	f3	d3	b3
Plate1	p5	n5	l5	j5	h5	f5	d5	b5
Plate1	p6	n6	l6	j6	h6	f6	d6	b6
Plate1	p7	n7	l7	j7	h7	f7	d7	b7
Plate1	p9	n9	l9	j9	h9	f9	d9	b9
Plate1	p10	n10	l10	j10	h10	f10	d10	b10
Plate1	p11	n11	l11	j11	h11	f11	d11	b11
Plate1					h13	f13	d13	b13
Plate1					h14	f14	d14	b14
Plate1					h15	f15	d15	b15
Plate2	p1	n1	l1	j1	h1	f1	d1	b1
Plate2	p2	n2	l2	j2	h2	f2	d2	b2
Plate2	p5	n5	l5	j5	h5	f5	d5	b5
Plate2	p6	n6	l6	j6	h6	f6	d6	b6
Plate2	p9	n9	l9	j9	h9	f9	d9	b9
Plate2	p10	n10	l10	j10	h10	f10	d10	b10
Plate2					h13	f13	d13	b13
Plate2					h14	f14	d14	b14
Plate3	p3	n3	l3	j3	h3	f3	d3	b3
Plate3	p7	n7	l7	j7	h7	f7	d7	b7
Plate3	p11	n11	l11	j11	h11	f11	d11	b11
Plate3					h15	f15	d15	b15

5. The dissociation plate was place into a Roche 480 lightcylcer, with the following reaction conditions:
    - Based on Brant's "Leafy Spurge Glycophosphate" program

Reaction Conditions: Based of Brant's "Leafy Spurge Glycosophate" program

Step	# cycles	Analysis Mode	Target °C	Acquisition Mode	Hold	Ramp Rate (°C/sec)
Pre-incubation	1	None	95°C	None	10 sec	4.8
			95°C	None	20 sec	4.8
Amplification	45	Quantification	55°C	None	10 sec	2.5
			70°C	Single	35 sec	4.8
			95°C	None	5 sec	4.8
Melting Curve	1	Melting Curve	65°C	None	1 min	2.5
			97°C	Continuous	-	0.11
Cooling	1	None	40°C	None	30 sec	2.5

6. To determine if the primers worker and were usable, we looked at both the absolute concentration and number of amplified products (Only one was warranted)

Protocol #8: Plates for expression(2 genes per plate)

Abstract

Documents

- Both templates(Primer &Master Mix template and cDNA template), loading plate, and reaction calculation

  Garett_Honeybee_Two_Gene_Layout_Feb_13_2017.doc

- Light layout for LightOne Pro

  Plate1-in01-Master_Ref_Gene_Plate_1_June_30_2014.csv

Materials

- 96 well plate
- PCR water
- SYBR Green Real-Time PCR Master mixes
- 384 well PCR microplate
- Pooled samples (RT+) from Protocol #5
- Primer working stocks from Protocol #6
- Calibrating gene working stocks

Equipment

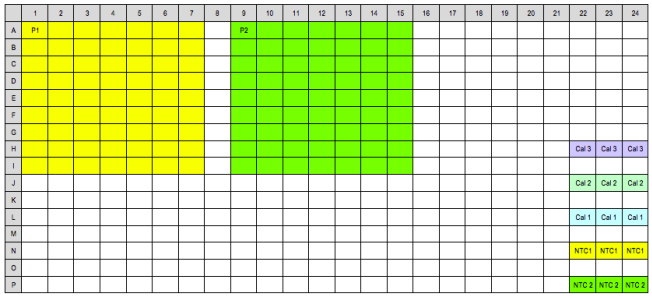
- Roche 480 LightCycler
- LightOne Pro

Protocol

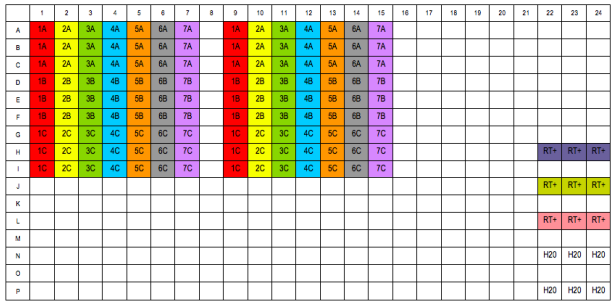
1. Two genes per plate were tested
    - Note: Two genes can be more or less depending upon number of samples
    - Note: Reference genes were tested first to determined if expression was consistent across both time and development. Depending on the Q-base program (analysis program), more or less than

3 reference genes are needed for the analysis. Because of this, plates with references genes need to be run first until acceptable references are found before experimental genes are run.

2. Plate layout for the two genes used
    1. **Primer and Master Mix Template-Yellow represents gene #1 and Green represents gene #2. Calibrating gene 1(Cal 3) was the light blue, Calibrating gene 2(Cal 2) was light green, and Calibrating gene 3(Cal3) was the darker blue color. NTC1 is the Non-template gene 1 and NTC2 is the non-template gene 2, which do not have RT +.**



    1. **cDNA Template-** This plate layout includes 3 replicates of the 21 samples, which was done seperately for each of the two genes.



1.

1A (40_A)	5A (190_A)
1B (40_B)	5B (190_B)
1C (40_C)	5C (190_C)
2A (70_A)	6A (240_A)
2B (70_B)	6B (240_B)
2C (70_C)	6C (240_C)
3A (100_A)	7A (Cont_A)
3B (100_B)	7B (Cont_B)
3C (100_C)	7C (Cont_C)
4A (130_A)	
4B (130_B)	
4C (130_C)	

        - Note: Three reference primers were chosen for the calibrating genes because they had a cp value below 20. These three genes were prepared by producing a workeing stock just for the calibrating genes. The working stocks were produced by admixing 40µl of both the Forward and Reverse primers with 720µl of PCR water.

