RNA reading evaluations and summary (after attaining RIN values of >8 for all RNAs on an Agilent Bioanalyzer 2100):

Sample ID	oznak	Nanodrop direct concentration (2x diluted for measurement)	real concentration	260/280	260/230	volume (ul)	Original sample remaining	after using:	dilute 1: 4	to get these ng/uL		contains 1000 ng		Water to get:	10 uL	use for RT	RT
1	wt-K	400.98	**801.96**	1.99	2.36	10	8	2 uL	8 uL	200.49 ng/uL	of this, take:	6.23 uL	+	3.77 uL	10 uL	: 8 uL	1000 ng RNA (per 10 uL)
2	wt-Cd	377.11	**754.22**	1.98	2.30	10	8	2 uL	8 uL	188.555 ng/uL	of this, take:	6.63 uL	+	3.37 uL	10 uL	: 8 uL	20 uL RT rxn
3	mu2-K	353.25	**706.50**	1.98	2.29	10	8	2 uL	8 uL	176.625 ng/uL	of this, take:	7.08 uL	+	2.92 uL	10 uL	: 8 uL	50 ng RNA/uL
4	mu2-Cd	335.91	**671.82**	1.98	2.28	10	8	2 uL	8 uL	167.955 ng/uL	of this, take:	7.44 uL	+	2.56 uL	10 uL	: 8 uL	
5	mu3-K	360.69	**721.38**	1.96	2.32	10	8	2 uL	8 uL	180.345 ng/uL	of this, take:	6.93 uL	+	3.07 uL	10 uL	: 8 uL	
6	mu3-Cd	416.04	**832.08**	1.89	1.94	10	8	2 uL	8 uL	208.02 ng/uL	of this, take:	6.01 uL	+	3.99 uL	10 uL	: 8 uL	
7	mu1-K	389.90	**779.80**	2.01	2.28	10	8	2 uL	8 uL	194.95 ng/uL	of this, take:	6.41 uL	+	3.59 uL	10 uL	: 8 uL	
8	mu1-Cd	396.70	**793.40**	1.97	2.23	10	8	2 uL	8 uL	198.35 ng/uL	of this, take:	6.30 uL	+	3.70 uL	10 uL	: 8 uL	
9	oe2-K	377.96	**755.92**	2.01	2.24	10	8	2 uL	8 uL	188.98 ng/uL	of this, take:	6.61 uL	+	3.39 uL	10 uL	: 8 uL	
10	oe2-Cd	372.90	**745.80**	1.94	2.19	10	8	2 uL	8 uL	186.45 ng/uL	of this, take:	6.70 uL	+	3.30 uL	10 uL	: 8 uL	
11	oe4-K	361.87	**723.74**	1.98	2.23	10	8	2 uL	8 uL	180.935 ng/uL	of this, take:	6.91 uL	+	3.09 uL	10 uL	: 8 uL	
12	oe4-Cd	378.00	**756.00**	1.96	2.23	10	8	2 uL	8 uL	189 ng/uL	of this, take:	6.61 uL	+	3.39 uL	10 uL	: 8 uL	
13	oe3-K	554.16	**1108.32**	2.04	2.26	15	13	2 uL	8 uL	277.08 ng/uL	of this, take:	4.51 uL	+	5.49 uL	10 uL	: 8 uL	
14	oe3-Cd	929.86	**1859.72**	1.99	2.05	15	13	2 uL	8 uL	464.93 ng/uL	of this, take:	2.69 uL	+	7.31 uL	10 uL	: 8 uL	
15	oe1-K	696.05	**1392.10**	2	2.04	15	13	2 uL	8 uL	348.025 ng/uL	of this, take:	3.59 uL	+	6.41 uL	10 uL	: 8 uL	
16	oe1-Cd	916.63	**1833.26**	1.98	1.99	15	13	2 uL	8 uL	458.315 ng/uL	of this, take:	2.73 uL	+	7.27 uL	10 uL	: 8 uL	
17	wt-K	944	**1888.00**	2.02	2.15	15	13	2 uL	8 uL	472 ng/uL	of this, take:	2.65 uL	+	7.35 uL	10 uL	: 8 uL	
18	wt-Cd	966.41	**1932.82**	1.99	2.05	12	10	2 uL	8 uL	483.205 ng/uL	of this, take:	2.59 uL	+	7.41 uL	10 uL	: 8 uL	

These 8 uL sample are used for RT...
(e.g.: each 8 uL contains 1000 ng of RNA)

Samples 17 and 18 are biological replicates of 1 and 2 (1 and 2 plants were grown at the same time as knocked out lines , whereas 17 and 18 were grown with overexpressing lines)

Just side Notes: 150 uL eluted from column
Turbo DNase treatment of 10-20 ug of each
using lowest Turbo DNase Enzyme amount suggested
Each 150 uL sample

approximate avg. sample dilution duiring TURBO

0.272727273

diluted this much during TURBO: 3.666666667

38.5 approximate recovered from 55 uL TURBO rxns.

reconcentration factor after TURBO:

reconcentrated ~this much during precipitation after TURBO: 3.208333333

Original overall sample dilution after precipitation since 150 uL column isolation: 0.875

or 1: 1.142857143

Final RNA dilutions arrived at so that 8 µl of each RNA can be added to 20 µl RT reactions such that the final RNA concentration in each reaction is 50 ng RNA/µl: with the reasonable condition met that the RNAs have each been diluted out far enough to effectively minimize inhibition of the RT enzyme during reverse transcription.

Original sample remaining		after using:	dilute 1: 4	to get these ng/uL		contains 1000 ng		Water to get:	10 uL	use for RT	RT
8	→	2 uL	→ 8 uL	200.49 ng/uL	of this, take:	6.23 uL	+	3.77 uL	10 uL	: 8 uL	→ 100
8	→	2 uL	→ 8 uL	188.555 ng/uL	of this, take:	6.63 uL	+	3.37 uL	10 uL	: 8 uL	2
8	→	2 uL	→ 8 uL	176.625 ng/uL	of this, take:	7.08 uL	+	2.92 uL	10 uL	: 8 uL	5
8	→	2 uL	→ 8 uL	167.955 ng/uL	of this, take:	7.44 uL	+	2.56 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	180.345 ng/uL	of this, take:	6.93 uL	+	3.07 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	208.02 ng/uL	of this, take:	6.01 uL	+	3.99 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	194.95 ng/uL	of this, take:	6.41 uL	+	3.59 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	198.35 ng/uL	of this, take:	6.30 uL	+	3.70 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	188.98 ng/uL	of this, take:	6.61 uL	+	3.39 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	186.45 ng/uL	of this, take:	6.70 uL	+	3.30 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	180.935 ng/uL	of this, take:	6.91 uL	+	3.09 uL	10 uL	: 8 uL	
8	→	2 uL	→ 8 uL	189 ng/uL	of this, take:	6.61 uL	+	3.39 uL	10 uL	: 8 uL	
13	→	2 uL	→ 8 uL	277.08 ng/uL	of this, take:	4.51 uL	+	5.49 uL	10 uL	: 8 uL	
13	→	2 uL	→ 8 uL	464.93 ng/uL	of this, take:	2.69 uL	+	7.31 uL	10 uL	: 8 uL	
13	→	2 uL	→ 8 uL	348.025 ng/uL	of this, take:	3.59 uL	+	6.41 uL	10 uL	: 8 uL	
13	→	2 uL	→ 8 uL	458.315 ng/uL	of this, take:	2.73 uL	+	7.27 uL	10 uL	: 8 uL	
13	→	2 uL	→ 8 uL	472 ng/uL	of this, take:	2.65 uL	+	7.35 uL	10 uL	: 8 uL	
10	→	2 uL	→ 8 uL	483.205 ng/uL	of this, take:	2.59 uL	+	7.41 uL	10 uL	: 8 uL	

These 8 uL sample are

nocked out lines , whereas 17 and 18 were grown with overexpressing lines)

(e.g.: each 8 uL contain

After you run your 20 µl, 50 ng RNA/µl RT reactions for each sample, dilute each RT reaction 1:5 with nuclease-free water, resulting in 18, 100-µl cDNA samples. From each of these 18, 100 µl, 1:5-diluted cDNA samples, steal 20 µl and mix together in a separate tube. This is your Stock I cDNA solution which will then be a 360 µl, ~10 ng/µl cDNA solution. Protect this Stock I with your life as it is the material for your Test Plate(s) (shown below), and is also the material to use for your final standard curves on your final plates.

For your Test Plate: take 185 µl of your Stock I and dilute 85 µl of it as follows:

1: 20.83	(uL) COMPREHESIVE SERIAL DILUTION TABLE (uL)					Achieved	Actual Final Dilutions Achieved
(Stock I) A	Total made	Stock I	Water	to Next	(FINAL VOL.)	Dilutions 1:	for Test Plate after used in-well:
B	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	2.2	1: 45.37116
C	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	4.7	1: 98.81003
D	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	10.3	1: 215.19
E	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	22.5	1: 468.6442
F	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	49.0	1: 1020.621
G	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	106.7	1: 2222.724
H	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	232.4	1: 4840.683
I	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	506.0	1: 10542.12
J	185.0 uL	85.0 uL	100.0 uL	85.0 uL	100.0 uL	1102.0	1: 22958.78
K	185.0 uL	85.0 uL	100.0 uL	0.0 uL	185.0 uL	2400.0	1: 50000

Realize that Tube A above contains 100 µl after 85 µl of it is removed to perform the serial dilutions of it for Tubes B thru K. These 11 dilutions are the 11 samples used on your Test Plate(s) shown below.

Test Plate MM set-up for your 9 genes (need 2 plates for 9 genes):

(NTC wells contain water instead of one of the 11 cDNA dilutions of Stock I prepared above)

NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene A
NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene B
NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene3
NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene4
NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene5
NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene6
NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene7
NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	gene8

NTC	1: 20.83	1: 45.4	1: 98.8	1: 215.2	1: 468.6	1: 1021	1: 2223	1: 4841	1: 10542	1: 22959	1: 50000	UbQ

Example Test Plate Master Mix Set-up for 9 targets: primers all used at 650 nM [final]

GOOD

Target Master Mix Set-up

25.00 uL prepared/Well	2X Master Mix:	2079.00 uL		Set 1/sample	101.33 uL	Well size prepared:	25.00 uL
20.00 uL used per Well	0X RT-Taq Solution:	0.00 uL		Set 1/MM ea	329.33 uL	RNA added/well:	6.00 uL
Depends on Machine/MM used	0X ROX or H2O:	0.00 uL				samples prepared	108
	Total MMRT prepared:	2079.00 uL			1	wells prepared	108
					Master adjust:	1.444444444	1
	Total MMRT needed:	1950.00 uL			Reset: "Ctrl r"		1.44444444

1			2		
gene A			gene B		
Fwd primer:	13.00 uL	split	Fwd primer:	13.00 uL	split
Rev primer:	13.00 uL	into	Rev primer:	13.00 uL	into
Probe:	0.00 uL	12	Probe:	0.00 uL	12
MMRT:	216.67 uL	19.00 uL amounts	MMRT:	216.67 uL	19.00 uL amounts
	0.00 uL	then add 6.00 uL		0.00 uL	then add 6.00 uL
water:	86.67 uL	Sample to each	water:	86.67 uL	Sample to each

129.00 uL xtra made

Test Plate(s)

Main Primer-Probe nM

300 nM FWD primer

300 nM REV primer

3			4		
gene3			gene4		
Fwd primer:	13.00 uL	split	Fwd primer:	13.00 uL	split
Rev primer:	13.00 uL	into	Rev primer:	13.00 uL	into
Probe:	0.00 uL	12	Probe:	0.00 uL	12
MMRT:	216.67 uL	19.00 uL amounts	MMRT:	216.67 uL	19.00 uL amounts
	0.00 uL	then add 6.00 uL		0.00 uL	then add 6.00 uL
water:	86.67 uL	Sample to each	water:	86.67 uL	Sample to each

SYBR Green Mix

Eurogentec
SYBR Master Mix
5 min. @ 95C
Then 40 to 50 cycles of:
15 sec@95C 1min @60C
then: Melt curve analysis

5			6		
gene5			gene6		
Fwd primer:	13.00 uL	split	Fwd primer:	13.00 uL	split
Rev primer:	13.00 uL	into	Rev primer:	13.00 uL	into
Probe:	0.00 uL	12	Probe:	0.00 uL	12
MMRT:	216.67 uL	19.00 uL amounts	MMRT:	216.67 uL	19.00 uL amounts
	0.00 uL	then add 6.00 uL		0.00 uL	then add 6.00 uL
water:	86.67 uL	Sample to each	water:	86.67 uL	Sample to each

7			8		
gene7			gene8		
Fwd primer:	13.00 uL	split	Fwd primer:	13.00 uL	split
Rev primer:	13.00 uL	into	Rev primer:	13.00 uL	into
Probe:	0.00 uL	12	Probe:	0.00 uL	12
MMRT:	216.67 uL	19.00 uL amounts	MMRT:	216.67 uL	19.00 uL amounts
	0.00 uL	then add 6.00 uL		0.00 uL	then add 6.00 uL
water:	86.67 uL	Sample to each	water:	86.67 uL	Sample to each

Total 50 mM MgSO4
0 uL
needed here
50 mM MgSO

0 uL Mg2+ solution per target | To get: 4 mM final in-well [Mg+2] | mM Mg+2 in Master Mix @ 1X: 4

9			10		
ubiquitin					
Fwd primer:	13.00 uL	split	Fwd primer:		
Rev primer:	13.00 uL	into	Rev primer:		
Probe:	0.00 uL	12	Probe:		
MMRT:	216.67 uL	19.00 uL amounts	MMRT:		
	0.00 uL	then add 6.00 uL			
water:	86.67 uL	Sample to each	water:		

After theTest Plate(s) are run, we then use (ISURF #03407) to identify the good sample and standard dilution ranges for each target, and then, set up your 5 final plates accordingly:

The 5 final plates would look like (all NTC, standards and samples performed in duplicate replicate):

NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene A
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	
NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene B
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	

NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene3
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	
NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene4
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	

NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene5
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	
NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene6
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	

NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene7
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	
NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	gene8
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	

NTC	NTC	Stnd1	Stnd1	Stnd2	Stnd2	Stnd3	Stnd3	Stnd4	Stnd4	Stnd5	Stnd5	UbQ
1	1	2	2	3	3	4	4	5	5	6	6	
7	7	8	8	9	9	10	10	11	11	12	12	
13	13	14	14	15	15	16	16	17	17	18	18	

(Thinking ahead a little here (to avoid running out of master mix during final analyses): The Master Mix set-up for your final 5 plates would thus look like:

GOOD	Target Master Mix Set-up(s)		xtra ea.made	Well size prepared:	25.00 uL
25.00 uL prepared/Well	2X Master Mix:	6426.00 uL	Extra/Target 101.33 uL	RNA added/well:	6.00 uL
25.00 uL used per Well	0X RT-Taq Solution:	0.00 uL	Total MM ea. 1013.33 uL	samples prepared	216
Depends on Machine/MM used	0X ROX or H2O:	0.00 uL	1	wells prepared	432
SYBR?: X	Total MMRT prepared:	6426.00 uL	Master adjust:	1.111111111	1
1	Total MMRT needed:	6000.00 uL	2	Reset: "Ctrl r"	1.11111111
	xtra made	426.00 uL			

