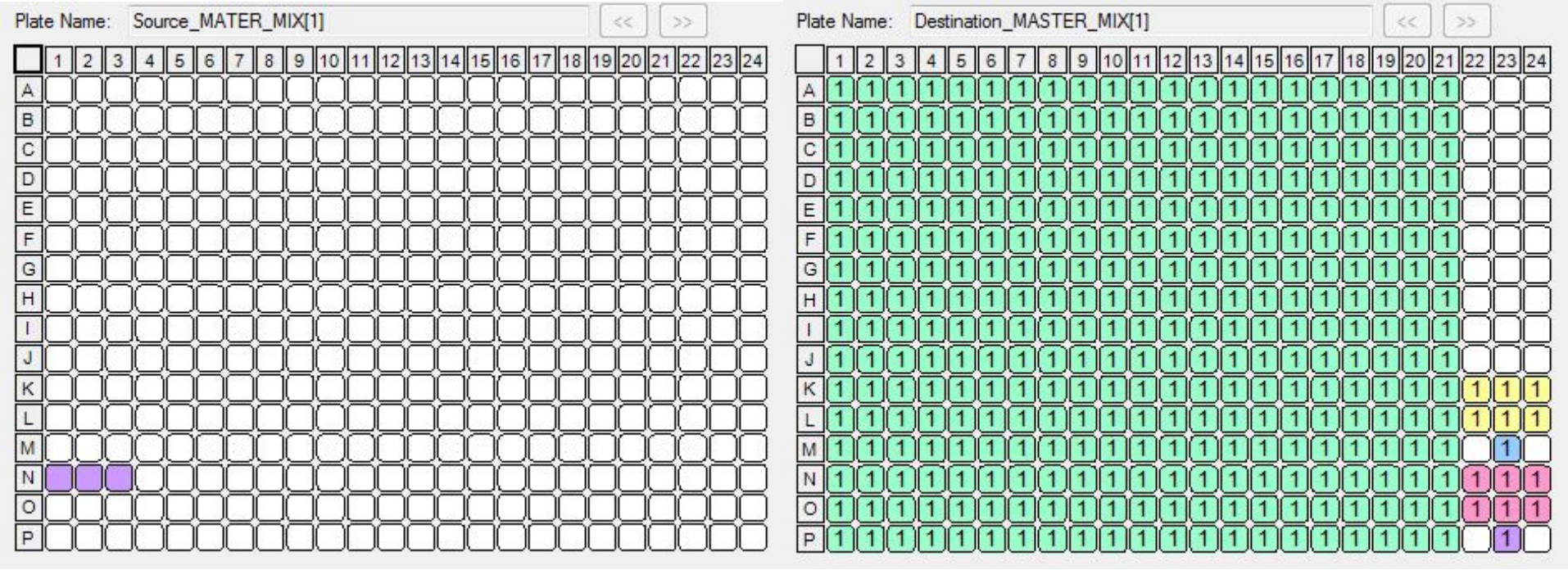
MASTER_MIX

350 nL in each well

Source

Destination



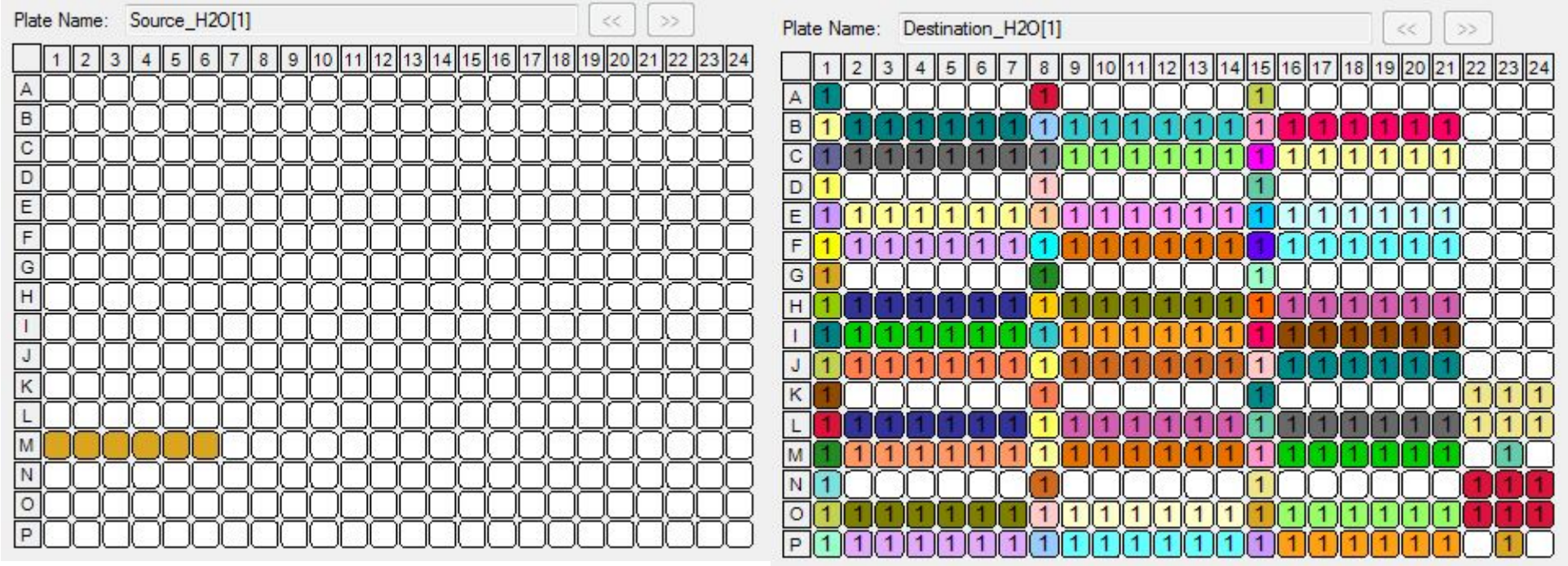
[60 µL in each well]

H_2O

25, 50, 75 or 125 nL in different wells

Source

Destination



[60 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)

