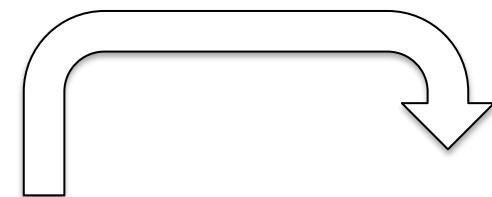


# MASTER\_MIX

350 nL in each well

Source

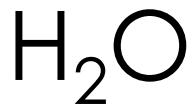
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J		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



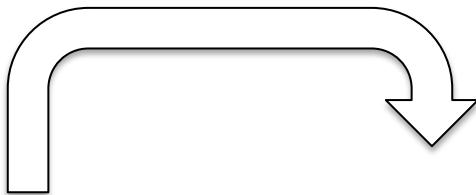
Destination

	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	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	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

[60 µL in each well]



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)**

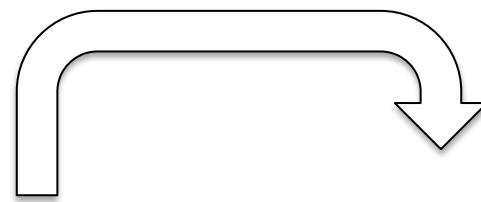
# RNA

25 nL in each well

Source

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



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

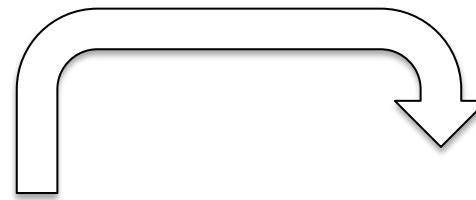
Destination

	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																								

**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]												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																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
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

**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\_1 (barcodes 1-70)

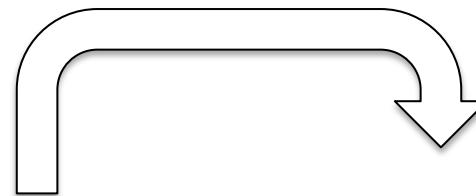
25, 50, or 100 nL (1, 2 or 4 drops)

C18-C21, F18-F21, I18-I21, L18-L21 → 4 drops  
D18-D21, G18-G21, J18-J21 → 2 drops  
E18-E21, H18-H21, K18-K21 → 1 drop

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

Source

Plate Name: Source_TSO2[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																								



Destination

Plate Name: Destination_TSO2[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		
B	1 1		
C	1 1		
D	1 1		
E	1 1		
F	1 1		
G	1 1		
H	1 1		
I	1 1		
J	1 1		
K	1 1		
L	1 1		
M	1 1		
N	1 1		
O	1 1		
P	1 1		

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

120 µM (A1-A21, K1-K7, N1-N7) – 60 µM (B1-B21, L1-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)

# TSO\_2 (barcodes 1-7+71-77+15-70)

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

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

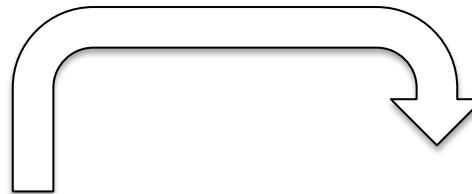
600 µM (rows A,B,C)

400 µM (rows D,E,F)

50 µM (rows G,H,I,J)

[20 µL in each well]

Row B was  
changed with  
barcodes 71-77 for  
troubleshooting !!