1 SnRK2.4			2 SnRK2.10		
Fwd primer:	40.00 uL	split	Fwd primer:	40.00 uL	split
Rev primer:	40.00 uL	into	Rev primer:	40.00 uL	into
Probe:	0.00 uL	24	Probe:	0.00 uL	24
MMRT:	666.67 uL	38.00 uL amounts	MMRT:	666.67 uL	38.00 uL amounts
	0.00 uL	then add 12.00 uL		0.00 uL	then add 12.00 uL
water:	266.67 uL	Sample to each	water:	266.67 uL	Sample to each

3 GDH1			4 NR1		
Fwd primer:	40.00 uL	split	Fwd primer:	40.00 uL	split
Rev primer:	40.00 uL	into	Rev primer:	40.00 uL	into
Probe:	0.00 uL	24	Probe:	0.00 uL	24
MMRT:	666.67 uL	38.00 uL amounts	MMRT:	666.67 uL	38.00 uL amounts
	0.00 uL	then add 12.00 uL		0.00 uL	then add 12.00 uL
water:	266.67 uL	Sample to each	water:	266.67 uL	Sample to each

5 IRT1			6 FIT		
Fwd primer:	40.00 uL	split	Fwd primer:	40.00 uL	split
Rev primer:	40.00 uL	into	Rev primer:	40.00 uL	into
Probe:	0.00 uL	24	Probe:	0.00 uL	24
MMRT:	666.67 uL	38.00 uL amounts	MMRT:	666.67 uL	38.00 uL amounts
	0.00 uL	then add 12.00 uL		0.00 uL	then add 12.00 uL
water:	266.67 uL	Sample to each	water:	266.67 uL	Sample to each

7 FRO2			8 PDF2		
Fwd primer:	40.00 uL	split	Fwd primer:	40.00 uL	split
Rev primer:	40.00 uL	into	Rev primer:	40.00 uL	into
Probe:	0.00 uL	24	Probe:	0.00 uL	24
MMRT:	666.67 uL	38.00 uL amounts	MMRT:	666.67 uL	38.00 uL amounts
	0.00 uL	then add 12.00 uL		0.00 uL	then add 12.00 uL
water:	266.67 uL	Sample to each	water:	266.67 uL	Sample to each

9 UBQ			10		
Fwd primer:	40.00 uL	split	Fwd primer:		
Rev primer:	40.00 uL	into	Rev primer:		
Probe:	0.00 uL	24	Probe:		
MMRT:	666.67 uL	38.00 uL amounts	MMRT:		
	0.00 uL	then add 12.00 uL			
water:	266.67 uL	Sample to each	water:		

Sample Plate(s)

Main Primer-Probe nM
300 nM FWD primer
300 nM REV primer

SYBR Green Mix
3 to 5 min 50°C
2 to 5 min 95°C
Then 40-50 cycles of:
[15s 95°C:30s 60°C]
Then melting curve analysis

Be certain to use the correct thermo protocol~!

mM $MgSO_4$

Total 50 mM MgSO4
0.00 uL
needed here
50

0 uL Mg2+ solution per target | To get: 3 mM final in-well [Mg+2] | mM Mg+2 in Master Mix @ 1X: 3

Remember the sub-master mix for each target is assembled in nuclease-free 1.5 mL tubes. Never assemble reagents directly into the 96-well plate itself, technical replicates should always be prepared in a tube and dispensed into the 96-well plate from the tube. As you set these things up – you will see what I mean …

Your RNAs are of perfect concentrations to get robust qPCR results

Test Plate Cq values pasted into the UMES.xls files region (for the first 7 targets) cells B197:H208, then run the Ctrl Shift C Macro – and then look at the points generated in the TestPlateResultsAnalysis.xls files.

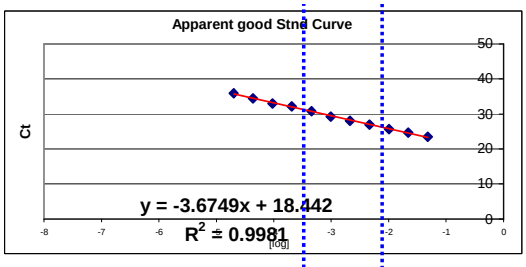
ENTER Test Plate Cts Here:

	SnRK2.4	SnRK2.10	GDH1	NR1	IRT1	FIT	UBQ
NTC	50.00	38.97	38.78	50.00	50.00	35.85	50.00
1	23.50	25.47	21.79	23.68	22.97	24.05	24.10
2	24.67	26.69	23.36	24.80	24.16	25.27	24.77
3	25.68	27.85	24.73	26.17	25.03	26.80	25.88
4	26.92	28.95	25.32	27.45	26.28	27.90	27.43
5	28.05	30.39	27.03	28.41	27.55	28.95	28.87
6	29.25	31.36	28.17	29.82	28.84	30.05	30.25
7	30.84	33.02	29.35	31.23	31.11	31.20	30.99
8	32.17	33.96	31.53	32.61	31.92	32.63	32.28
9	33.00	35.23	33.83	33.04	32.73	34.07	33.96
10	34.48	35.22	50.00	35.11	50.00	36.12	35.81
11	35.93	36.66	50.00	50.00	50.00	34.99	36.36

Extra targets (beyond 14 targets) are processed using auxillary Test Plate analysis files …

Sample	Type	in-well Dilution 1:	Primer/Probe	Paste Cts
A1	NTC	FRO2	FRO2	50
A2	UNKN	20.833333	FRO2	22.63
A3	UNKN	45.37116	FRO2	23.19
A4	UNKN	98.810025	FRO2	24.71
A5	UNKN	215.19002	FRO2	25.36
A6	UNKN	468.64421	FRO2	26.27
A7	UNKN	1020.6207	FRO2	28.06
A8	UNKN	2222.7238	FRO2	28.57
A9	UNKN	4840.6829	FRO2	29.87
A10	UNKN	10542.115	FRO2	30.99
A11	UNKN	22958.784	FRO2	31.58
A12	UNKN	50000	FRO2	30.61
B1	NTC	PDF2	PDF2	50
B2	UNKN	20.833333	PDF2	23.87
B3	UNKN	45.37116	PDF2	24.9
B4	UNKN	98.810025	PDF2	26.14
B5	UNKN	215.19002	PDF2	26.97
B6	UNKN	468.64421	PDF2	28.63
B7	UNKN	1020.6207	PDF2	29.32
B8	UNKN	2222.7238	PDF2	30.8
B9	UNKN	4840.6829	PDF2	30.94
B10	UNKN	10542.115	PDF2	33.2
B11	UNKN	22958.784	PDF2	50
B12	UNKN	50000	PDF2	50

TEST PLATE RESULTS NEXT:



gene A

start useful stnd curve at 1:

serial 1:

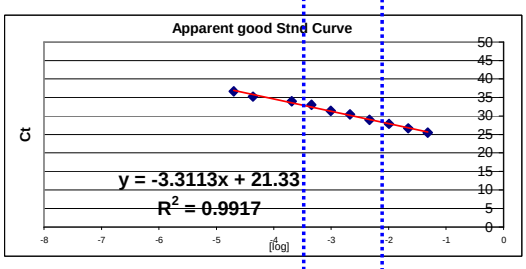
Efficiency:	87.12%	104.23%

Correlation: -0.999

b = 18.442

m = -3.6749

Ctrl+Shift+B to run both



gene B

start useful stnd curve at 1:

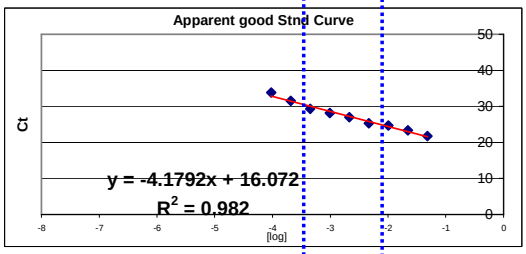
serial 1:

Efficiency:	100.45%	117.08%

Correlation: -0.996

b = 21.330

m = -3.3113



gene3

start useful stnd curve at 1:

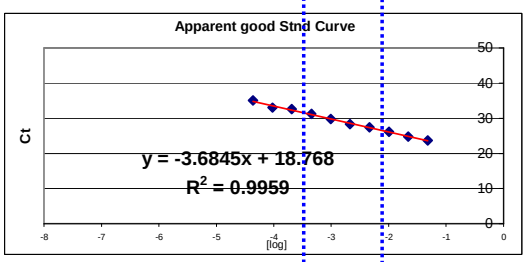
serial 1:

Efficiency:	73.49%	91.70%

Correlation: -0.991

b = 16.072

m = -4.1792



gene4

start useful stnd curve at 1:

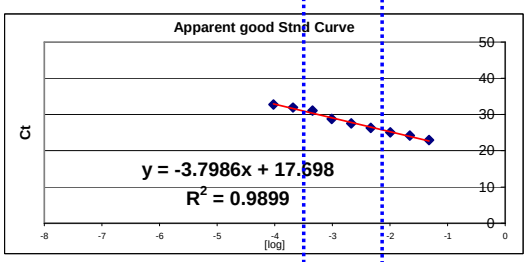
serial 1:

Efficiency:	86.81%	100.36%

Correlation: -0.998

b = 18.768

m = -3.6845



gene5

start useful stnd curve at 1:

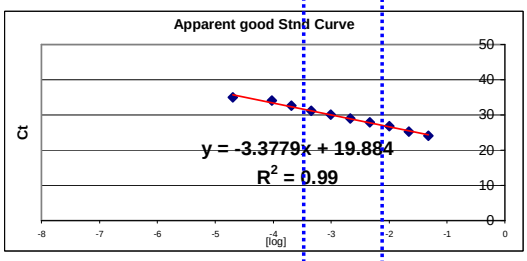
serial 1:

Efficiency:	83.34%	112.90%

Correlation: -0.995

b = 17.698

m = -3.7986



gene6

start useful stnd curve at 1:

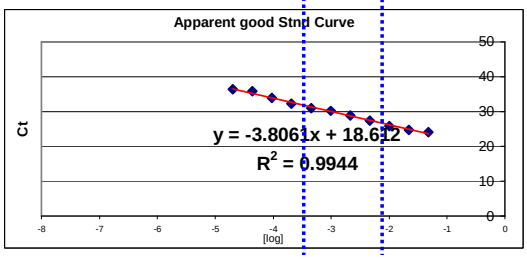
serial 1:

Efficiency:	97.71%	113.88%

Correlation: -0.995

b = 19.884

m = -3.3779



UbQ

start useful stnd curve at 1:

serial 1:

Efficiency:	83.12%	101.62%

Correlation: -0.997

b = 18.612

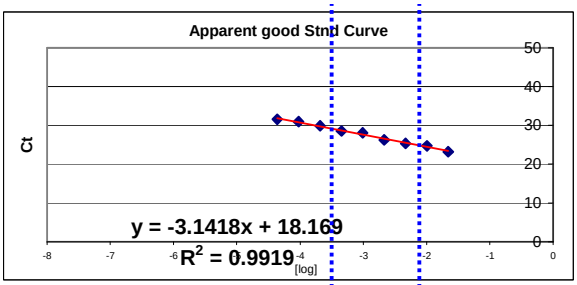
m = -3.8061

Great Test Plate results from 9 genes on the first try (Dr. Agnieszka Zmienko, Poland).

All 9 primer sets worked beautifully using the PQ-Method

And the good working range for all samples (and targets) was chosen to be between 1:120 and 1:3000.

Working within this range, you will get strong, LOG linear amplification for all 9 targets – and you won't run out of Stock I or sample cDNA by the time the entire experiment has been run. (An important parameter calculated by PQ is whether or not you will run out of one or more samples or Stock I by the time the entire run is complete.

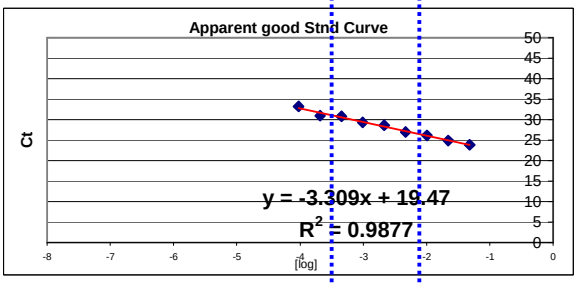


FRO2

start useful stnd

Efficiency: 108.11%
Correlation: -0.996
b = 18.169
m = -3.1418

Ctrl+Shift



PDF2

start useful stnd

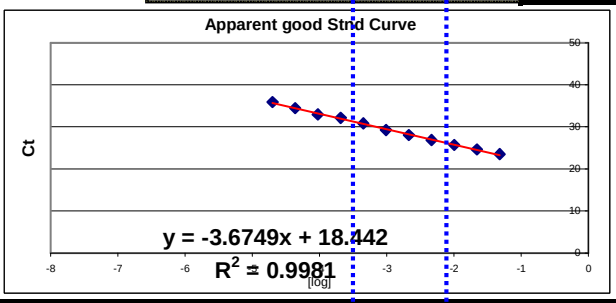
Efficiency: 100.54%
Correlation: -0.994
b = 19.470
m = -3.3090

Picking through the points a little further … the following is obtained (and the proper name of each of the targets is now included for each):

With some points thrown out – we focus in on the region between 1:120 and 1:3000 (the region between the blue dotted lines) in-well dilution range:

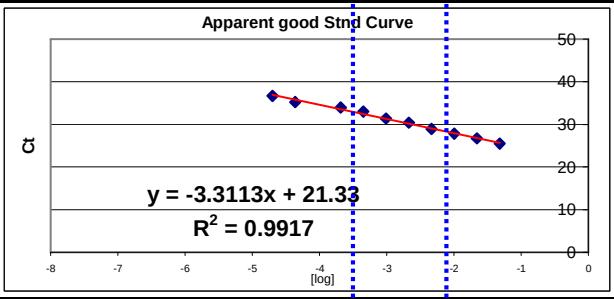
each case.