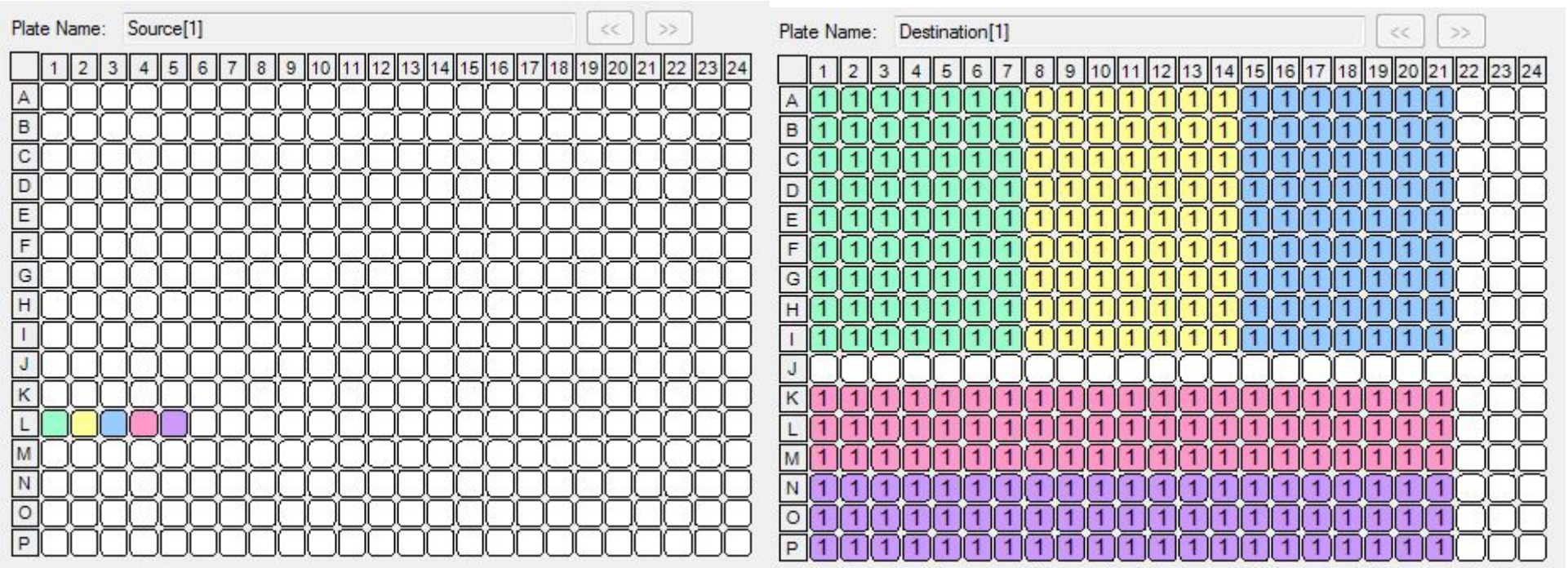
RNA

25 nL in each well

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

Source

Destination



4 µg/µL [L1] **– 400 ng/µL** [L2] **– 40 ng/µL** [L3] **– 