transfection efficiency test setup

	R007	FM	PP	FP
cell_volume	100 ul	206.731 ul	195.455 ul	193.5 ul
cells	150,000 cells	206,731 cells	390,909 cells	387,000 cells
dna_mass	0.5 ug	0.258413 ug	0.195455 ug	0.325758 ug
dna_volume	5 ul	4.13462 ul	9.77273 ul	65.1515 ul
plasmid_reagent_complex_volume	15 ul	8.26923 ul	19.5455 ul	65.803 ul
reagent_volume	10 ul	0.258413 ul	0.586364 ul	0.651515 ul

FACTORS

	Rep	Reagent	Media	Cells per well	Initial cell volume	Initial cell concentration	Final cell concentration	DNA per well	DNA volume	Initial DNA concentration	Reagent per well	DNA:Reagent ratio
2	3	R007	CD_CHO	150,000 cells	100 ul	1,500,000 cells/ml	697,674 cells/ml	250 ng	5 ul	50 ng/ul	10 ul	1:40
5	3	R007	CD_CHO	300,000 cells	100 ul	3,000,000 cells/ml	1,395,349 cells/ml	250 ng	5 ul	50 ng/ul	10 ul	1:40
8	3	R007	CD_CHO	150,000 cells	100 ul	1,500,000 cells/ml	697,674 cells/ml	500 ng	5 ul	100 ng/ul	10 ul	1:20
11	3	R007	CD_CHO	300,000 cells	100 ul	3,000,000 cells/ml	1,395,349 cells/ml	500 ng	5 ul	100 ng/ul	10 ul	1:20
14	3	R007	CD_CHO	150,000 cells	100 ul	1,500,000 cells/ml	697,674 cells/ml	1,000 ng	5 ul	200 ng/ul	10 ul	1:10
17	3	R007	CD_CHO	300,000 cells	100 ul	3,000,000 cells/ml	1,395,349 cells/ml	1,000 ng	5 ul	200 ng/ul	10 ul	1:10
20	3	R007	Freestyle_EM_CHO	150,000 cells	100 ul	1,500,000 cells/ml	697,674 cells/ml	250 ng	5 ul	50 ng/ul	10 ul	1:40
23	3	R007	Freestyle_EM_CHO	300,000 cells	100 ul	3,000,000 cells/ml	1,395,349 cells/ml	250 ng	5 ul	50 ng/ul	10 ul	1:40
26	3	R007	Freestyle_EM_CHO	150,000 cells	100 ul	1,500,000 cells/ml	697,674 cells/ml	500 ng	5 ul	100 ng/ul	10 ul	1:20
29	3	R007	Freestyle_EM_CHO	300,000 cells	100 ul	3,000,000 cells/ml	1,395,349 cells/ml	500 ng	5 ul	100 ng/ul	10 ul	1:20
32	3	R007	Freestyle_EM_CHO	150,000 cells	100 ul	1,500,000 cells/ml	697,674 cells/ml	1,000 ng	5 ul	200 ng/ul	10 ul	1:10
35	3	R007	Freestyle_EM_CHO	300,000 cells	100 ul	3,000,000 cells/ml	1,395,349 cells/ml	1,000 ng	5 ul	200 ng/ul	10 ul	1:10
38	3	FM	CD_CHO	206,731 cells	207 ul	1,000,000 cells/ml	961,538 cells/ml	129 ng	4 ul	31 ng/ul	258 nl	1:2
41	3	FM	CD_CHO	413,462 cells	207 ul	2,000,000 cells/ml	1,923,077 cells/ml	129 ng	4 ul	31 ng/ul	258 nl	1:2
44	3	FM	CD_CHO	206,731 cells	207 ul	1,000,000 cells/ml	961,538 cells/ml	258 ng	4 ul	62 ng/ul	258 nl	1:1
47	3	FM	CD_CHO	413,462 cells	207 ul	2,000,000 cells/ml	1,923,077 cells/ml	258 ng	4 ul	62 ng/ul	258 nl	1:1
50	3	FM	CD_CHO	206,731 cells	207 ul	1,000,000 cells/ml	961,538 cells/ml	517 ng	4 ul	125 ng/ul	258 nl	1:0.5
53	3	FM	CD_CHO	413,462 cells	207 ul	2,000,000 cells/ml	1,923,077 cells/ml	517 ng	4 ul	125 ng/ul	258 nl	1:0.5
56	3	FM	Freestyle_EM_CHO	206,731 cells	207 ul	1,000,000 cells/ml	961,538 cells/ml	129 ng	4 ul	31 ng/ul	258 nl	1:2
59	3	FM	Freestyle_EM_CHO	413,462 cells	207 ul	2,000,000 cells/ml	1,923,077 cells/ml	129 ng	4 ul	31 ng/ul	258 nl	1:2
62	3	FM	Freestyle_EM_CHO	206,731 cells	207 ul	1,000,000 cells/ml	961,538 cells/ml	258 ng	4 ul	62 ng/ul	258 nl	1:1