Chosen apparent Inhibition Threshold 1: 25



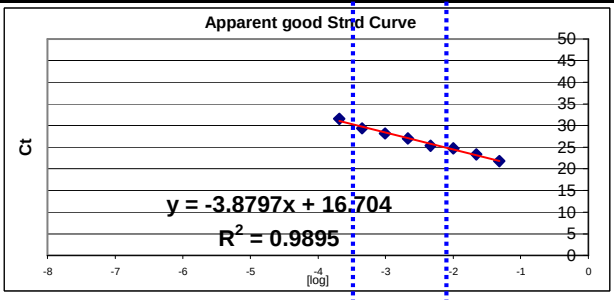
SnRK2.4	adj.upper: 5.76	Stnd. Curve "in-well"
	adj.lower: 1	
	start useful stnd curve at 1: 120	120.0
	serial 1: 2.924018	351
Efficiency: 87.12%	104.23% (Better E)	1026
Correlation: -0.999		3000
b = 18.442	adjust dil: 0.218395	4 points
m = -3.6749	des.start: 120	1
3-point	des.end: 1	1
4-point	desdilfact: 2.92401774	1
1	OK	

Ctrl q to put in desired starts and ends, Ctrl e to put in desired dilution factors

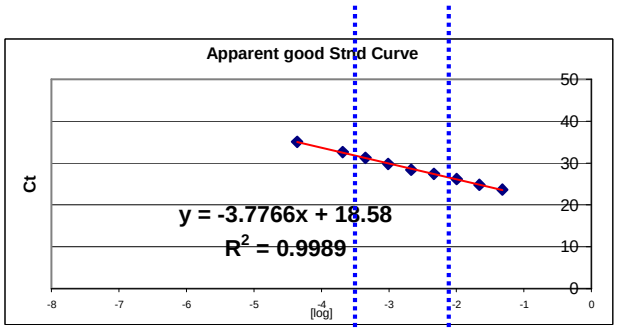


SnRK2.10	adj.upper: 5.76	Stnd. Curve "in-well"
	adj.lower: 1	
	start useful stnd curve at 1: 120	120.0
	serial 1: 2.924018	351
Efficiency: 100.45%	117.08% (Better E)	1026
Correlation: -0.996		3000
b = 21.330	adjust dil: 0.218395	4 points
m = -3.3113	des.start: 120	1
3-point	des.end: 1	1
4-point	desdilfact: 2.92401774	1
1	OK	

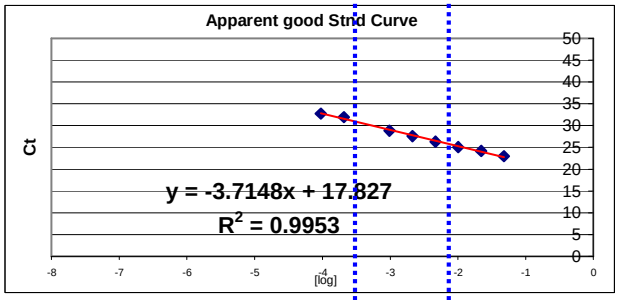
Ctrl z to accept automatic file calculations for all target optimal ranges



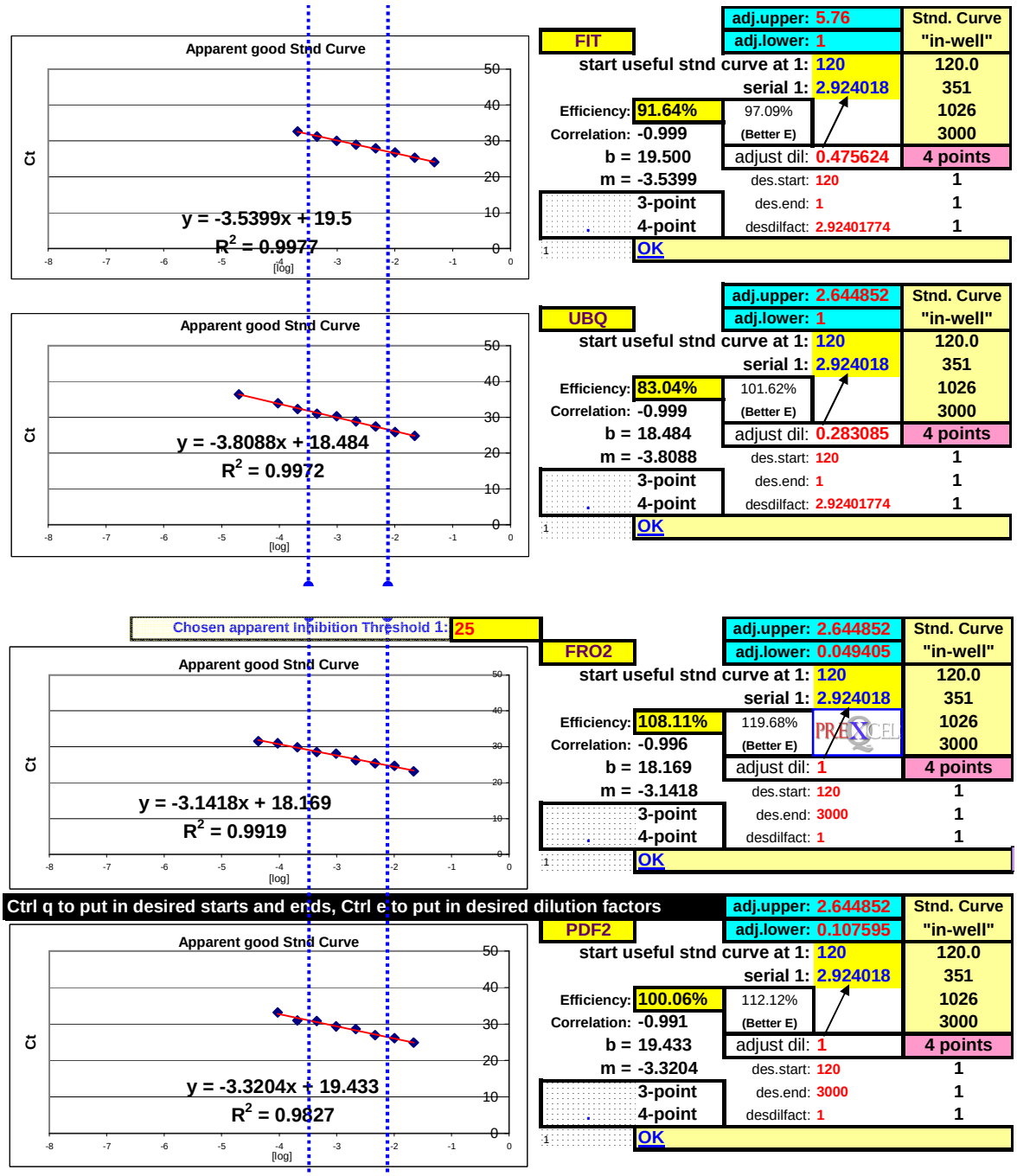
GDH1	adj.upper: 5.76	Stnd. Curve "in-well"
	adj.lower: 1	
	start useful stnd curve at 1: 120	120.0
	serial 1: 2.924018	351
Efficiency: 81.03%	91.70% (Better E)	1026
Correlation: -0.995		3000
b = 16.704	adjust dil: 0.475624	4 points
m = -3.8797	des.start: 120	1
3-point	des.end: 1	1
4-point	desdilfact: 2.92401774	1
1	OK	



NR1	adj.upper: 5.76	Stnd. Curve "in-well"
	adj.lower: 1	
	start useful stnd curve at 1: 120	120.0
	serial 1: 2.924018	351
Efficiency: 83.99%	100.36% (Better E)	1026
Correlation: -0.999		3000
b = 18.580	adjust dil: 0.283085	4 points
m = -3.7766	des.start: 120	1
3-point	des.end: 1	1
4-point	desdilfact: 2.92401774	1
1	OK	



IRT1	adj.upper: 5.76	Stnd. Curve "in-well"
	adj.lower: 1	
	start useful stnd curve at 1: 120	120.0
	serial 1: 2.924018	351
Efficiency: 85.86%	112.90% (Better E)	1026
Correlation: -0.998		3000
b = 17.827	adjust dil: 0.366936	4 points
m = -3.7148	des.start: 120	1
3-point	des.end: 1	1
4-point	desdilfact: 2.92401774	1
1	OK	



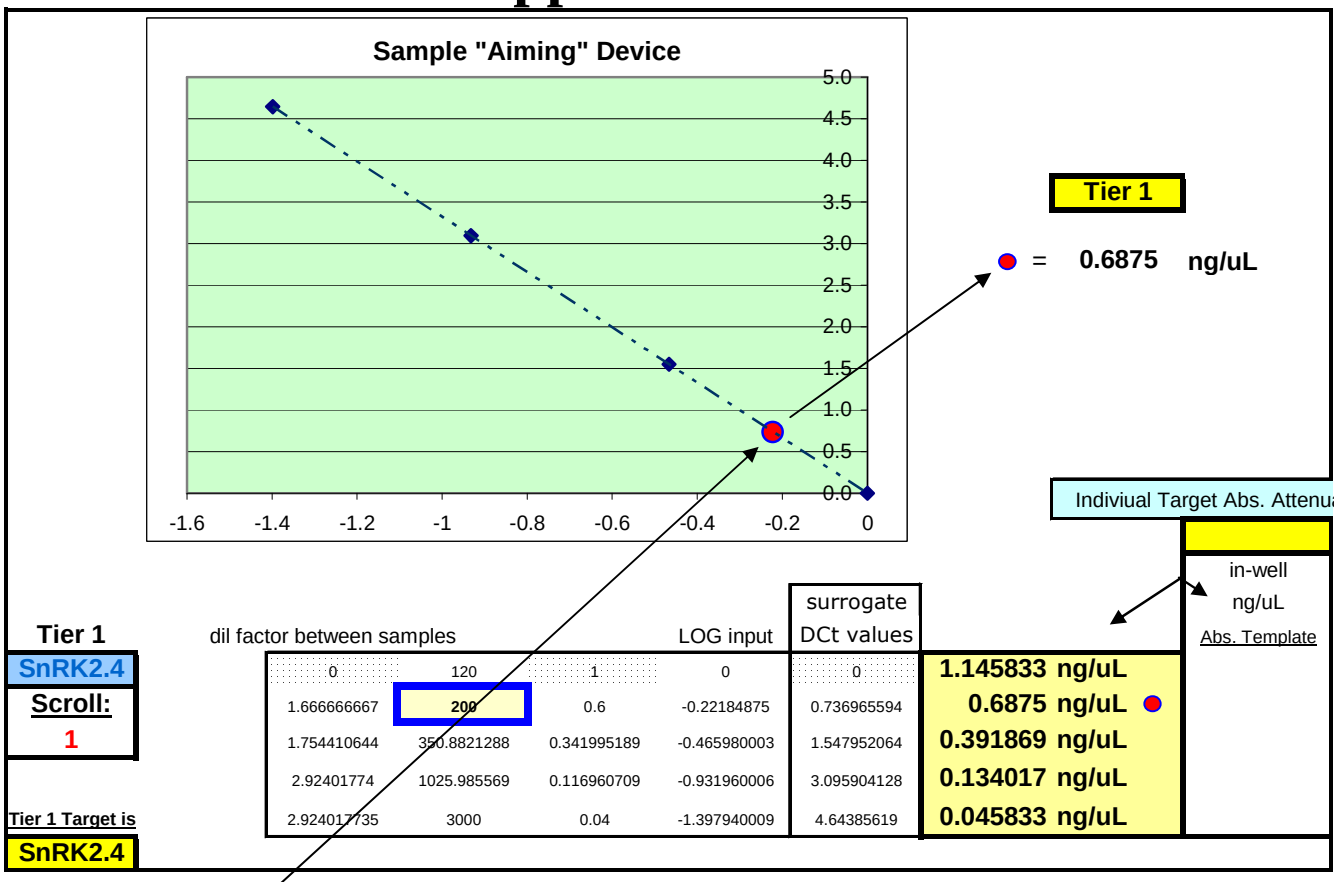
So, based on these parameters, the final set-up will be as follows:

Standards are prepared from your Stock I as shown:

	(uL) COMPREHESIVE SERIAL DILUTION TABLE (uL)				Achieved		Actual Final Dilutions Achieved
	Total made	Stock I	Water	to Next	(FINAL VOL.)	Dilutions 1:	for Final Plates after used in-well:
A	419.8	72.9	346.9	219.8	200 uL	5.76	1: 120
B	366.3	219.8	146.5	166.3	200 uL	9.6	1: 200
C	291.8	166.3	125.5	91.8	200 uL	16.84234218	1: 350.8821288
D	268.4	91.8	176.6	68.4	200 uL	49.24730732	1: 1025.985569
E	200.0	68.4	131.6		200 uL	144	1: 3000

This will result in 5 tubes each containing 200 µl of a standard.

The 5 standards will appear like this:



But, the "Red Dot" here, also represents where all the cDNA samples will be diluted to (by the time they are in-well; or "in tube). To get the cDNA samples to the appropriate dilution to achieve the "red dot" dilution, each sample cDNA will be diluted as shown on the next page:

Further pre-qPCR sample cDNA dilutions:

Sample	1:5 cDNA	Water	1st total
1	50.1 uL	124.9 uL	175 uL
2	50.1 uL	124.9 uL	175 uL
3	50.1 uL	124.9 uL	175 uL
4	50.1 uL	124.9 uL	175 uL
5	50.1 uL	124.9 uL	175 uL
6	50.1 uL	124.9 uL	175 uL
7	50.1 uL	124.9 uL	175 uL
8	50.1 uL	124.9 uL	175 uL
9	50.1 uL	124.9 uL	175 uL
10	50.1 uL	124.9 uL	175 uL
11	50.1 uL	124.9 uL	175 uL
12	50.1 uL	124.9 uL	175 uL
13	50.1 uL	124.9 uL	175 uL
14	50.1 uL	124.9 uL	175 uL
15	50.1 uL	124.9 uL	175 uL
16	50.1 uL	124.9 uL	175 uL
17	50.1 uL	124.9 uL	175 uL
18	50.1 uL	124.9 uL	175 uL

So – notice that the red dot dilution is mathematically obtained as follows:

Currently, we assume that your "1:5: cDNA samples are each theoretically ~10 ng/µl each (since they each came from ~50 ng RNA/µl RT rxns) – thus, when assuming ~100% efficiency during reverse transcription, 50 ng RNA/µl = 50 ng cDNA/µl. When diluted 1:5, this becomes ~10 ng/µl. Then, the dilutions above will result in cDNA samples that are each 2.86458 ng/µl, which, when 6 µl of them are placed into 25 µl rxns., will result in the "red-dot dilution of 0.6875 ng cDNA/µl in each case.

Master Mix set-up for your final tubes for 18 samples and 9 targets:

GOOD	Target Master Mix Set-up(s)		xtra ea.made	Well size prepared:	25.00 uL
25.00 uL prepared/Well	2X Master Mix:	6426.00 uL	Extra/Target 101.33 uL	RNA added/well:	6.00 uL
25.00 uL used per Well	0X RT-Taq Solution:	0.00 uL	Total MM ea. 1013.33 uL	samples prepared	216
Depends on Machine/MM used	0X ROX or H2O:	0.00 uL	1	wells prepared	432
SYBR?: X	Total MMRT prepared:	6426.00 uL	Master adjust:	1.111111111	1
1	Total MMRT needed:	6000.00 uL	2	Reset: "Ctrl r"	1.11111111
	xtra made	426.00 uL			

SnRK2.4				SnRK2.10			
Fwd primer:	40.00 uL	split		Fwd primer:	40.00 uL	split	
Rev primer:	40.00 uL	into		Rev primer:	40.00 uL	into	
Probe:	0.00 uL	24		Probe:	0.00 uL	24	
MMRT:	666.67 uL	38.00 uL	amounts	MMRT:	666.67 uL	38.00 uL	amounts
	0.00 uL	then add	12.00 uL		0.00 uL	then add	12.00 uL
water:	266.67 uL		Sample to each	water:	266.67 uL		Sample to each

Sample Plate(s)

Main Primer-Probe nM
300 nM FWD primer
300 nM REV primer

SYBR Green Mix
3 to 5 min 50°C
2 to 5 min 95°C
Then 40-50 cycles of:
[15s 95°C:30s 60°C]
Then melting curve analysis

3				4			
GDH1				NR1			
Fwd primer:	40.00 uL	split		Fwd primer:	40.00 uL	split	
Rev primer:	40.00 uL	into		Rev primer:	40.00 uL	into	
Probe:	0.00 uL	24		Probe:	0.00 uL	24	
MMRT:	666.67 uL	38.00 uL	amounts	MMRT:	666.67 uL	38.00 uL	amounts
	0.00 uL	then add	12.00 uL		0.00 uL	then add	12.00 uL
water:	266.67 uL		Sample to each	water:	266.67 uL		Sample to each

Be certain to use the correct thermo protocol~!