Destination

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

120 µM (A1-A21, K1-K7, N1-N7) – 60 µM (B1-B21, L1-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 <sub>2</sub> O	3,75	-	-	2,16
<b>TOTAL</b>	<b>350</b>			<b>201,25</b>

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

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

**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 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				

**(2)**

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	71 72 73 74 75 76 77	71 72 73 74 75 76 77	71 72 73 74 75 76 77	71 72 73 74 75 76 77	
C	15 16 17 18 19 20 21	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	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	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	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	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	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	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	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	71 72 73 74 75 76 77 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	71 72 73 74 75 76 77 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 ( $\mu$ M)						
		0	0,5	1	2	4	8	16
TSO concentration ( $\mu$ M)	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
	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
	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

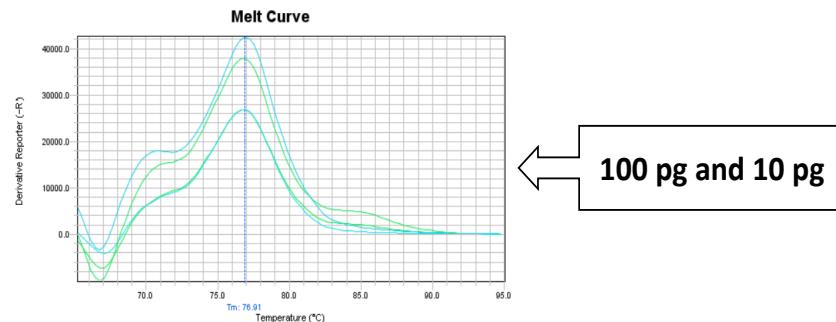
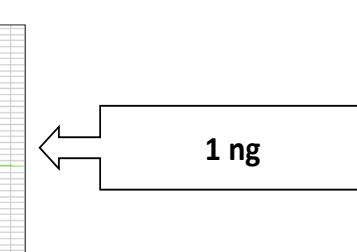
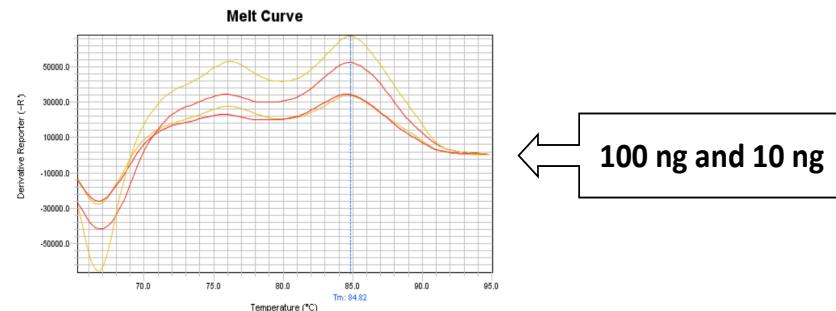
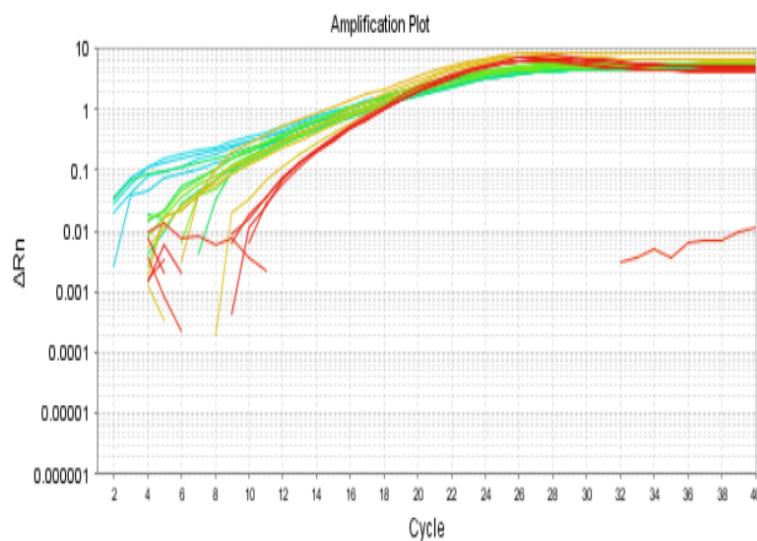
- ABI 7900 HT qPCR system used for the RT
- RT conditions SSIII (nanoCAGE):
  - 22°C, 10 min.
  - 50°C, 30 min.
  - 70°C, 15 min.
  - 4°C, Hold