4 ng/µL** [L4] **– 400 pg/µL** [L5]
[20 µL in each well]

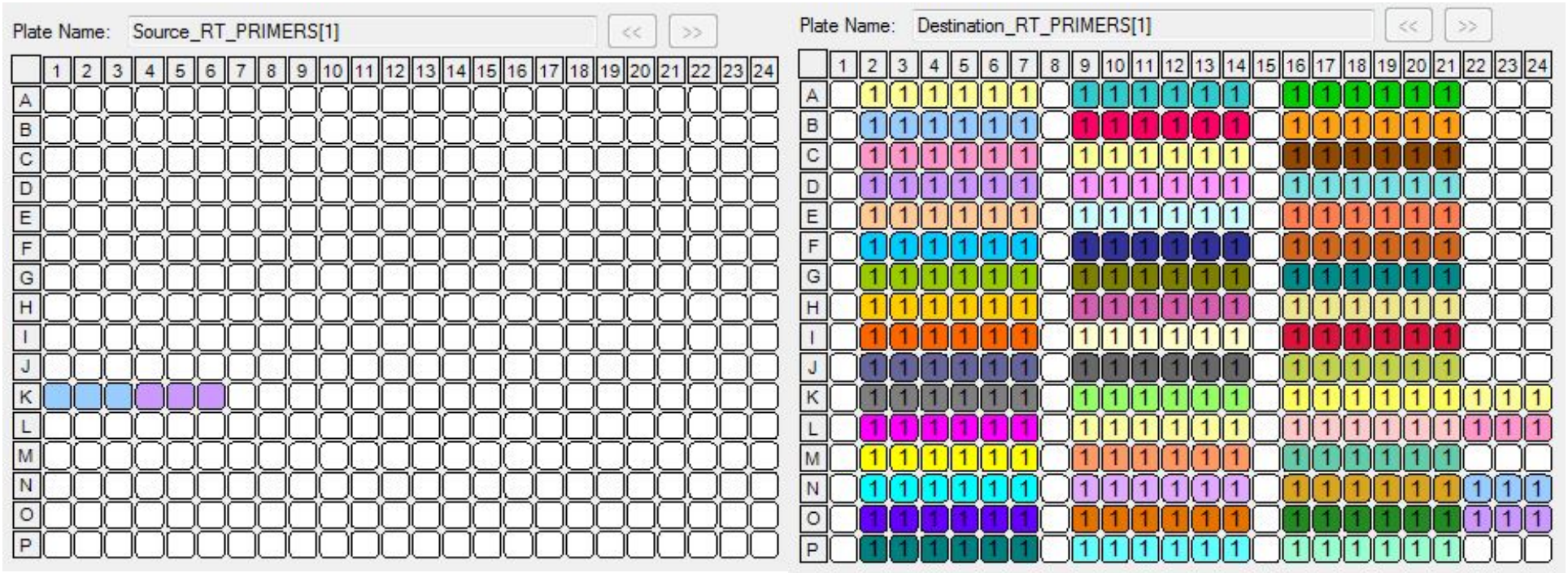
100 ng [A1-I7] **– 10 ng** [A8-I14] **– 1 ng** [A16-I21] **– 100 pg** [K1-M21] **– 10 pg** [N1-P21]