5				6			
IRT1				FIT			
Fwd primer:	40.00 uL	split		Fwd primer:	40.00 uL	split	
Rev primer:	40.00 uL	into		Rev primer:	40.00 uL	into	
Probe:	0.00 uL	24		Probe:	0.00 uL	24	
MMRT:	666.67 uL	38.00 uL	amounts	MMRT:	666.67 uL	38.00 uL	amounts
	0.00 uL	then add	12.00 uL		0.00 uL	then add	12.00 uL
water:	266.67 uL		Sample to each	water:	266.67 uL		Sample to each

7				8			
FRO2				PDF2			
Fwd primer:	40.00 uL	split		Fwd primer:	40.00 uL	split	
Rev primer:	40.00 uL	into		Rev primer:	40.00 uL	into	
Probe:	0.00 uL	24		Probe:	0.00 uL	24	
MMRT:	666.67 uL	38.00 uL	amounts	MMRT:	666.67 uL	38.00 uL	amounts
	0.00 uL	then add	12.00 uL		0.00 uL	then add	12.00 uL
water:	266.67 uL		Sample to each	water:	266.67 uL		Sample to each

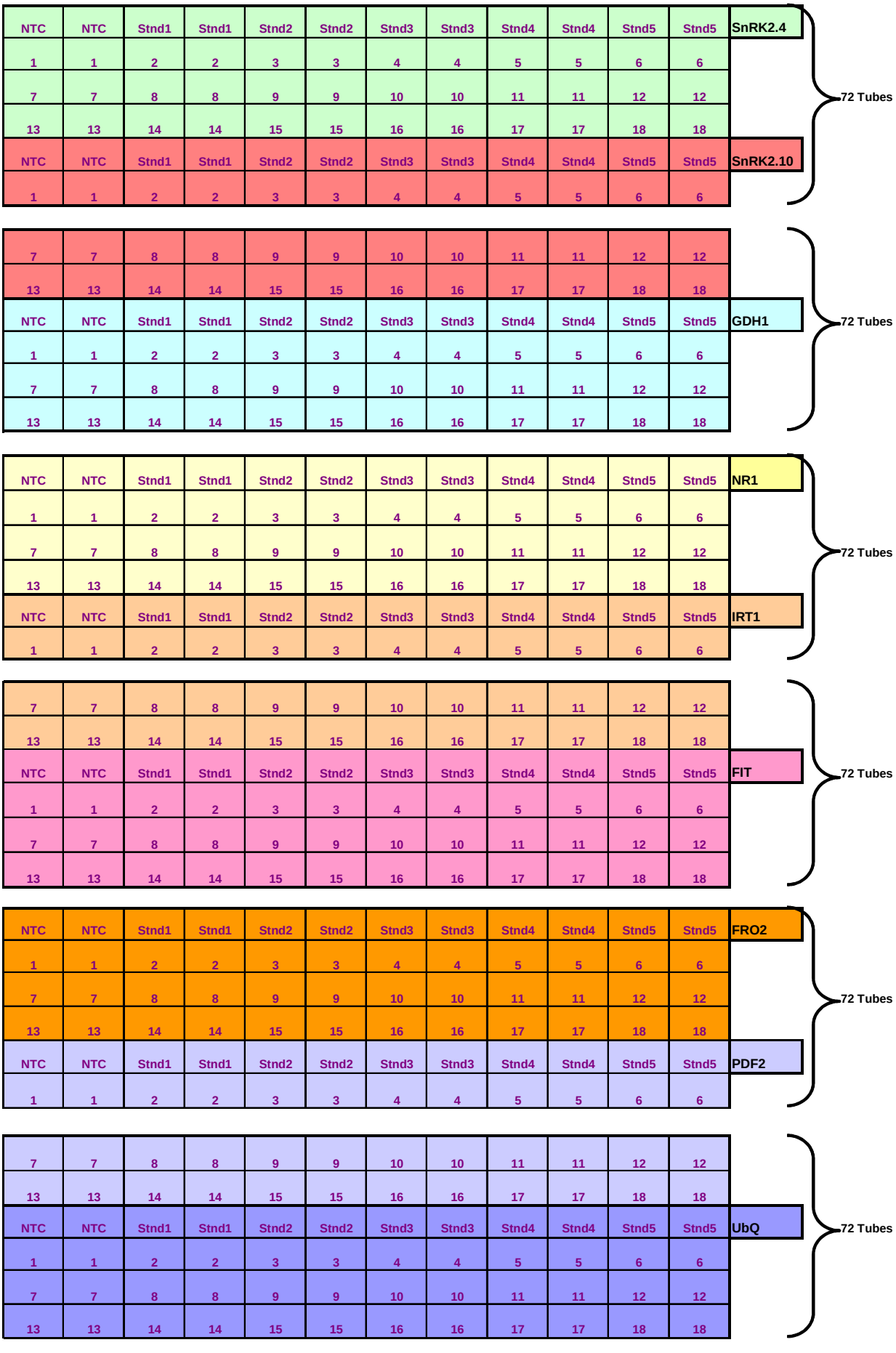
mM $MgSO_4$

Total 50 mM MgSO4
0.00 uL
needed here
50

0 uL Mg2+ solution per target	To get: 3 mM final in-well [Mg+2]	mM Mg+2 in Master Mix @ 1X:	3

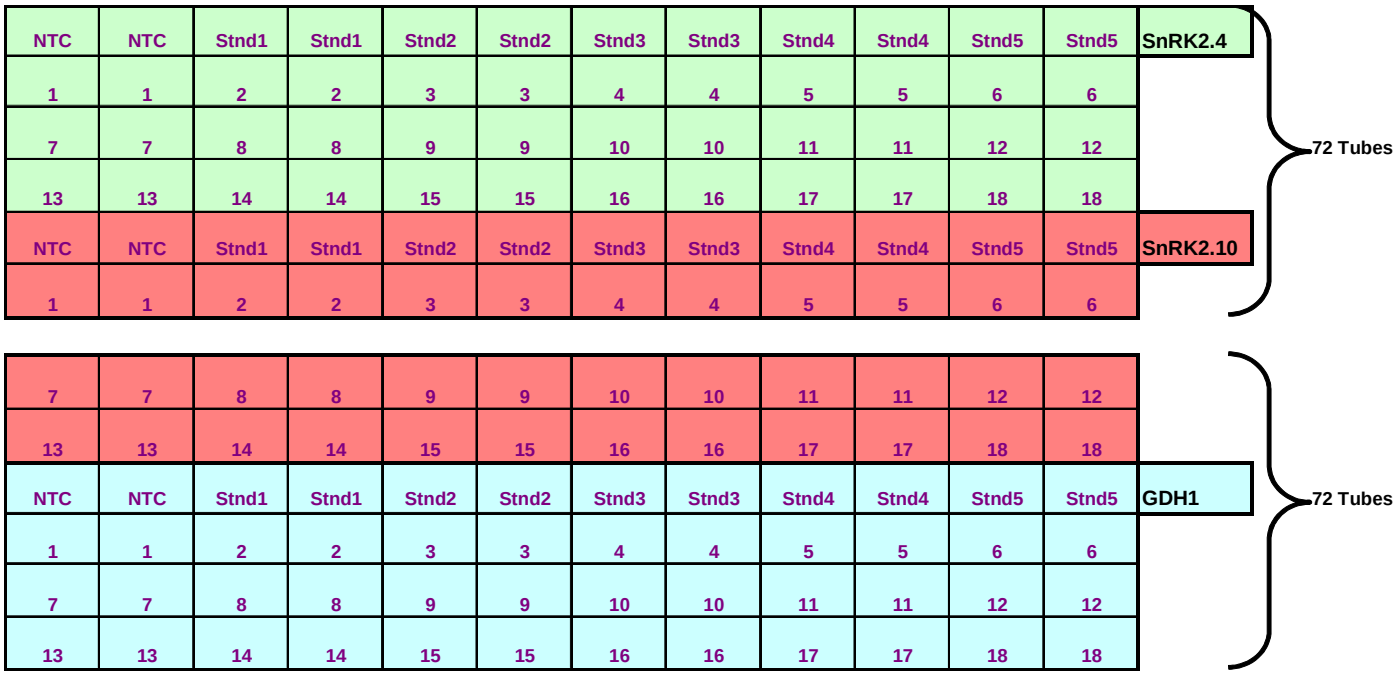
9				10			
UBQ							
Fwd primer:	40.00 uL	split		Fwd primer:			
Rev primer:	40.00 uL	into		Rev primer:			
Probe:	0.00 uL	24		Probe:			
MMRT:	666.67 uL	38.00 uL	amounts	MMRT:			
	0.00 uL	then add	12.00 uL				
water:	266.67 uL		Sample to each	water:			

6 FINAL ROUNDS of 72-Tube runs:

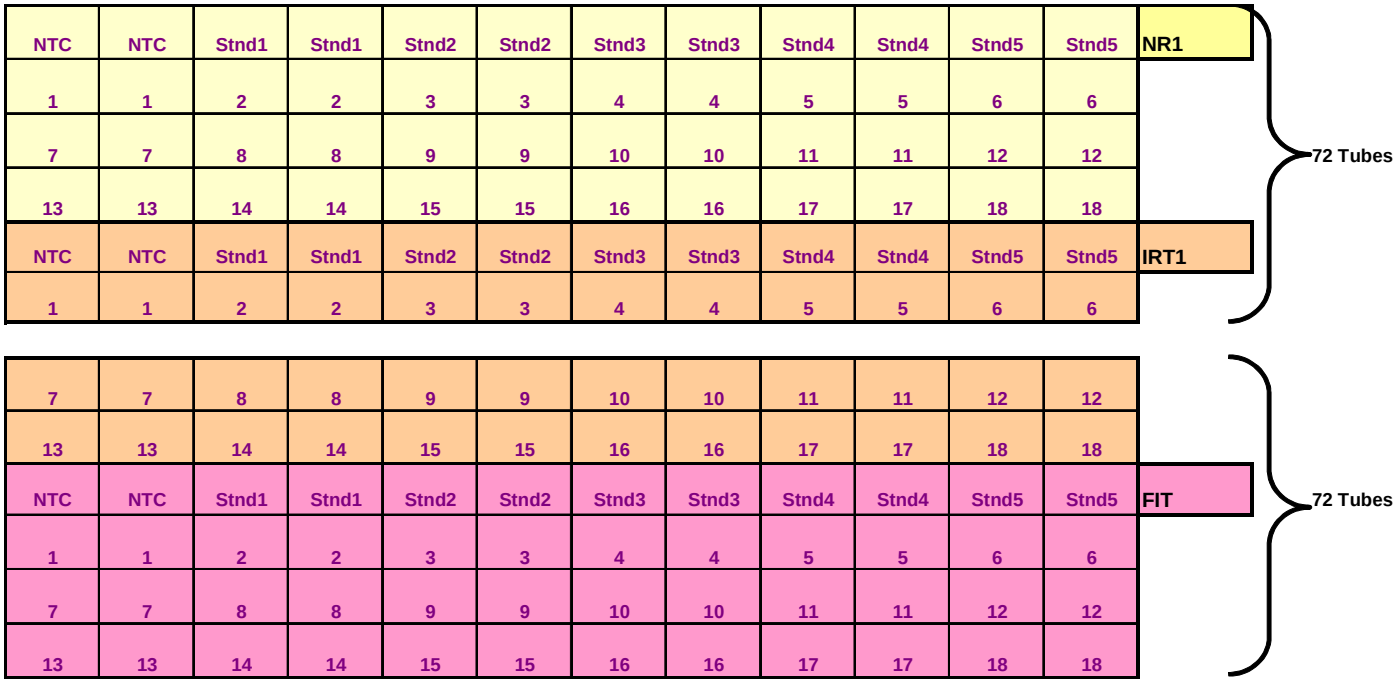


Or… Take it 3 targets at a time:

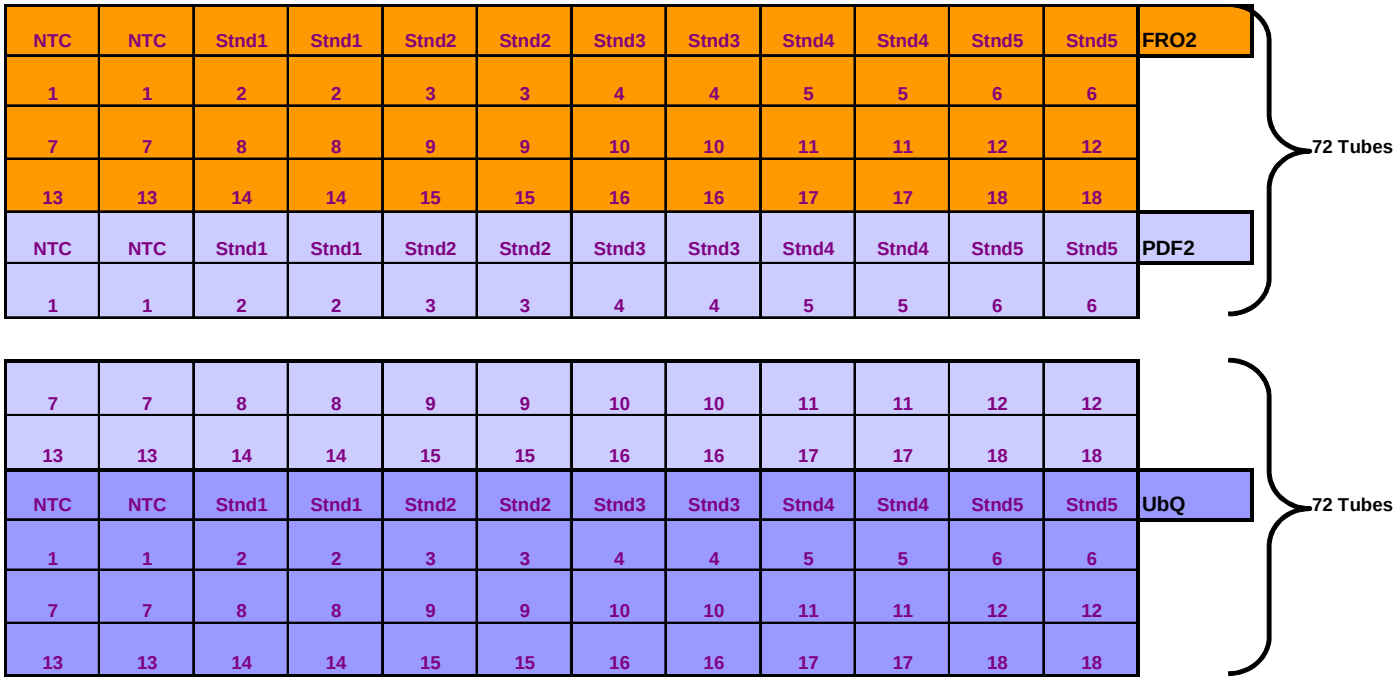
Round 1:



Round 2:



Round 3:



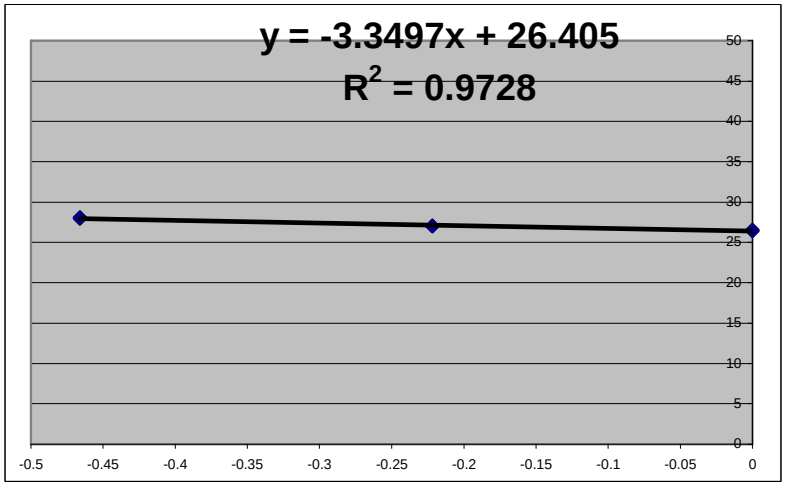
Where the Master Mix would be prepared for only 3 targets at a time (instead of the entire 9 targets) for 18 samples:

GOOD			Target Master Mix Set-up			xtra ea.made			Well size prepared:	25.00 uL	
25.00 uL	prepared/Well		2X Master Mix:	2160.00 uL		Set 1/sample	101.33 uL		RNA added/well:	6.00 uL	
25.00 uL	used per Well		0X RT-Taq Solution:	0.00 uL		Set 1/MM ea	1013.33 uL		samples prepared	72	
Depends on Machine/MM used			0X ROX or H2O:	0.00 uL				1	wells prepared	144	
			Total MMRT prepared:	2160.00 uL				Master adjust:	1.111111111	1	
1			Total MMRT needed:	2000.00 uL		2		Reset: "Ctrl r"		1.11111111	
			xtra made	160.00 uL							
Target 1						Target 2					
Fwd primer:	40.00 uL	split				Fwd primer:	40.00 uL	split			
Rev primer:	40.00 uL	into				Rev primer:	40.00 uL	into			
Probe:	0.00 uL	24				Probe:	0.00 uL	24			
MMRT:	666.67 uL	38.00 uL	amounts	Sample Plate(s)		MMRT:	666.67 uL	38.00 uL	amounts		
	0.00 uL	then add	12.00 uL				0.00 uL	then add	12.00 uL		
water:	266.67 uL		Sample to each	Main Primer-Probe nM		water:	266.67 uL		Sample to each		
				300 nM FWD primer							
3				300 nM REV primer		4					
Target 3											
Fwd primer:	40.00 uL	split		SYBR Green Mix		Fwd primer:					
Rev primer:	40.00 uL	into		Eurogentec		Rev primer:					
Probe:	0.00 uL	24		SYBR Master Mix		Probe:					
MMRT:	666.67 uL	38.00 uL	amounts	5 min. @ 95C		MMRT:					
	0.00 uL	then add	12.00 uL	Then 40 to 50 cycles of:							
water:	266.67 uL		Sample to each	15 sec@95C 1min @60C		water:					
				then: Melt curve analysis							

Choose your own option… and always label tubes, program the machine, etc., a day before the qPCR.

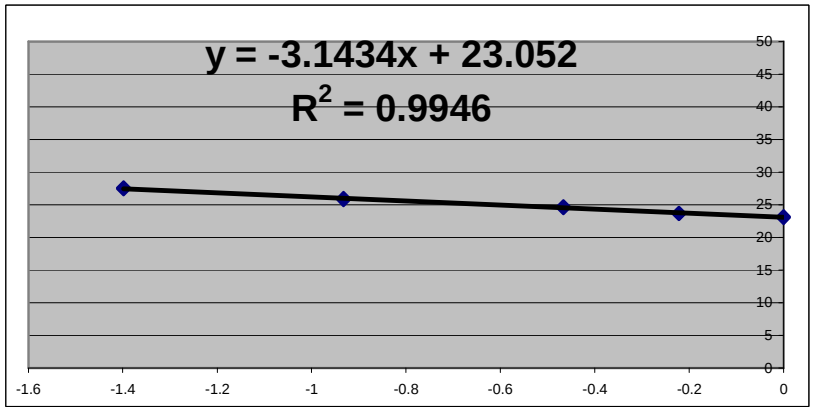
Final Standard Curves on the Final Plates

GDH1



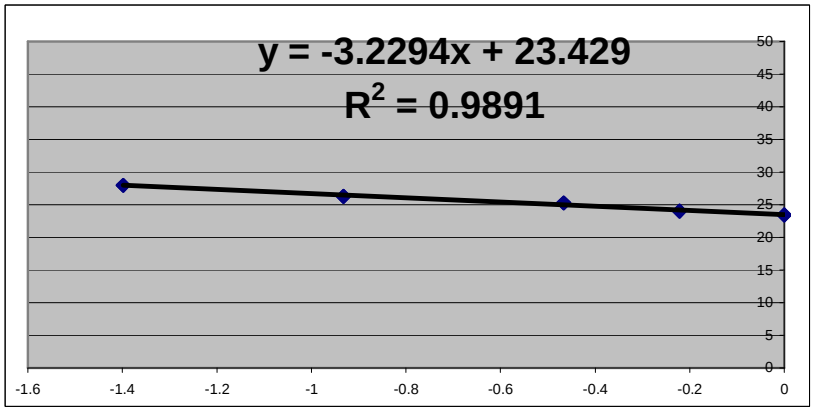
m = **-3.349722**
b = **26.40532**
Efficiency = **98.85%**
E_{AMP} = **1.988531**

NR1



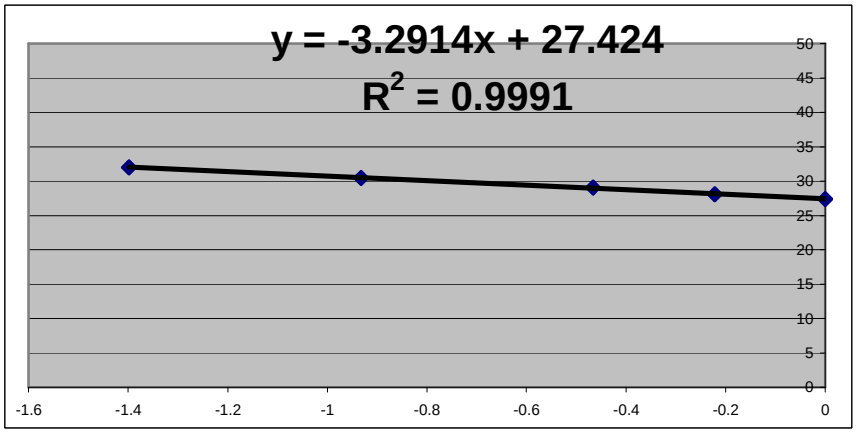
m = **-3.143399**
b = **23.05181**
Efficiency = **108.03%**
E_{AMP} = **2.080305**

IRT1



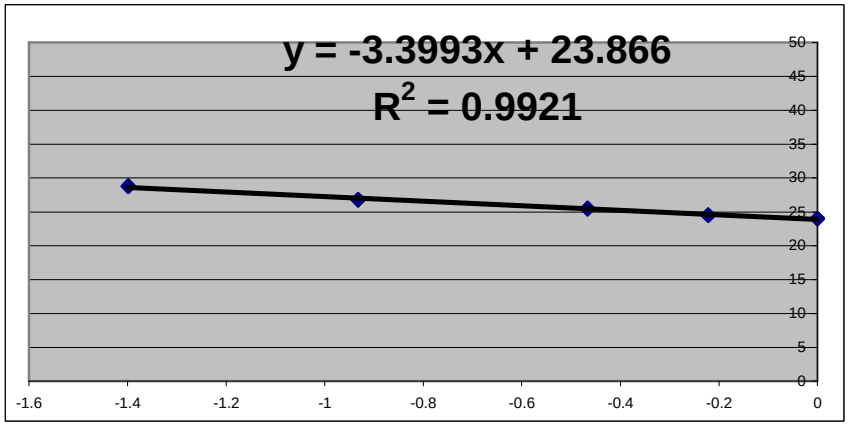
m = **-3.229445**
b = **23.42888**
Efficiency = **104.01%**
E_{AMP} = **2.040097**

SnRK2.4



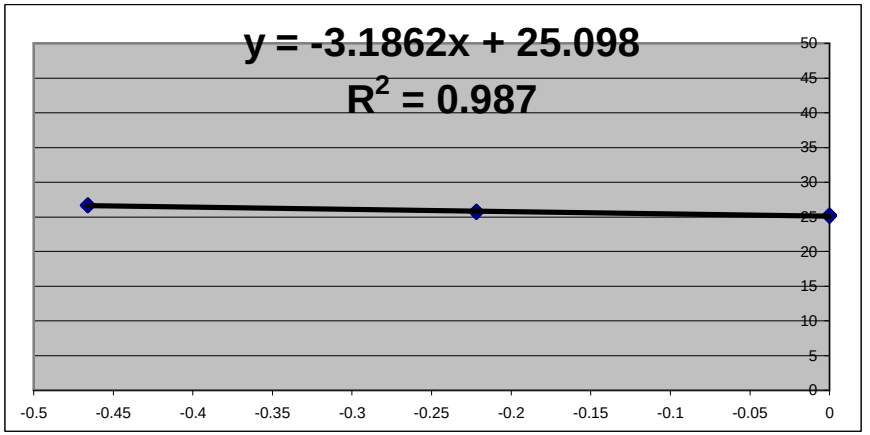
m = **-3.291411**
b = **27.42415**
Efficiency = **101.29%**
E_{AMP} = **2.012895**

SnRK2.10



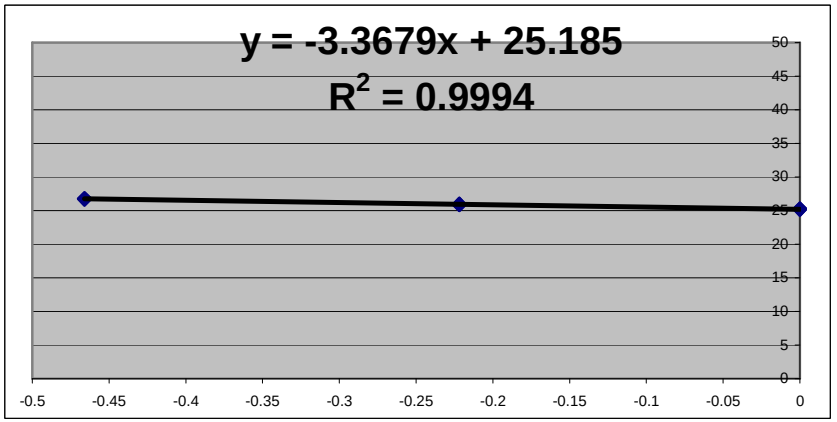
m = **-3.399335**
b = **23.86635**
Efficiency = **96.87%**
E_{AMP} = **1.96868**

FIT



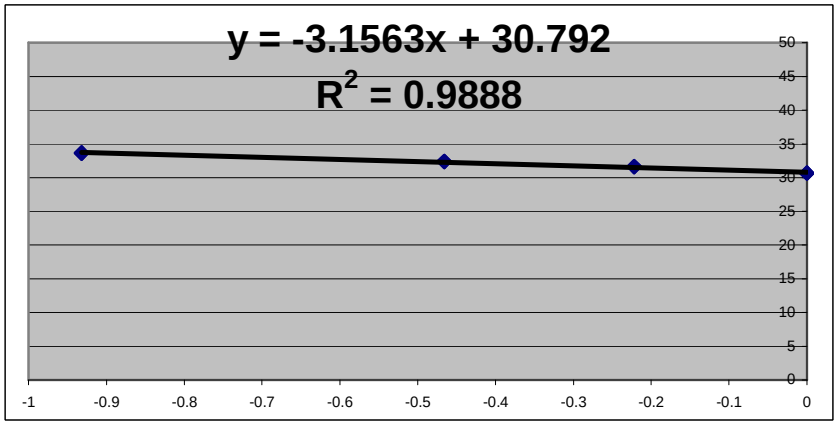
m = **-3.186216**
b = **25.09837**
Efficiency = **105.99%**
E_{AMP} = **2.059928**

FRO2



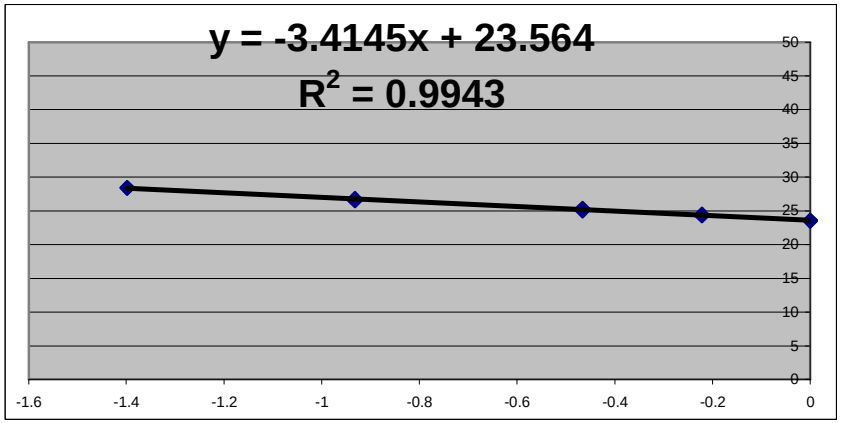
m = **-3.367945**
b = **25.18503**
Efficiency = **98.11%**
E_{AMP} = **1.981148**

PDF2



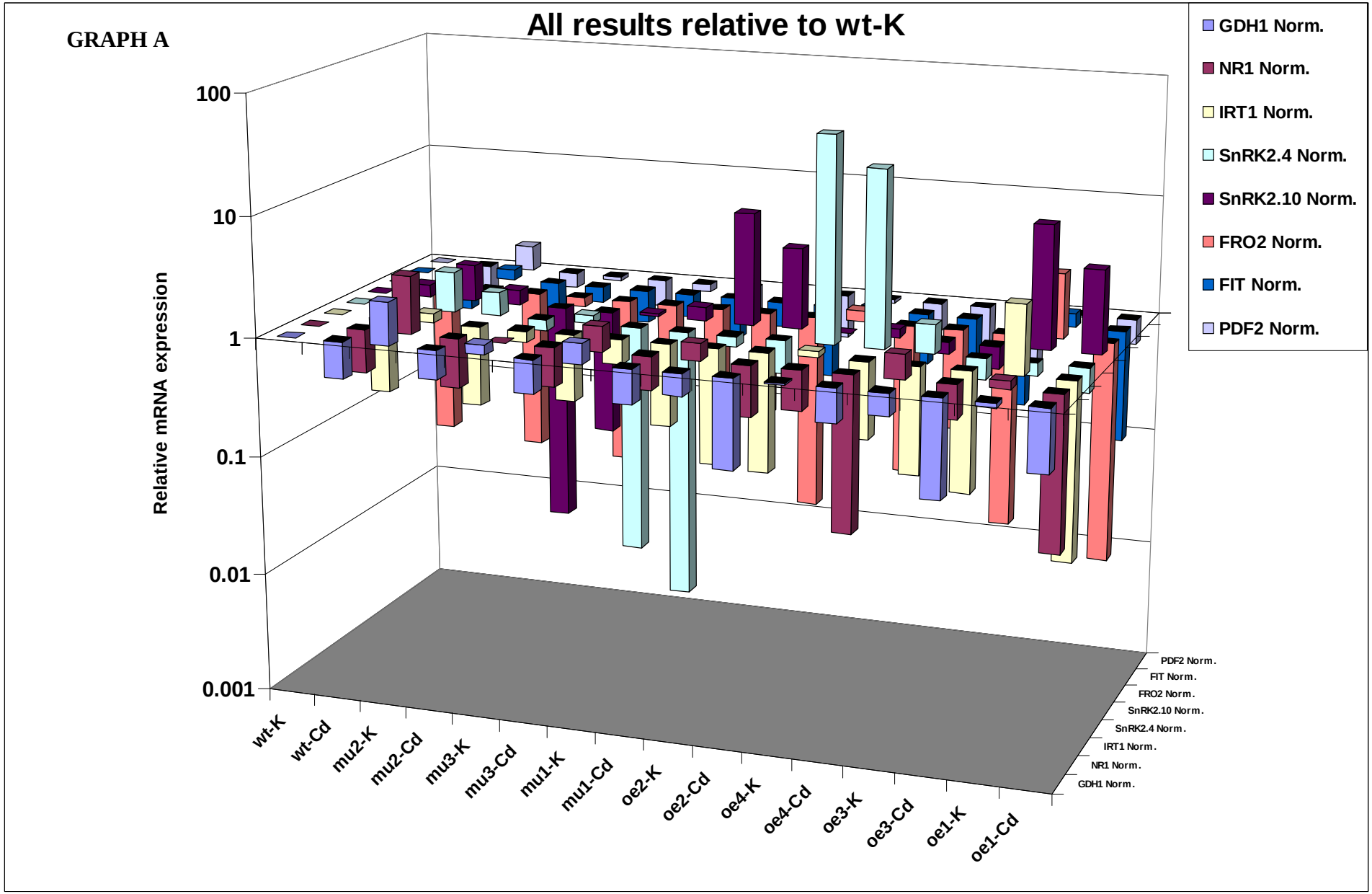
m = **-3.156312**
b = **30.79186**
Efficiency = **107.41%**
E_{AMP} = **2.07408**