# RT SAMPLES PURIFICATION

- 5  $\mu\text{L}$  of water added in 7 wells, then RT products from 70 wells were collected (5 RNA concentration tested)
- Dilute the pool of RT products (~70  $\mu\text{L}$ ) 2 times in water (+ ~140  $\mu\text{L}$   $\text{H}_2\text{O}$ )
- AMPure XP purification : 1x
- Elution in 100  $\mu\text{L}$
- AMPure XP purification : 1x
- Elution in 30  $\mu\text{L}$

# qPCR

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



100 ng and 10 ng

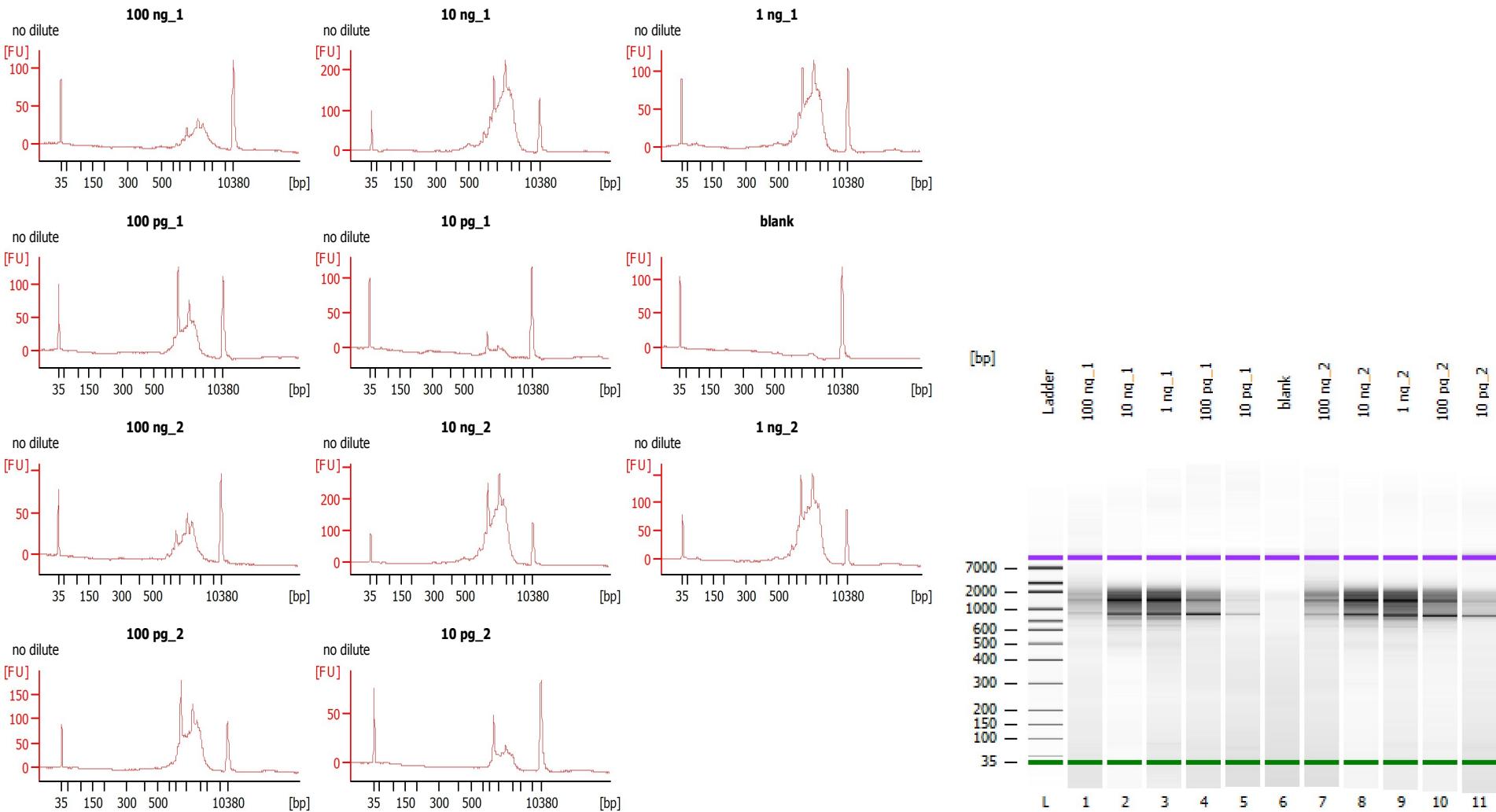
1 ng

100 pg and 10 pg

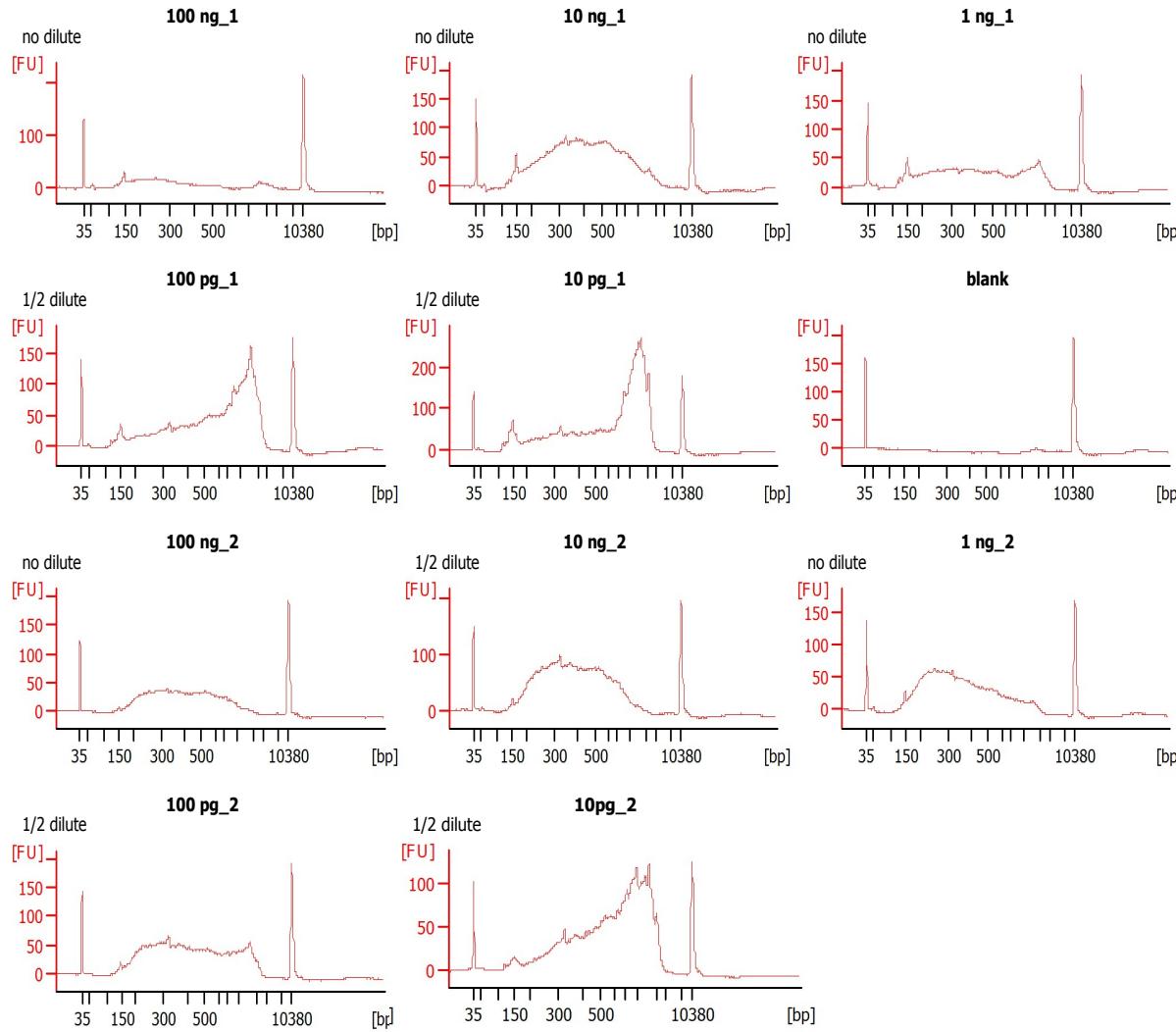
# cDNA PCR

- Kapa HiFi Hot Start
- 15, 18, 21, 24, and 27 amplification cycles respectively applied for 100-ng, 10-ng, 1-ng, 100-pg and 10-pg samples
- AMPure XP purification : 1x
- Elution in 20  $\mu\text{L}$   $\text{H}_2\text{O}$