3. To prepare loading plates(which are used for transfer into microplates), a chart was used to calculate volumes needed for transfer
    1. 10µl reactions (RT + template and primer)

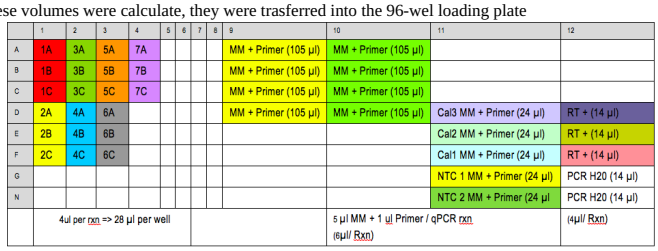
10-µL-reactions (RT+ Template and primer)

1358

Primer + Master Mix			No Template Master Mix			Cal 1	Cal 2	Cal 3
For 70	For 1		For 8	For 1		RT+	RT+	RT+
						p2032	p6928	p0922
-	See below	cDNA Template (1/4X stock)	-	-	cDNA Template (1/4x stock)	See below	See below	See below
350 ul	5ul	Master Mix (2X)	40ul	5ul	Master Mix (2X)	15ul	15	15
-	-	ddH20 (roche lightcycler kit)	32ul	4ul	ddH20 (roche lightcycler kit)	-	-	-
70 ul	1ul	Primer (Fwd +Rev)(10uM ea)	-	See below	Primer (Fwd +Rev)(10uM ea)	3ul	3	3
420 ul	6 ul / rxn		72	9 ul / rxn		18 ul	18	18
÷4 =		Add 4ul of ¼ x template to each	÷2 =		Add 1ul primer (1358) to well after adding mix	÷ 3=	÷ 3=	÷ 3=
105 ul per well			36 ul / 2 well		(or add 4ul primer to 36ul master mix in ea. Tube)	6ul/well	6ul/well	6ul/well
						(Add 4ul of ¼ x template to each rxn well)		

1.

4. After these volumes were calculate, they were trasferred into the 96-wel loading plate



1.

5. Using a multi-chanel pippet, these components were transferred into a PCR microplate according to both the **Primer and Master mix template** and the **cDNA template** noted above.
    - To guide transfer from the 96-well loading plate to the microplate, we used the LightOne Pro. The light layout is listed below

Plate	Wells							
Plate1	a1	a3	a5	a7	a9	a11	a13	a15
Plate1	b1	b3	b5	b7	b9	b11	b13	b15
Plate1	c1	c3	c5	c7	c9	c11	c13	c15
Plate1	d1	d3	d5	d7	d9	d11	d13	d15
Plate1	e1	e3	e5	e7	e9	e11	e13	e15
Plate1	f1	f3	f5	f7	f9	f11	f13	f15
Plate1	g1	g3	g5	g7	g9	g11	g13	g15
Plate1	h1	h3	h5	h7	h9	h11	h13	h15
Plate1	i1	i3	i5	i7	i9	i11	i13	i15
Plate1	a2	a4	a6	a8	a10	a12	a14	
Plate1	b2	b4	b6	b8	b10	b12	b14	
Plate1	c2	c4	c6	c8	c10	c12	c14	
Plate1	d2	d4	d6	d8	d10	d12	d14	
Plate1	e2	e4	e6	e8	e10	e12	e14	
Plate1	f2	f4	f6	f8	f10	f12	f14	
Plate1	g2	g4	g6	g8	g10	g12	g14	
Plate1	h2	h4	h6	h8	h10	h12	h14	
Plate1	i2	i4	i6	i8	i10	i12	i14	
Plate1	p22	n22	l22	j22	h22			
Plate1	p23	n23	l23	j23	h23			
Plate1	p24	n24	l24	j24	h24			
Plate2	a1	a3	a5	a7	a9	a11	a13	a15
Plate2	b1	b3	b5	b7	b9	b11	b13	b15
Plate2	c1	c3	c5	c7	c9	c11	c13	c15
Plate2	d1	d3	d5	d7	d9	d11	d13	d15
Plate2	e1	e3	e5	e7	e9	e11	e13	e15
Plate2	f1	f3	f5	f7	f9	f11	f13	f15
Plate2	g1	g3	g5	g7	g9	g11	g13	g15
Plate2	h1	h3	h5	h7	h9	h11	h13	h15
Plate2	i1	i3	i5	i7	i9	i11	i13	i15
Plate2	a2	a4	a6	a8	a10	a12	a14	
Plate2	b2	b4	b6	b8	b10	b12	b14	
Plate2	c2	c4	c6	c8	c10	c12	c14	
Plate2	d2	d4	d6	d8	d10	d12	d14	
Plate2	e2	e4	e6	e8	e10	e12	e14	
Plate2	f2	f4	f6	f8	f10	f12	f14	
Plate2	g2	g4	g6	g8	g10	g12	g14	
Plate2	h2	h4	h6	h8	h10	h12	h14	
Plate2	i2	i4	i6	i8	i10	i12	i14	
Plate2	p22	n22	l22	j22	h22			
Plate2	p23	n23	l23	j23	h23			
Plate2	p24	n24	l24	j24	h24			

6. Once the plates are finished, the data was analyzed using qBase.

References

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