RT_PRIMERS

25 nL in each well

Source

Destination



10 µM [K1] **– 20 µM** [K2] **– 40 µM** [K3] **– 80 µM** [K4] **– 160 µM** [K5] **– 240 µM** [K6]
[20 µL in each well]

0.5 µM [col.2,9,16,K22,N22] **– 1 µM** [col. 3,10,17,K23,N23] **– 2 µM** [col.4,11,18,K24, N24] **– 4 µM** [col.5,12,19,L22, O22] **– 8 µM** [col. 6,13,20,L23,O23] **– 12 µM** [col. 7,14,21,L24, O24]

TSO (barcodes 1-70)

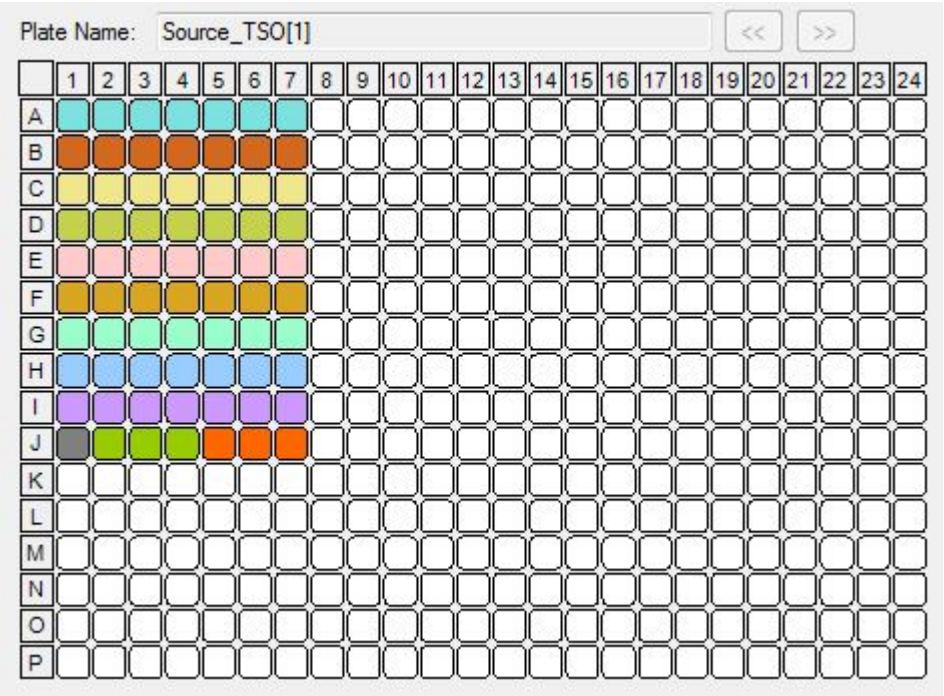
25, 50, or 100 nL (1, 2 or 4 drops)