UbQ



m = **-3.41449173**
b = **23.56419801**
Efficiency = **96.28%**
E_{AMP} = **1.962769757**

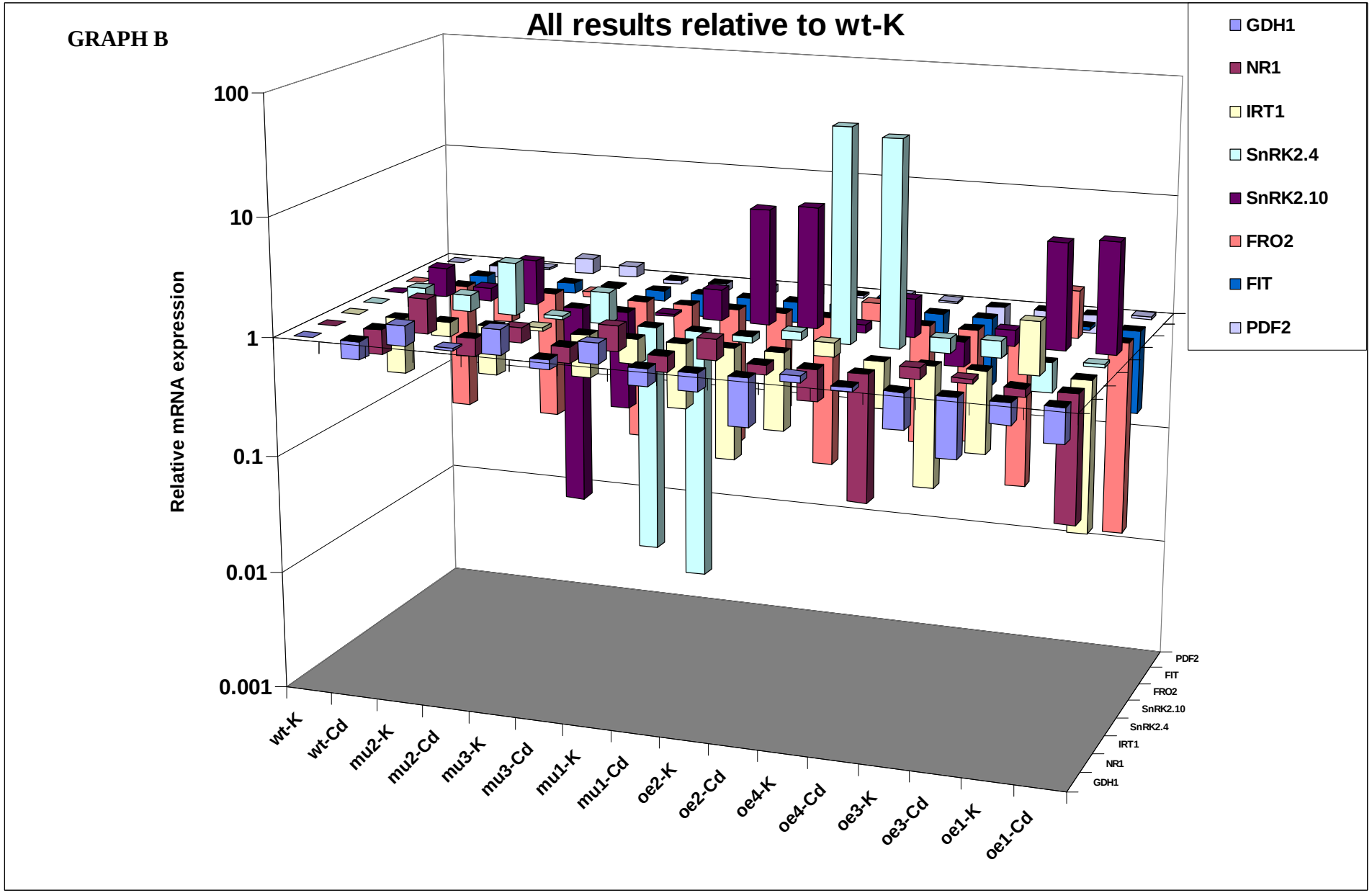
GRAPH A



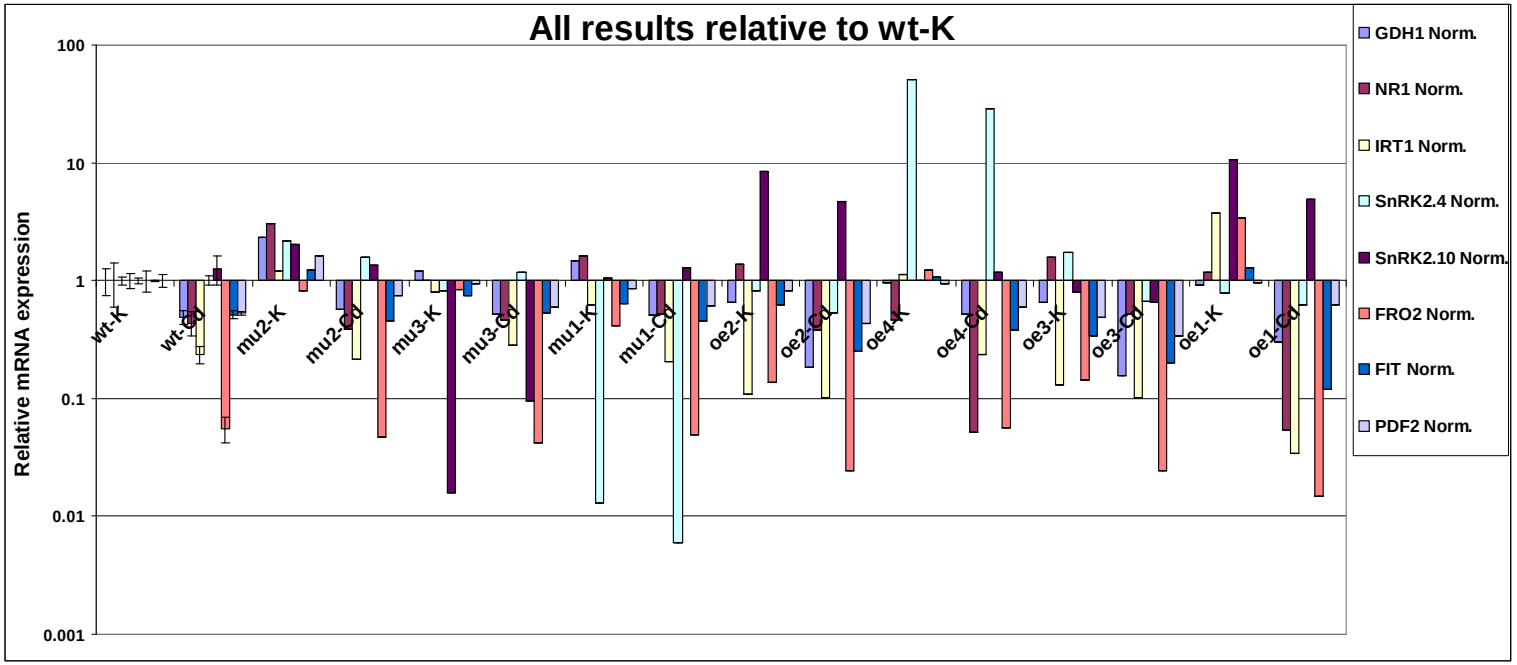
LOG Transformed Graph: (after all data is divided by wt-K quantities, including the wt-K by itself, the wt-K values thus become "1", and then LOG-transforming turns those values into "0", since the LOG of 1 = 0. All other signals are thus above or below "0" (the control level of target). In this graph, all targets have been normalized to reference gene UbQ.

In this graph, all targets are NOT normalized to reference gene, UbQ:

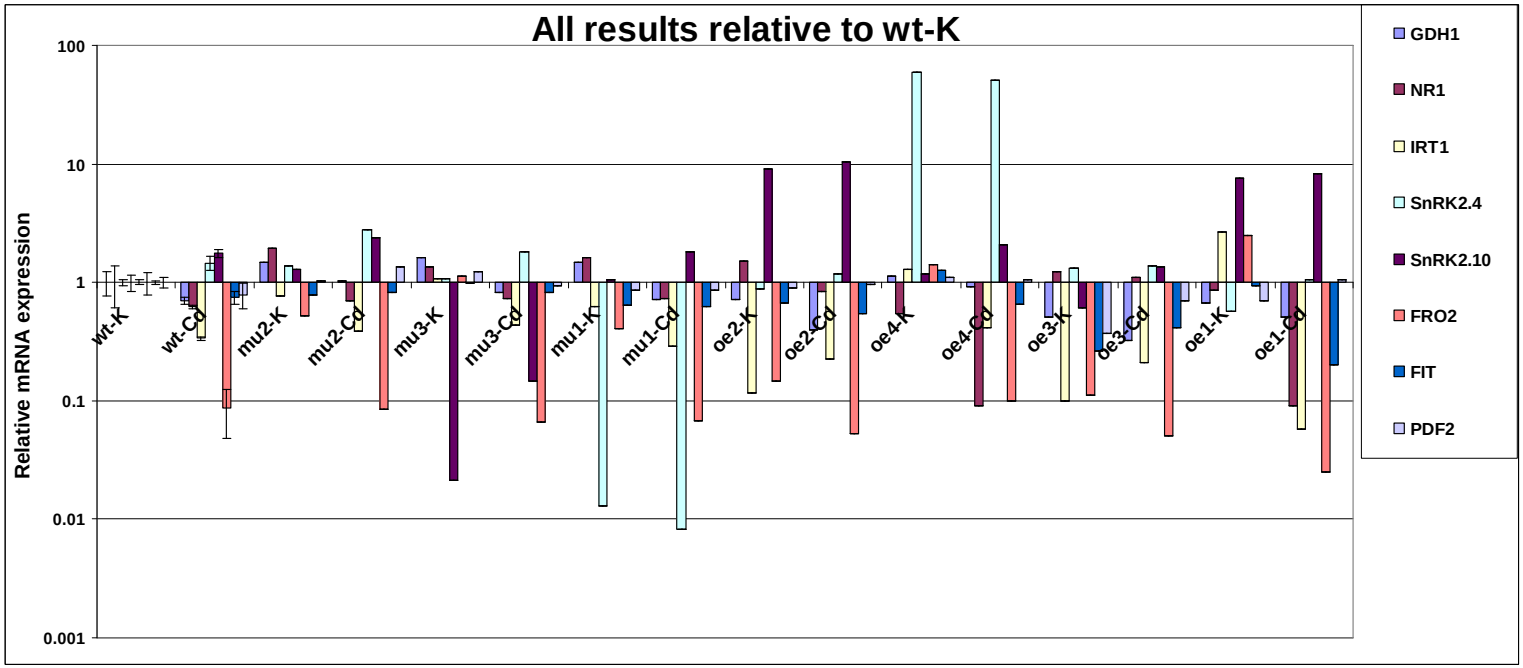
GRAPH B



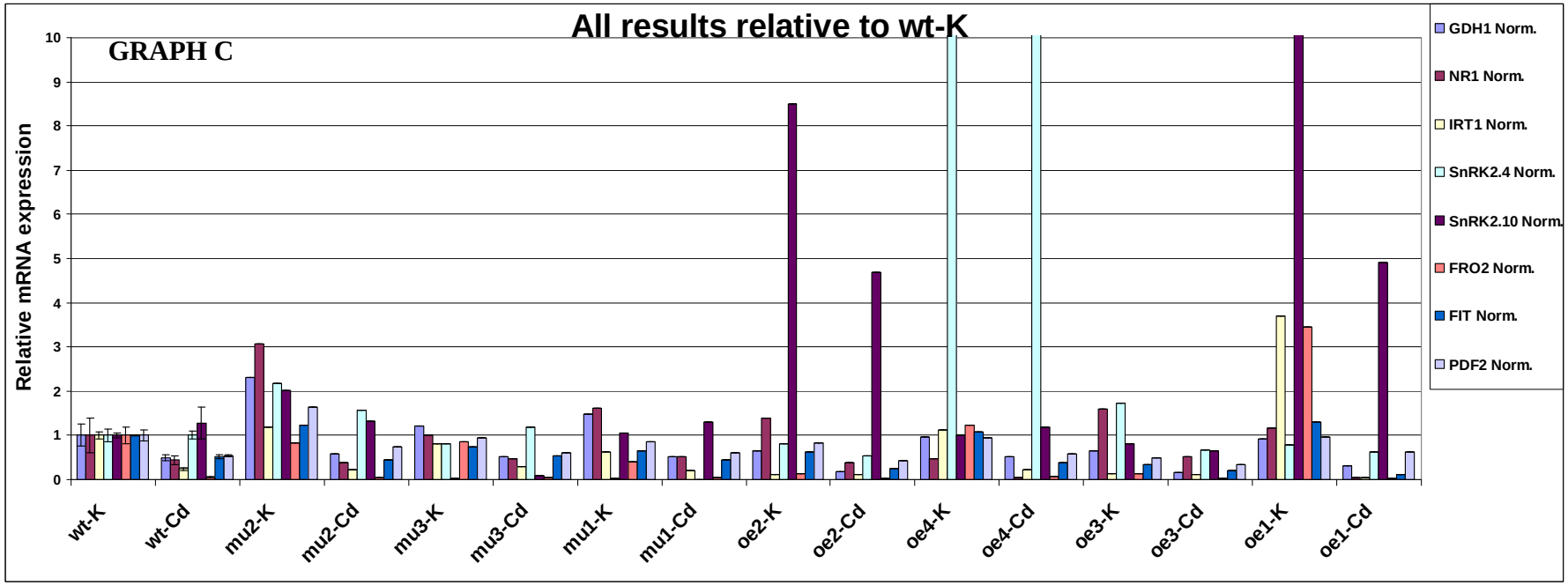
GRAPH A as a bar graph: notice, since wt-K has also been divided by itself then logged, it is "0" here since Log of 1 = 0