65	3	FM	Freestyle_EM_CHO	413,462 cells	207 ul	2,000,000 cells/ml	1,923,077 cells/ml	258 ng	4 ul	62 ng/ul	258 nl	1:1
68	3	FM	Freestyle_EM_CHO	206,731 cells	207 ul	1,000,000 cells/ml	961,538 cells/ml	517 ng	4 ul	125 ng/ul	258 nl	1:0.5
71	3	FM	Freestyle_EM_CHO	413,462 cells	207 ul	2,000,000 cells/ml	1,923,077 cells/ml	517 ng	4 ul	125 ng/ul	258 nl	1:0.5
74	3	PP	CD_CHO	390,909 cells	195 ul	2,000,000 cells/ml	1,818,182 cells/ml	98 ng	10 ul	10 ng/ul	586 nl	1:6
77	3	PP	CD_CHO	781,818 cells	195 ul	4,000,000 cells/ml	3,636,364 cells/ml	98 ng	10 ul	10 ng/ul	586 nl	1:6
80	3	PP	CD_CHO	390,909 cells	195 ul	2,000,000 cells/ml	1,818,182 cells/ml	195 ng	10 ul	20 ng/ul	586 nl	1:3
83	3	PP	CD_CHO	781,818 cells	195 ul	4,000,000 cells/ml	3,636,364 cells/ml	195 ng	10 ul	20 ng/ul	586 nl	1:3
86	3	PP	CD_CHO	390,909 cells	195 ul	2,000,000 cells/ml	1,818,182 cells/ml	391 ng	10 ul	40 ng/ul	586 nl	1:1.5
89	3	PP	CD_CHO	781,818 cells	195 ul	4,000,000 cells/ml	3,636,364 cells/ml	391 ng	10 ul	40 ng/ul	586 nl	1:1.5
92	3	PP	Freestyle_EM_CHO	390,909 cells	195 ul	2,000,000 cells/ml	1,818,182 cells/ml	98 ng	10 ul	10 ng/ul	586 nl	1:6
95	3	PP	Freestyle_EM_CHO	781,818 cells	195 ul	4,000,000 cells/ml	3,636,364 cells/ml	98 ng	10 ul	10 ng/ul	586 nl	1:6
98	3	PP	Freestyle_EM_CHO	390,909 cells	195 ul	2,000,000 cells/ml	1,818,182 cells/ml	195 ng	10 ul	20 ng/ul	586 nl	1:3
101	3	PP	Freestyle_EM_CHO	781,818 cells	195 ul	4,000,000 cells/ml	3,636,364 cells/ml	195 ng	10 ul	20 ng/ul	586 nl	1:3
104	3	PP	Freestyle_EM_CHO	390,909 cells	195 ul	2,000,000 cells/ml	1,818,182 cells/ml	391 ng	10 ul	40 ng/ul	586 nl	1:1.5
107	3	PP	Freestyle_EM_CHO	781,818 cells	195 ul	4,000,000 cells/ml	3,636,364 cells/ml	391 ng	10 ul	40 ng/ul	586 nl	1:1.5
110	3	FP	CD_CHO	387,000 cells	194 ul	2,000,000 cells/ml	1,800,000 cells/ml	163 ng	65 ul	2 ng/ul	652 nl	1:4
113	3	FP	CD_CHO	774,000 cells	194 ul	4,000,000 cells/ml	3,600,000 cells/ml	163 ng	65 ul	2 ng/ul	652 nl	1:4
116	3	FP	CD_CHO	387,000 cells	194 ul	2,000,000 cells/ml	1,800,000 cells/ml	326 ng	65 ul	5 ng/ul	652 nl	1:2
119	3	FP	CD_CHO	774,000 cells	194 ul	4,000,000 cells/ml	3,600,000 cells/ml	326 ng	65 ul	5 ng/ul	652 nl	1:2
122	3	FP	CD_CHO	387,000 cells	194 ul	2,000,000 cells/ml	1,800,000 cells/ml	652 ng	65 ul	10 ng/ul	652 nl	1:1
125	3	FP	CD_CHO	774,000 cells	194 ul	4,000,000 cells/ml	3,600,000 cells/ml	652 ng	65 ul	10 ng/ul	652 nl	1:1
128	3	FP	Freestyle_EM_CHO	387,000 cells	194 ul	2,000,000 cells/ml	1,800,000 cells/ml	163 ng	65 ul	2 ng/ul	652 nl	1:4
131	3	FP	Freestyle_EM_CHO	774,000 cells	194 ul	4,000,000 cells/ml	3,600,000 cells/ml	163 ng	65 ul	2 ng/ul	652 nl	1:4
134	3	FP	Freestyle_EM_CHO	387,000 cells	194 ul	2,000,000 cells/ml	1,800,000 cells/ml	326 ng	65 ul	5 ng/ul	652 nl	1:2
137	3	FP	Freestyle_EM_CHO	774,000 cells	194 ul	4,000,000 cells/ml	3,600,000 cells/ml	326 ng	65 ul	5 ng/ul	652 nl	1:2
140	3	FP	Freestyle_EM_CHO	387,000 cells	194 ul	2,000,000 cells/ml	1,800,000 cells/ml	652 ng	65 ul	10 ng/ul	652 nl	1:1
143	3	FP	Freestyle_EM_CHO	774,000 cells	194 ul	4,000,000 cells/ml	3,600,000 cells/ml	652 ng	65 ul	10 ng/ul	652 nl	1:1