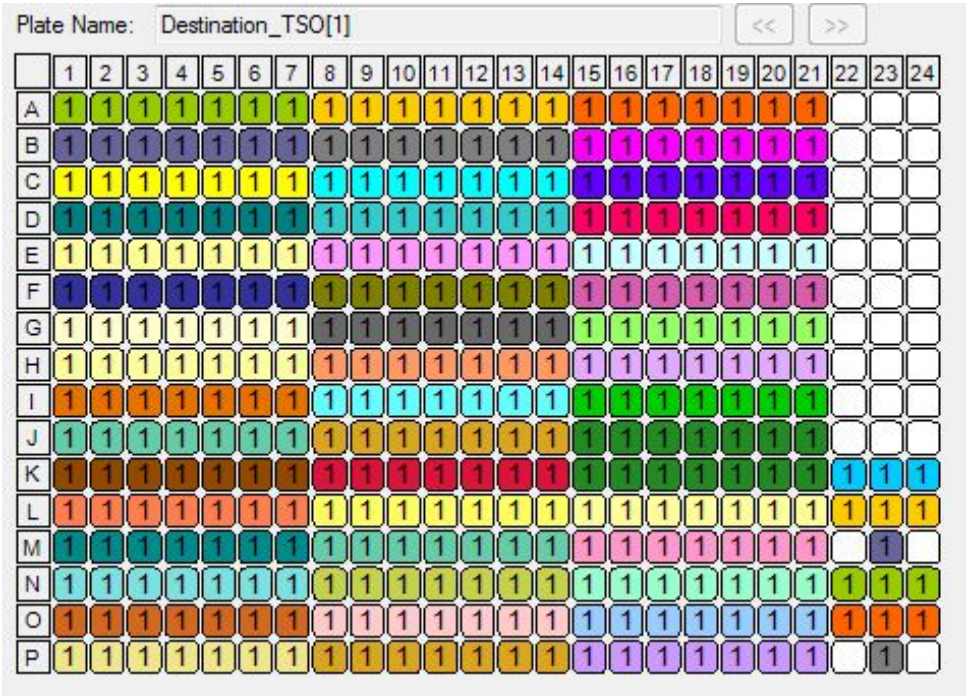
A1-A7, D1-D7, G1-G7, J1-J21 ➔ **4 drops**
B1-B7, E1-E7, H1-H7 ➔ **2 drops**
C1-C7, F1-F7, I1-I7 ➔ **1 drop**

(K22-K24, L22-L24, M23 ➔ **4 drops**
N22-N24, O22-O24, P23 ➔ **4 drops...)**

Source

Destination





600 µM (rows A,B,C)
400 µM (rows D,E,F)
50 µM (rows G,H,I,J)
[20 µL in each well]

120 µM (A1–A21, K1–K7, N1–N7) **– 60 µM** (B1–B21, L–1–L7, O1–O7) **– 30 µM** (C1–C21, M1–M7, P1–P7) **– 80 µM** (D1–D21, K8–K14, N8–N14, J1–J21, K22–K24, L22–L24, M23, N22–N24, O22–O24, P23) **– 40 µM** (E1–E21, L8–L14, O8–O14) **– 20 µM** (F1–F21, M8–M14, P8–P14) – **10 µM** (G1–G21, K15–K21, N15–N21) – **5 µM** (H1–H21, L15–L21, O15–O21) – **2.5 µM** (I1–I21, M15–M21, P1–P21)

MASTER_MIX PREPARATION (SSIII, nanoCAGE)

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_2O	3,75	-	-	2,16
TOTAL	**350**			**201,25**