- Picogreen quantitation and BioAnalyzer HS DNA chip

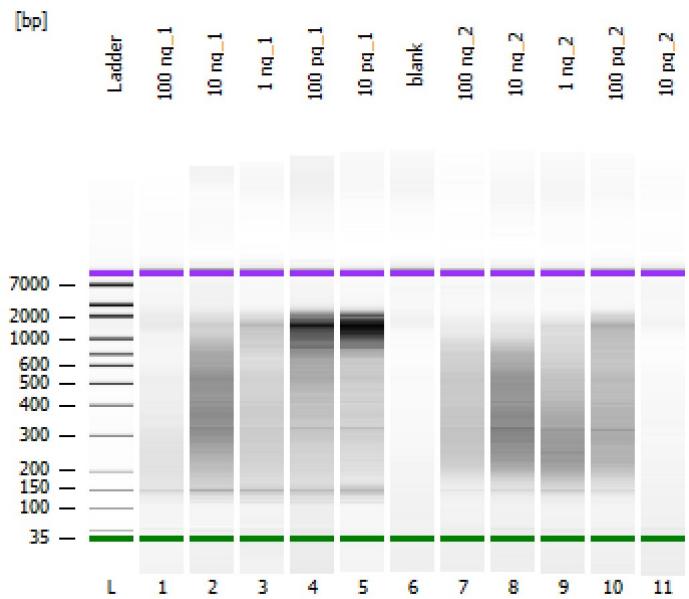
# cDNA PCR products QC



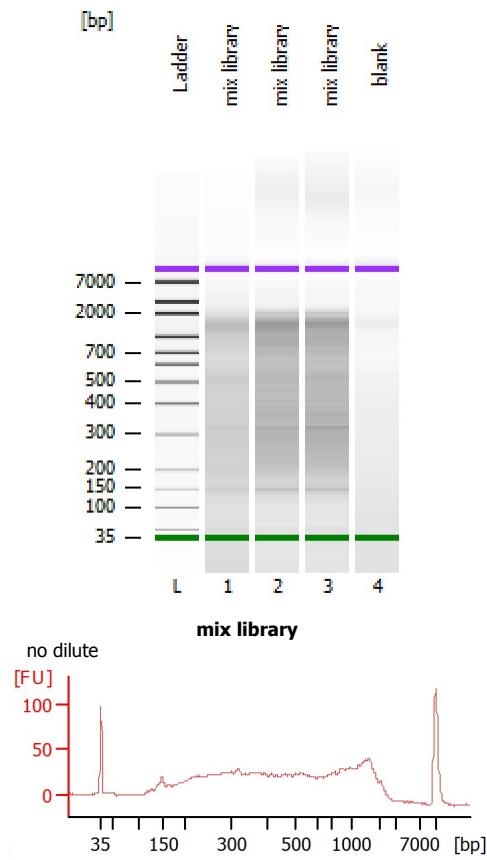
# Tagmentation and QC



There seems to be less/no artefacts for the plate 2 although the profiles of cDNA PCR products purified from plate 1 and plate 2 seems to be the same...  
Why ???



# Sequencing



# Comments