Protocol introduction

```
Reagents:
        CHO-S Cells
        GFP plasmid
        OptiPRO SFM (for FM protocol)
        Deionized water (for R007 protocol)
        Hoects
        PI
        Transfection reagents (['R007', 'FM', 'PP', 'FP'])
        medias (['CD_CHO', 'Freestyle_EM_CHO'])
Equipment:
        half deel well plates (4)
        96 well plates (4)
        shake flasks (2)
        tubes (?)
        Celigo
        Electronic pipettes
```

T=-1: Seed cells in multiple media at proper density

```
Protocol:
Grow CHO-S in the following media: ['CD_CHO', 'Freestyle_EM_CHO']
The day before transfection (t=-1) you should passage cells for each media to ~2e+06 cells/ml in >15.3 ml
```

cell seed calculator

```
measured_cell_density = {
    'CD_CHO': 1.5E7 * (u.cells/u.mL),
    'Freestyle_EM_CHO': 5E6 * (u.cells/u.mL)
}

cells_extra_factor = 1.5

for media in medias:
    print(media)
    suggested_amount_of_cells = needed_cells_per_media*cells_extra_factor
    print(f"\tYou need ~{suggested_amount_of_cells:~gP}")
    volume_to_spin = suggested_amount_of_cells/measured_cell_density[media]
    print(f"\tSo spin down ~{volume_to_spin.to_compact():~gP} and resuspend in {(suggested_amount_of_cells/(
max_cell_concentration/2)).to_compact():~gP} {media}")
```

```
CD_CHO
        You need ~4.59529e+07 cells
        So spin down ~3.06353 ml and resuspend in 22.9765 ml CD_CHO
Freestyle_EM_CHO
        You need ~4.59529e+07 cells
        So spin down ~9.19058 ml and resuspend in 22.9765 ml Freestyle_EM_CHO
```

T=0: Transfection with multiple reagents

Brief overview:

- Do each reagent on its own in 1 plate
- Prepare DNA
- Prepare cells
- Create DNA/reagent complexes
- Add to cells

36 wells per reagent in total spread over 2 medias, 3 levels of DNA and 2 levels of cell density for a total of 12 different setups and 3 replicates
We will use 1 plate per transfection reagent
Use C2->F10 for the experiment and C11-F11 for WT controls
That means no edge wells will be used. Fill edge wells with 215 ul uL media

	1	2	3	4	5	6	7	8	9	10	11	12
A	.	.	.	.	.	.	.	.	.	.	.	.
B	.	.	.	.	.	.	.	.	.	.	.	.
C	.	M1+D1+P1	M1+D1+P1	M1+D1+P1	M1+D1+P2	M1+D1+P2	M1+D1+P2	M1+D1+P3	M1+D1+P3	M1+D1+P3	WT	.
D	.	M1+D2+P1	M1+D2+P1	M1+D2+P1	M1+D2+P2	M1+D2+P2	M1+D2+P2	M1+D2+P3	M1+D2+P3	M1+D2+P3	WT	.
E	.	M2+D1+P1	M2+D1+P1	M2+D1+P1	M2+D1+P2	M2+D1+P2	M2+D1+P2	M2+D1+P3	M2+D1+P3	M2+D1+P3	WT	.
F	.	M2+D2+P1	M2+D2+P1	M2+D2+P1	M2+D2+P2	M2+D2+P2	M2+D2+P2	M2+D2+P3	M2+D2+P3	M2+D2+P3	WT	.
G	.	.	.	.	.	.	.	.	.	.	.	.
H	.	.	.	.	.	.	.	.	.	.	.	.