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

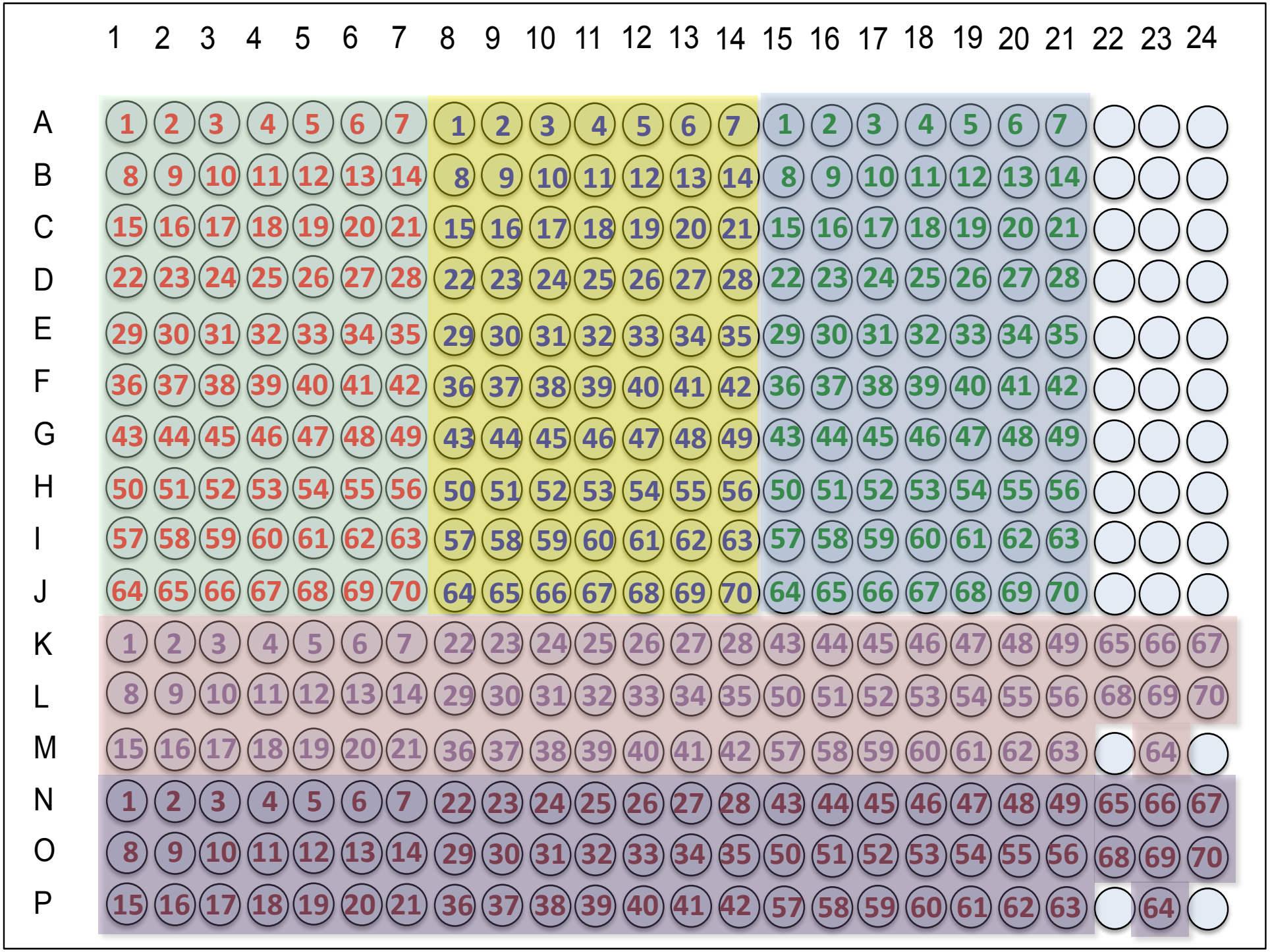
➔ 3x 65 μL wells required to effectively fill 384 wells

MASTER_MIX PREPARATION (SSIV, manufacturer)

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 IV Reaction Buffer	100	5x	1x	57,5
DTT	25	0,1 M	0,005 M	14,375
dNTPs	31,25	10 mM	0,625 mM	17,97
Betain	75	5 M	0,75 M	43,13
SuperScript IV	25	200 U/µL	10 U/uL	14,375
H_2O	53,75	-	-	30,90625
TOTAL	**350**			**201,25**