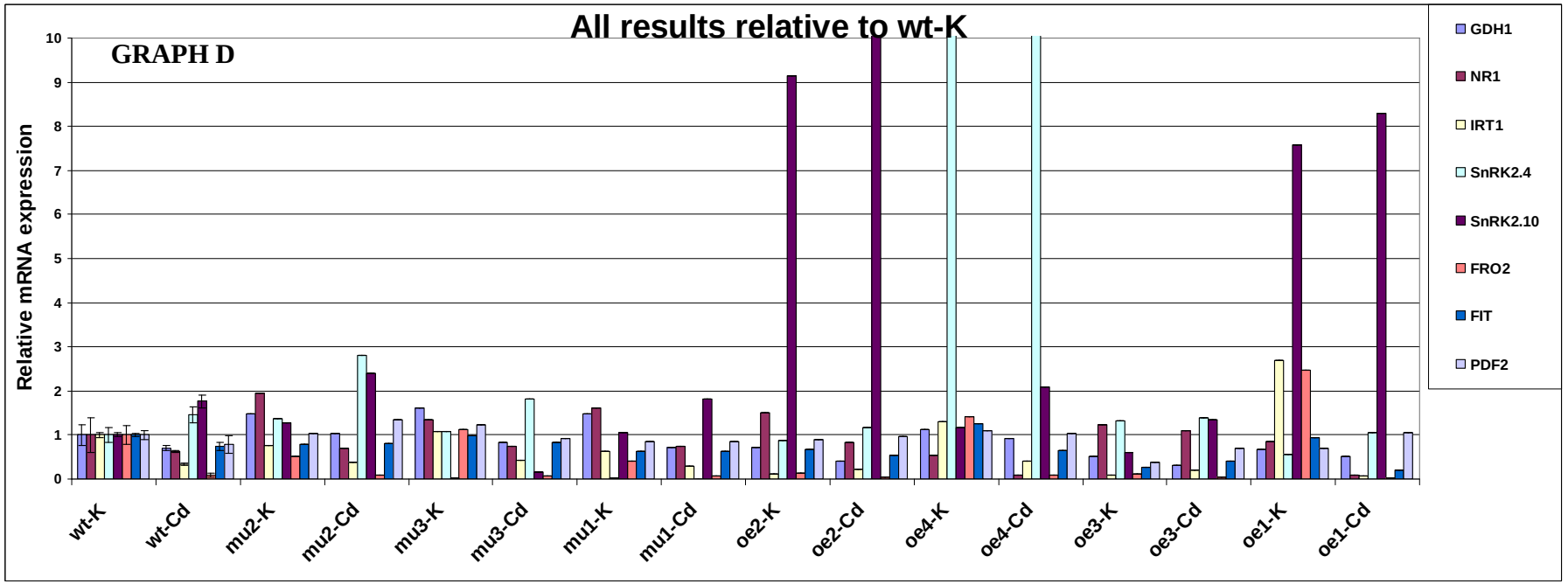
GRAPH B as a bar graph: notice, since wt-K has also been divided by itself then logged, it is "0" here since Log of 1 = 0



Different graphs of same results: Targets all normalized to UbQ, then normalized to wt-K, without LOG-transformation of data.



Targets NOT normalized to UbQ, but normalized to wt-K, without LOG-transformation of data. Notice control (wt-K = "1").



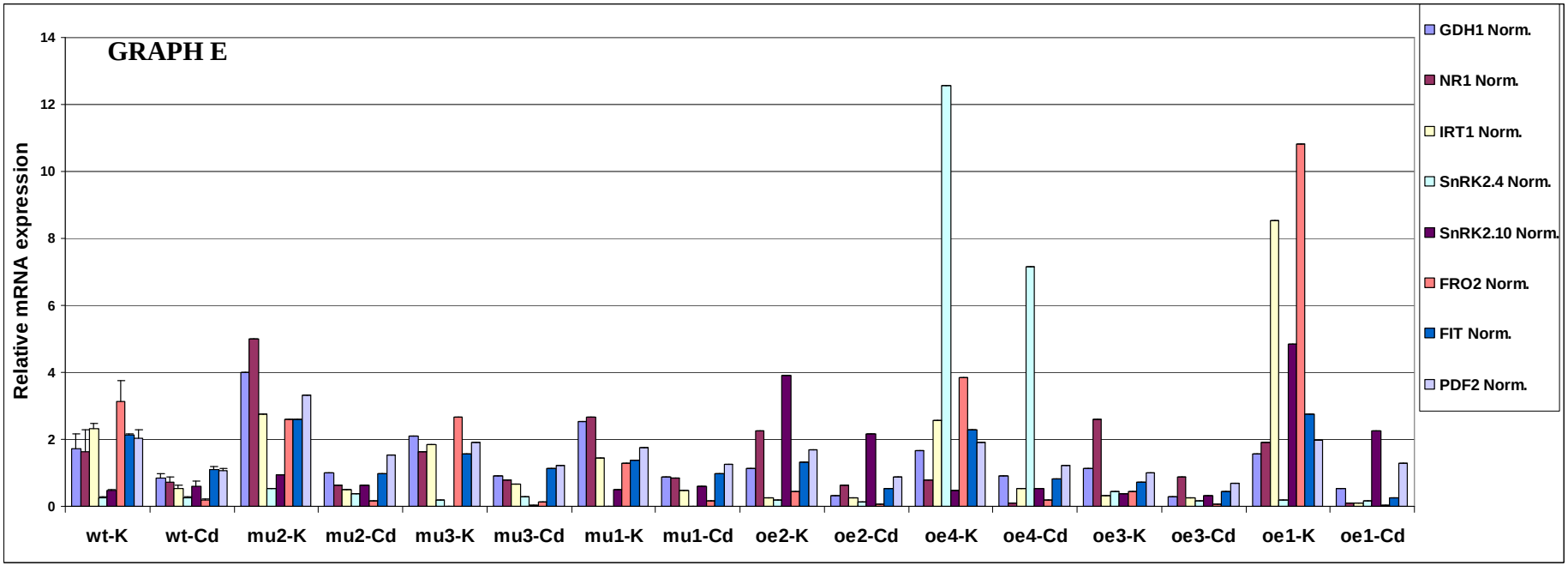
Data for **Graph C**

grp avgs:		GDH1 Norm.	NR1 Norm.	IRT1 Norm.	SnRK2.4 Norm.	SnRK2.10 Norm.	FRO2 Norm.	FIT Norm.	PDF2 Norm.
	wt-K	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	wt-Cd	0.490	0.439	0.236	1.010	1.273	0.056	0.513	0.528
	mu2-K	2.320	3.064	1.198	2.172	2.019	0.827	1.229	1.631
	mu2-Cd	0.576	0.391	0.217	1.571	1.334	0.047	0.457	0.748
	mu3-K	1.215	1.000	0.805	0.811	0.016	0.842	0.742	0.931
	mu3-Cd	0.526	0.469	0.281	1.179	0.095	0.042	0.531	0.597
	mu1-K	1.476	1.619	0.623	0.013	1.055	0.413	0.643	0.861
	mu1-Cd	0.513	0.525	0.206	0.006	1.303	0.049	0.452	0.615
	oe2-K	0.657	1.382	0.109	0.818	8.501	0.136	0.619	0.827
	oe2-Cd	0.182	0.378	0.103	0.536	4.689	0.024	0.251	0.436
	oe4-K	0.968	0.469	1.120	51.003	1.002	1.228	1.079	0.944
	oe4-Cd	0.525	0.052	0.234	29.014	1.183	0.057	0.376	0.593
	oe3-K	0.655	1.586	0.131	1.737	0.797	0.144	0.341	0.485
	oe3-Cd	0.156	0.526	0.102	0.672	0.654	0.025	0.200	0.337
	oe1-K	0.913	1.172	3.708	0.790	10.502	3.442	1.299	0.968
	oe1-Cd	0.304	0.053	0.034	0.628	4.917	0.015	0.120	0.623
st dev:	wt-K	0.348	0.567	0.105	0.202	0.087	0.277	0.020	0.176
	wt-Cd	0.096	0.147	0.058	0.116	0.512	0.020	0.057	0.032
	mu2-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu2-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu3-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu3-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu1-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu1-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe2-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe2-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe4-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe4-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe3-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe3-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe1-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe1-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
n	wt-K	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	wt-Cd	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	mu2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
s.e.m.	wt-K	0.246059232	0.401029117	0.074098242	0.142704368	0.061205935	0.19556279	0.014136387	0.124725909
	wt-Cd	0.067918345	0.104022045	0.040821852	0.082377225	0.361853997	0.014092196	0.040325768	0.022580815
	mu2-K	0	0	0	0	0	0	0	0
	mu2-Cd	0	0	0	0	0	0	0	0
	mu3-K	0	0	0	0	0	0	0	0
	mu3-Cd	0	0	0	0	0	0	0	0
	mu1-K	0	0	0	0	0	0	0	0
	mu1-Cd	0	0	0	0	0	0	0	0
	oe2-K	0	0	0	0	0	0	0	0
	oe2-Cd	0	0	0	0	0	0	0	0
	oe4-K	0	0	0	0	0	0	0	0
	oe4-Cd	0	0	0	0	0	0	0	0
	oe3-K	0	0	0	0	0	0	0	0
	oe3-Cd	0	0	0	0	0	0	0	0
	oe1-K	0	0	0	0	0	0	0	0
	oe1-Cd	0	0	0	0	0	0	0	0

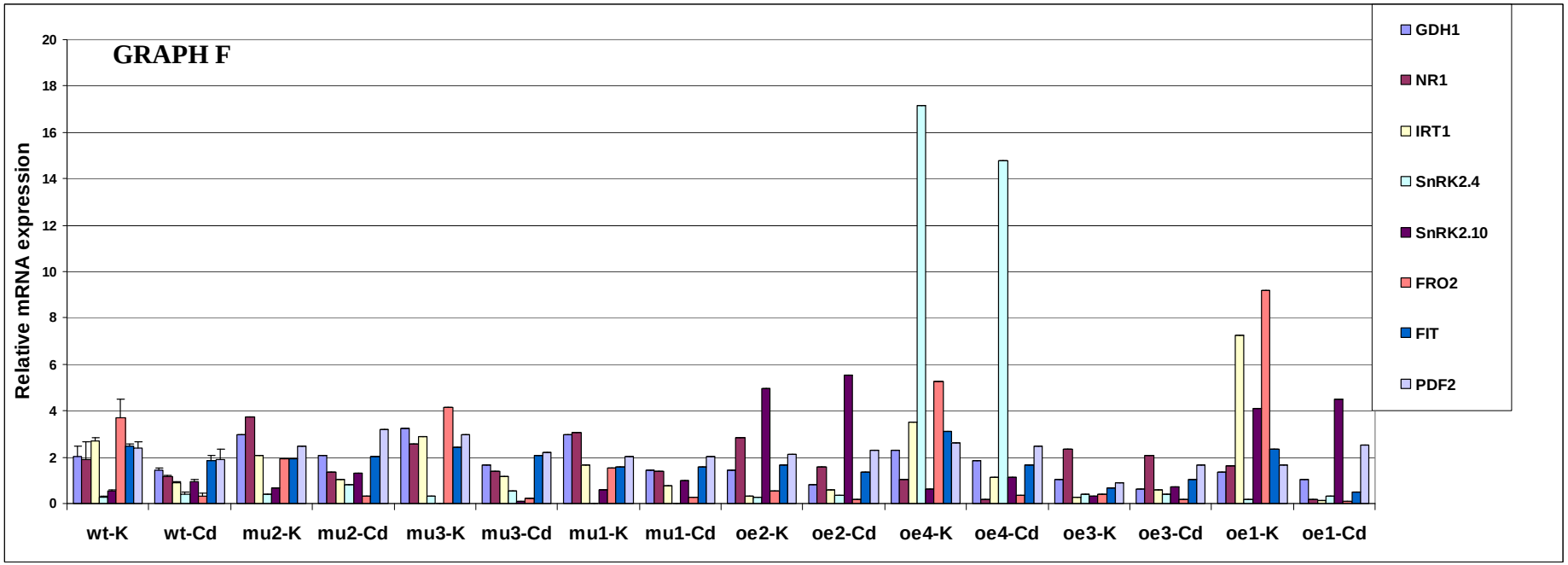
Data for **Graph D**

grp avgs:		GDH1	NR1	IRT1	SnRK2.4	SnRK2.10	FRO2	FIT	PDF2
	wt-K	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	wt-Cd	0.705	0.619	0.336	1.459	1.767	0.086	0.743	0.786
	mu2-K	1.477	1.956	0.761	1.373	1.281	0.522	0.779	1.036
	mu2-Cd	1.035	0.704	0.388	2.802	2.388	0.084	0.817	1.341
	mu3-K	1.618	1.336	1.068	1.072	0.021	1.113	0.984	1.238
	mu3-Cd	0.819	0.733	0.436	1.825	0.148	0.065	0.823	0.929
	mu1-K	1.470	1.617	0.619	0.013	1.048	0.408	0.638	0.855
	mu1-Cd	0.717	0.735	0.287	0.008	1.813	0.068	0.629	0.857
	oe2-K	0.710	1.497	0.117	0.877	9.153	0.145	0.666	0.891
	oe2-Cd	0.401	0.834	0.225	1.169	10.270	0.053	0.549	0.956
	oe4-K	1.128	0.548	1.301	59.043	1.165	1.420	1.252	1.099
	oe4-Cd	0.924	0.091	0.411	50.739	2.076	0.100	0.660	1.041
	oe3-K	0.506	1.229	0.101	1.332	0.613	0.111	0.262	0.374
	oe3-Cd	0.323	1.093	0.211	1.382	1.349	0.050	0.412	0.697
	oe1-K	0.662	0.852	2.680	0.569	7.589	2.476	0.938	0.701
	oe1-Cd	0.515	0.091	0.058	1.055	8.287	0.025	0.201	1.051
st dev:	wt-K	0.323	0.545	0.079	0.227	0.061	0.301	0.046	0.151
	wt-Cd	0.069	0.029	0.016	0.261	0.208	0.053	0.136	0.274
	mu2-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu2-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu3-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu3-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu1-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	mu1-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe2-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe2-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe4-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe4-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe3-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe3-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe1-K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	oe1-Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
n	wt-K	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	wt-Cd	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	mu2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
s.e.m.	wt-K	0.22871286	0.385489866	0.055793445	0.16066373	0.042873382	0.213177289	0.032508727	0.106589485
	wt-Cd	0.04882632	0.020333083	0.011588041	0.184368754	0.147276778	0.037540176	0.096131694	0.193779486
	mu2-K	0	0	0	0	0	0	0	0
	mu2-Cd	0	0	0	0	0	0	0	0
	mu3-K	0	0	0	0	0	0	0	0
	mu3-Cd	0	0	0	0	0	0	0	0
	mu1-K	0	0	0	0	0	0	0	0
	mu1-Cd	0	0	0	0	0	0	0	0
	oe2-K	0	0	0	0	0	0	0	0
	oe2-Cd	0	0	0	0	0	0	0	0
	oe4-K	0	0	0	0	0	0	0	0
	oe4-Cd	0	0	0	0	0	0	0	0
	oe3-K	0	0	0	0	0	0	0	0
	oe3-Cd	0	0	0	0	0	0	0	0
	oe1-K	0	0	0	0	0	0	0	0
	oe1-Cd	0	0	0	0	0	0	0	0

Raw Relative Quantity Results; targets normalized to UbQ:



Raw Relative Quantity Results; targets NOT normalized to UbQ:



Data for **Graph E**

grp avgs:		GDH1 Norm.	NR1 Norm.	IRT1 Norm.	SnRK2.4 Norm.	SnRK2.10 Norm.	FRO2 Norm.	FIT Norm.	PDF2 Norm.
	wt-K	1.721	1.633	2.297	0.247	0.461	3.138	2.118	2.034
	wt-Cd	0.844	0.717	0.543	0.249	0.586	0.175	1.086	1.073
	mu2-K	3.994	5.005	2.753	0.535	0.930	2.594	2.604	3.318
	mu2-Cd	0.992	0.638	0.498	0.387	0.615	0.149	0.968	1.521
	mu3-K	2.091	1.633	1.849	0.200	0.007	2.643	1.573	1.894
	mu3-Cd	0.905	0.766	0.645	0.291	0.044	0.133	1.125	1.215
	mu1-K	2.541	2.644	1.431	0.003	0.486	1.296	1.363	1.750
	mu1-Cd	0.883	0.858	0.474	0.001	0.600	0.155	0.958	1.252
	oe2-K	1.131	2.257	0.250	0.202	3.917	0.426	1.312	1.682
	oe2-Cd	0.314	0.618	0.236	0.132	2.160	0.077	0.532	0.887
	oe4-K	1.666	0.766	2.572	12.573	0.462	3.852	2.286	1.921
	oe4-Cd	0.903	0.084	0.538	7.152	0.545	0.179	0.797	1.205
	oe3-K	1.127	2.591	0.300	0.428	0.367	0.453	0.723	0.987
	oe3-Cd	0.269	0.860	0.235	0.166	0.301	0.077	0.424	0.686
	oe1-K	1.572	1.915	8.520	0.195	4.839	10.801	2.753	1.970
	oe1-Cd	0.524	0.087	0.079	0.155	2.265	0.046	0.253	1.267
st dev:	wt-K	0.599	0.926	0.241	0.050	0.040	0.868	0.042	0.359
	wt-Cd	0.165	0.240	0.133	0.029	0.236	0.063	0.121	0.065
	mu2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
n	wt-K	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	wt-Cd	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	mu2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
s.e.m.	wt-K	0.423480299	0.655052965	0.170227743	0.035178293	0.028199257	0.613627193	0.029945658	0.253689963
	wt-Cd	0.116890883	0.169912722	0.093781061	0.020306948	0.166716085	0.044217793	0.085423637	0.045928919
	mu2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Data for **Graph F**

		GDH1	NR1	IRT1	SnRK2.4	SnRK2.10	FRO2	FIT	PDF2
grp avgs:	**wt-K**	2.016	1.908	2.699	0.291	0.541	3.705	2.493	2.388
	wt-Cd	1.421	1.182	0.908	0.424	0.957	0.319	1.853	1.877
	mu2-K	2.978	3.732	2.053	0.399	0.694	1.934	1.942	2.474
	mu2-Cd	2.087	1.343	1.048	0.815	1.293	0.313	2.038	3.202
	mu3-K	3.263	2.548	2.884	0.312	0.011	4.122	2.453	2.955
	mu3-Cd	1.651	1.397	1.177	0.531	0.080	0.243	2.053	2.218
	mu1-K	2.964	3.084	1.670	0.004	0.567	1.512	1.590	2.042
	mu1-Cd	1.445	1.403	0.775	0.002	0.982	0.253	1.567	2.047
	oe2-K	1.431	2.856	0.316	0.255	4.956	0.539	1.660	2.128
	oe2-Cd	0.808	1.590	0.608	0.340	5.561	0.197	1.369	2.284
	oe4-K	2.275	1.046	3.513	17.170	0.631	5.260	3.121	2.623
	oe4-Cd	1.863	0.174	1.109	14.755	1.124	0.369	1.645	2.486
	oe3-K	1.020	2.344	0.272	0.387	0.332	0.410	0.654	0.893
	oe3-Cd	0.651	2.086	0.569	0.402	0.730	0.187	1.028	1.665
	oe1-K	1.335	1.626	7.235	0.165	4.109	9.173	2.338	1.673
	oe1-Cd	1.037	0.173	0.157	0.307	4.487	0.092	0.502	2.511
st dev:	**wt-K**	0.652	1.040	0.213	0.066	0.033	1.117	0.115	0.360
	wt-Cd	0.139	0.055	0.044	0.076	0.113	0.197	0.339	0.654
	mu2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
n	**wt-K**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	wt-Cd	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	mu2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	mu1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe2-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe4-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe3-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-K	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	oe1-Cd	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
s.e.m.	**wt-K**	0.461048348	0.73541306	0.150606848	0.046722279	0.023215285	0.789857285	0.081047423	0.254504338
	wt-Cd	0.09842601	0.038790163	0.031280348	0.053615887	0.079748137	0.139092591	0.239665679	0.462688413
	mu2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	mu1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe2-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe4-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe3-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	oe1-Cd	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Dr. Zmienko's graphing of the results

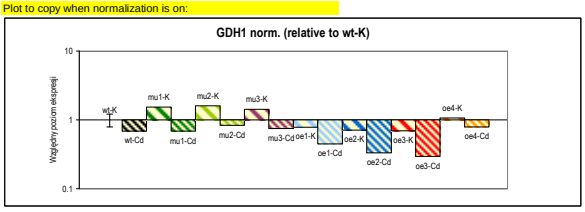
Use Geometric mean of both PDF2 and UbQ to normalize: x ("x" for yes)

TURN OFF UbQ: 0

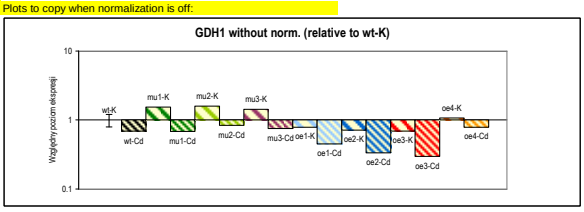
Plot to copy when normalization is on:

Plots to copy when normalization is off:

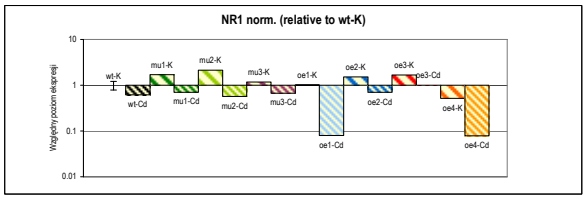
GDH1 norm. (relative to wt-K)



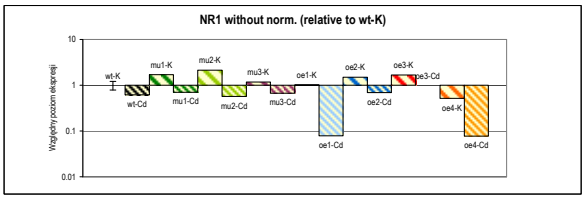
GDH1 without norm. (relative to wt-K)



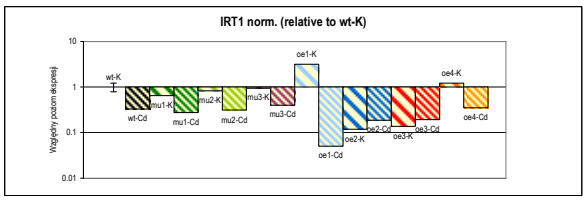
NR1 norm. (relative to wt-K)



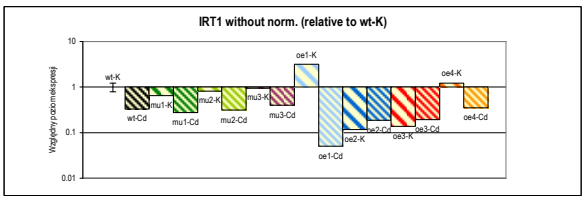
NR1 without norm. (relative to wt-K)



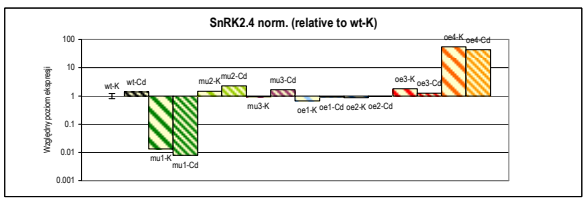
IRT1 norm. (relative to wt-K)



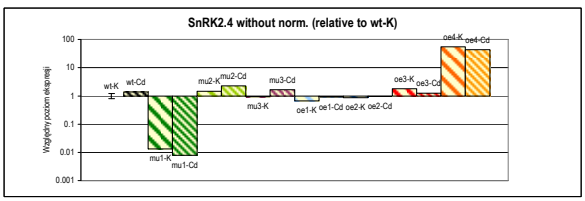
IRT1 without norm. (relative to wt-K)



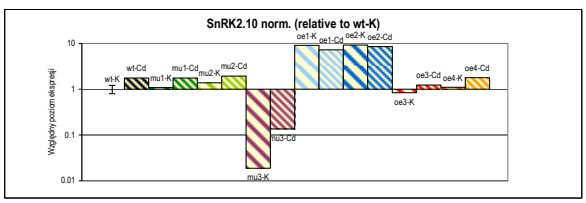
SnRK2.4 norm. (relative to wt-K)



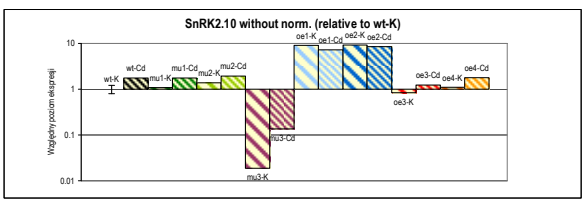
SnRK2.4 without norm. (relative to wt-K)



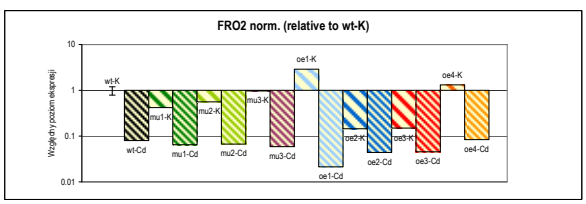
SnRK2.10 norm. (relative to wt-K)



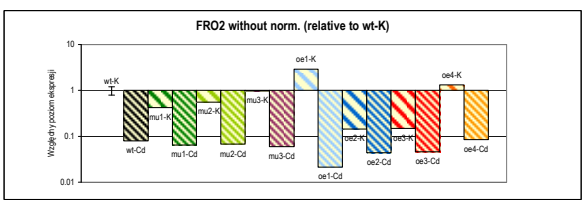
SnRK2.10 without norm. (relative to wt-K)



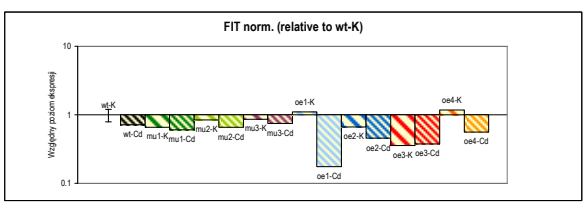
FRO2 norm. (relative to wt-K)



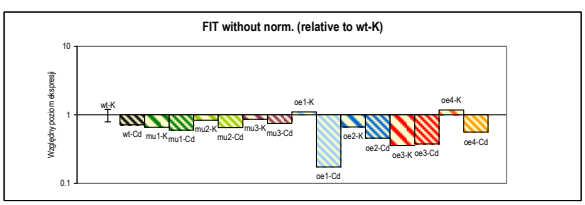
FRO2 without norm. (relative to wt-K)



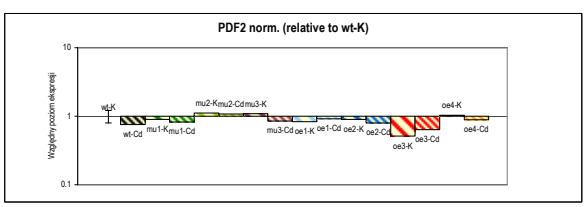
FIT norm. (relative to wt-K)



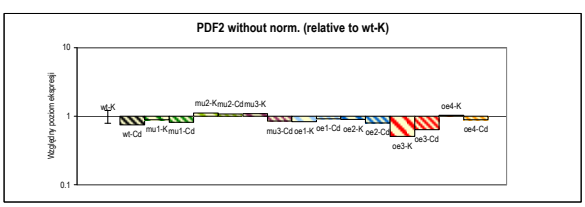
FIT without norm. (relative to wt-K)



PDF2 norm. (relative to wt-K)



PDF2 without norm. (relative to wt-K)



Efficiency of each target amplification according to each of their standard curves
(prepared from "Stock I" in each case; which is a mixture of a small portion of each sample – to which PREXCEL-Q parameters have been previously applied).

GDH1: 98.85% (First 3 standard points used)

NR1: 108.03% (All 5 standard points used)

IRT1: 104.01% (All 5 standard points used)

SnRK2.4: 101.29% (All 5 standard points used)

SnRK2.10: 96.87% (All 5 standard points used)

FIT: 105.99% (First 3 standard points used)

FRO2: 98.11% (First 3 standard points used) dimer signal suspected >Cq of 26.77

PDF2: 107.41% (First 4 standard points used)

UbQ: 96.28% (All 5 standard points used)

These are excellent amplification efficiencies for all targets – and, since all unknowns were diluted to the second standard curve point (ng/µl concentration), all targets in all samples have been assessed within the best part of each of their respective standard curves. This study was carried out phenomenally well and represents very extensive care taken on the part of Dr. Zmienko.

Things to remember: each sample's quantification cycle (Cq) value (formerly known as "Ct" value) is processed as follows:

Example: **GDH1 sample 1** gave duplicate Ct values of 25.2 and 24.98 and ithe GDH1 standard curve had values of:

m = **-3.3497**
b = **26.4053**
Efficiency = **98.85%**
E_{AMP} = **1.98853**

E_{AMP} is Efficiency + 1 and tells us how close the system got to doubling template every cycle.

So… to continue: from the standard curve plot of Ct vs. log(x) where x = the relative dilution factor or relative Qty of target template, we use the equation of a line to figure out how to calculate "x" or "relative quantity" that is represented by each Cq value.

So, y = mx + b for qPCR thus becomes:

Cq = m*log(x) + b

Thus Cq-b = m*log(x)

And

(Cq-b)/m = log(x)

Then, take the inverse log of both sides to get:

$$10^{((Ct-b)/m)} = x$$

And x = relative target quantity.

So … for GDH1 Cq value of 25.2, the relative Qty expression becomes: $\mathbf{10^{((25.2 - 26.4053)/-3.3497)}}$
and for the replicate GDH1 Cq value of 24.98, the relative Qty expression becomes: $\mathbf{10^{((24.98 - 26.4053)/-3.3497)}}$
(a) Thus: $\mathbf{10^{((25.2 - 26.4053)/-3.3497)} = 2.2899574}$
(b) And: $\mathbf{10^{((24.98 - 26.4053)/-3.3497)} = 2.6638177}$

And the **reference gene UbQ** Sample 1 duplicate qPCR wells gave Cq values of 23.39 and 23.31, respectively. So the UbQ relative Qty calculations for Sample 1 were (using the UbQ stnd. Curve components):
(c) Thus: $\mathbf{10^{((23.39 - 23.564198)/-3.414492)} = 1.12464967}$
(d) And: $\mathbf{10^{((23.31 - 23.564198)/ -3.414492)} = 1.186989313}$

Then, to properly normalize GDH1 rel. Qty values to the ref. gene UbQ Qty values, all possible divisions are performed:

$[(a)/(c) + (a)/(d) + (b)/(c) + (b)/(d)]/4$ = GDH1 target normalized to UbQ for sample #1.

Do this for each and every sample, for each and every target – and then graph with and without log transformation (either LOG base 10 or LOG base 2 is fine if LOG transforming).

Since Efficiency = $[10^{(-1/slope)}] - 1$, you can already see that efficiency in each case here is being taken into account since the slope of the standard curve is used in each target's respective relative Qty. calculation.

So, what you are ending up with here is the "**Efficiency-corrected E to the delta delta Cq method of qPCR quantification**". It is the strongest way to calculate qPCR results.