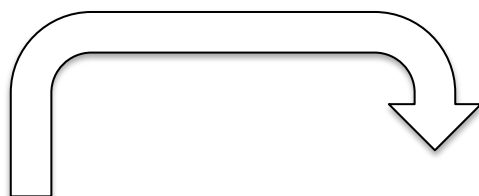


MASTER_MIX

350 nL in each well



Source

Destination

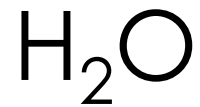
Plate Name: Source_MATER_MIX[1] << >>

	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																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

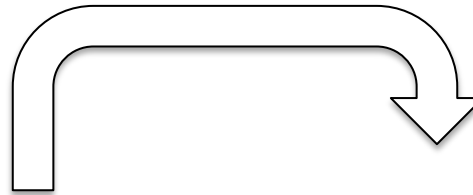
Plate Name: Destination_MASTER_MIX[1] << >>

	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	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
G	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
I	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
J	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
K	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
O	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

[65 μ L in each well]



25, 50, 75 or 125 nL in different wells



Source

Destination

Plate Name: Source_H2O[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																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

Plate Name: Destination_H2O[1]

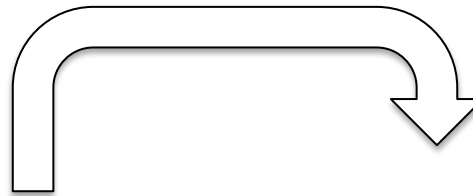
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1							1							1									
B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
D	1							1							1									
E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1	1	1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

[65 µL in each well]

25 nL (J2-J7, J9-J14, J18-J21, K22-K24, L22-L24, N22-N24, O22-O24, A1, A8, A15, D1, D8, D15, G1, G8, G15, K1, K8, K15, N1, N8, N15)
 50 nL (B2-B7, B9-B14, B18-B21, E2-E7, E9-E14, E18-E21, H2-H7, H9-H14, H18-H21, L2-L7, L9-L14, L18-L21, O2-O7, O9-O14, O18-O21, M23, P23, J1, J8, J15)
 75 nL (C2-C7, C9-C14, C18-C21, F2-F7, F9-F14, F18-F21, I2-I7, I9-I14, I18-I21, M2-M7, M9-M14, M18-M21, P2-P7, P9-P14, P18-P21, B1, B8, B15, E1, E8, E15, H1, H8, H15, L1, L8, L15, O1, O8, O15)
 100 nL (C1, C8, C15, F1, F8, F15, I1, I8, I15, M1, M8, M15, P1, P8, P15)

RNA

25 nL in each well



J1-J21, K22-24, L22-L24, M23,
N22-N24, O22-O24, P23
= RNA- CONTROLS

Source

Destination

Plate Name: Source[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																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

**4 $\mu\text{g}/\mu\text{L}$ [L1] – 400 $\text{ng}/\mu\text{L}$ [L2] – 40 $\text{ng}/\mu\text{L}$ [L3]
– 4 $\text{ng}/\mu\text{L}$ [L4] – 400 $\text{pg}/\mu\text{L}$ [L5]
[20 μL in each well]**

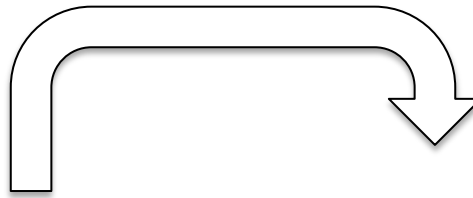
Plate Name: Destination[1]

	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	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
I	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
J																								
K	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
O	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

**100 ng [A1-I7] – 10 ng [A8-I14] – 1 ng [A16-
I21] – 100 pg [K1-M21] – 10 pg [N1-P21]**

RT_PRIMERS

25 nL in each well



Source

Destination

Plate Name: Source_RT_PRIMERS[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																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

**2,5 μ M [K1] – 5 μ M [K2] – 10 μ M [K3]
– 20 μ M [K4] – 40 μ M [K5] – 80 μ M [K6]
[20 μ L in each well]**

Plate Name: Destination_RT_PRIMERS[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		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
B		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
C		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
D		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
E		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
F		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
G		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
H		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
I		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
J		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
K		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
L		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
M		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
N		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
O		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			
P		1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1			

**0,125 μ M [col.2,9,16,K22,N22] – 0,25 μ M [col.
3,10,17,K23,N23] – 0,5 μ M [col.4,11,18,K24, N24]
– 1 μ M [col.5,12,19,L22, O22] – 2 μ M [col.
6,13,20,L23,O23] – 4 μ M [col. 7,14,21,L24, O24]**

TSO (barcodes 1-70)

25, 50, or 100 nL (1, 2 or 4 drops)

A1-A7, D1-D7, G1-G7, J1-J21 → 4 drops
 K22-K24, L22-L24, M23 → 4 drops
 N22-N24, O22-O24, P23 → 4 drops
 B1-B7, E1-E7, H1-H7 → 2 drops
 C1-C7, F1-F7, I1-I7 → 1 drop



Source

Destination

Plate Name: Source_TSO[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	1	1	1	1	1	1	1																	
B	1	1	1	1	1	1	1																	
C	1	1	1	1	1	1	1																	
D	1	1	1	1	1	1	1																	
E	1	1	1	1	1	1	1																	
F	1	1	1	1	1	1	1																	
G	1	1	1	1	1	1	1																	
H	1	1	1	1	1	1	1																	
I	1	1	1	1	1	1	1																	
J	1	1	1	1	1	1	1																	
K																								
L																								
M																								
N																								
O																								
P																								

400 μ M (rows A,B,C)
50 μ M (rows D,E,F)
6,25 μ M (rows G,H,I)
[20 μ L in each well]

Plate Name: Destination_TSO[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	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
G	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
I	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
J	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
K	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
L	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
O	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
P	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			

80 μ M (A1-A21, K1-K7, N1-N7) – **40 μ M** (B1-B21, L1-L7, O1-O7) – **20 μ M** (C1-C21, M1-M7, P1-P7) – **10 μ M** (D1-D21, K8-K14, N8-N14, J1-J21, K22-K24, L22-L24, M23, N22-N24, O22-O24, P23) – **5 μ M** (E1-E21, L8-L14, O8-O14) – **2,5 μ M** (F1-F21, M8-M14, P8-P14) – **1, 25 μ M** (G1-G21, K15-K21, N15-N21) – **0,625 μ M** (H1-H21, L15-L21, O15-O21) – **0,3125 μ M** (I1-I21, M15-M21, P1-P21)

MASTER_MIX PREPARATION

Reagent	Volume for 1 reaction (nL)	Stock conc.	Final conc.	Master_Mix for 384 reactions (201,25 µL)
Sorbitol/ Trehalose	40	0,66 M/3,3 M	0,0528M/0,264M	23
SuperScript III Reaction Buffer	100	5x	1x	57,5
DTT	50	0,1 M	0,01 M	28,75
dNTPs	31,25	10 mM	0,625 mM	17,97
Betain	75	5 M	0,75 M	43,13
SuperScript III	50	200 U/µL	20 U/uL	28,75
H ₂ O	3,75	-	-	2,16
TOTAL	350			201,25

$(65 - 15) / 350 = 142$ destination wells filled per source well

➔ 3x 65 µL wells required to effectively fill 384 wells