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

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


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 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7
B 8 9 10 11 12 13 14 8 9 10 11 12 13 14 8 9 10 11 12 13 14
C 15 16 17 18 19 20 21 15 16 17 18 19 20 21 15 16 17 18 19 20 21
D 22 23 24 25 26 27 28 22 23 24 25 26 27 28 22 23 24 25 26 27 28
E 29 30 31 32 33 34 35 29 30 31 32 33 34 35 29 30 31 32 33 34 35
F 36 37 38 39 40 41 42 36 37 38 39 40 41 42 36 37 38 39 40 41 42
G 43 44 45 46 47 48 49 43 44 45 46 47 48 49 43 44 45 46 47 48 49
H 50 51 52 53 54 55 56 50 51 52 53 54 55 56 50 51 52 53 54 55 56
I 57 58 59 60 61 62 63 57 58 59 60 61 62 63 57 58 59 60 61 62 63
J 64 65 66 67 68 69 70 64 65 66 67 68 69 70 64 65 66 67 68 69 70
K 1 2 3 4 5 6 7 22 23 24 25 26 27 28 43 44 45 46 47 48 49 65 66 67
L 8 9 10 11 12 13 14 29 30 31 32 33 34 35 50 51 52 53 54 55 56 68 69 70
M 15 16 17 18 19 20 21 36 37 38 39 40 41 42 57 58 59 60 61 62 63 64
N 1 2 3 4 5 6 7 22 23 24 25 26 27 28 43 44 45 46 47 48 49 65 66 67
O 8 9 10 11 12 13 14 29 30 31 32 33 34 35 50 51 52 53 54 55 56 68 69 70
P 15 16 17 18 19 20 21 36 37 38 39 40 41 42 57 58 59 60 61 62 63 64

		Random primer concentration (µM)						
		0	0,5	1	2	4	8	16
TSO	160	no_RT_PRIMERS	320,000	160,000	80,000	40,000	20,000	10,000
	80	no_RT_PRIMERS	160,000	80,000	40,000	20,000	10,000	5,000
	40	no_RT_PRIMERS	80,000	40,000	20,000	10,000	5,000	2,500
	60	no_RT_PRIMERS	120,000	60,000	30,000	15,000	7,500	3,750
	30	no_RT_PRIMERS	60,000	30,000	15,000	7,500	3,750	1,875
concentration	20	no_RT_PRIMERS	40,000	20,000	10,000	5,000	2,500	1,250
	10	no_RT_PRIMERS	20,000	10,000	5,000	2,500	1,250	0,625
	5	no_RT_PRIMERS	10,000	5,000	2,500	1,250	0,625	0,313
	2,5	no_RT_PRIMERS	5,000	2,500	1,250	0,625	0,313	0,156
(µM)	10	no_RT_PRIMERS	20,000	10,000	5,000	2,500	1,250	0,625

close to sc-nanoCAGE conditions

nanoCAGE conditions

no RNA

no RT primers

RT REACTION

- ABI 7900 HT qPCR system used for the RT

- RT conditions SSIII (nanoCAGE):

  - 22°C, 10 min.
  - 50°C, 30 min.
  - 70°C, 15 min.
  - 4°C, Hold

- RT conditions SSIV (manufacturer):

  - 23°C, 10 min.
  - 50°C, 10 min.
  - 80°C, 10 min.
  - 4°C, Hold

RT SAMPLES PURIFICATION

- 5 µL of water added in 7 wells, then RT products from 70 wells were collected (5 RNA concentration tested)
- 2x AMPure XP purification : 1.2x
- Elutions in 30 µL and 20 µL H_2O

qPCR

- Kapa Sybr Fast kit
- StepOne qPCR system
- Purified cDNA samples analysed in triplicates

cDNA PCR

- Kapa HiFi Hot Start
- amplification cycles for each sample
- AMPure XP purification : 1x
- Elution in 20 µL H_2O
- Picogreen quantitation and BioAnalyzer HS DNA chip