R007 protocol

Detailed steps for R007

R007 Prepare DNA

```
stock_dna_concentration = 1 * (u.ug/u.uL)
plasmid_extra_factor = 1.5
```

DNA:
We have 3 levels of DNA
	For 50 ng/ul
		Take 4.5 ul from stock and add 85.5 ul
	For 100 ng/ul
		Take 9 ul from stock and add 81 ul
	For 200 ng/ul
		Take 18 ul from stock and add 72 ul

R007 Prepare Cells and add them to wells

```
cell_extra_factor = 1.5
```

Cells:
We have 2 levels of cell densities and 2 medias
Repeat for each media!
We assume that on T=0 the cell densitity for both medias are 4e+06 cells/ml
	For 1.5e+06 cells/ml
		Take ~1.0 ml from stock and add ~1.7 ml
	For 3e+06 cells/ml
		Take ~2.0 ml from stock and add ~675.0 ul

Add 100 ul 1.5e+06 cells/ml CD_CHO to C2-C11
Add 100 ul 3e+06 cells/ml CD_CHO to D2-D11
Add 100 ul 1.5e+06 cells/ml Freestyle_EM_CHO to E2-E11
Add 100 ul 3e+06 cells/ml Freestyle_EM_CHO to F2-F11

R007 Make complexes

```
reagent_extra_factor = 1.1
```

```
Prepare 1x working solution from 4x stock solution:
        Mix 148.5 ul reagent with 445.5 ul deionized water (total = 594 ul 1x solution)
For 50 ng/ul plasmid:
        Add 180 ul 1x R007 to the 90 ul 50 ng/ul plasmid
        Pipette up/down 5 times (no vortex) and wait 5 min
        Then pipette (while gently shaking plate) 15 ul complex into wells C2-F4
For 100 ng/ul plasmid:
        Add 180 ul 1x R007 to the 90 ul 100 ng/ul plasmid
        Pipette up/down 5 times (no vortex) and wait 5 min
        Then pipette (while gently shaking plate) 15 ul complex into wells C5-F7
For 200 ng/ul plasmid:
        Add 180 ul 1x R007 to the 90 ul 200 ng/ul plasmid
        Pipette up/down 5 times (no vortex) and wait 5 min
        Then pipette (while gently shaking plate) 15 ul complex into wells C8-F10
Then put them into the incubator for 1.5 hours and then add 100 uL fresh media
```

FM protocol

```
Detailed steps for FM
```

FM Prepare DNA

```
stock_dna_concentration = 1 * (u.ug/u.uL)
plasmid_extra_factor = 1.5
```

```
DNA:
We have 3 levels of DNA
        For 31.25 ng/ul
                Take 2.3 ul from stock and add 72.1 ul OptiPRO SFM
        For 62.5 ng/ul
                Take 4.7 ul from stock and add 69.8 ul OptiPRO SFM
        For 125 ng/ul
                Take 9.3 ul from stock and add 65.1 ul OptiPRO SFM
```

FM Prepare Cells and add them to wells

```
cell_extra_factor = 1.5
```

```
Cells:
We have 2 levels of cell densities and 2 medias
Repeat for each media!
We assume that on T=0 the cell densitity for both medias are 4e+06 cells/ml
        For 1e+06 cells/ml
                Take ~1.4 ml from stock and add ~4.2 ml media
        For 2e+06 cells/ml
                Take ~2.8 ml from stock and add ~2.8 ml media



Add 206.7 ul 1e+06 cells/ml CD_CHO to C2-C11
Add 206.7 ul 2e+06 cells/ml CD_CHO to D2-D11
Add 206.7 ul 1e+06 cells/ml Freestyle_EM_CHO to E2-E11
Add 206.7 ul 2e+06 cells/ml Freestyle_EM_CHO to F2-F11
```

FM Make complexes

```
reagent_extra_factor = 1.1
```

```
Prepare 1x working solution by:
        Mix 15.3 ul FM with 245.6 ul OptiPRO SFM (total = 260.9 ul 1x solution)
Wait 5 minutes (more than 5 min is bad)
For 31.25 ng/ul plasmid:
        Add 74.4 ul 1x FM to the 74.4 ul 31.25 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 20-30 min
        Then pipette 8.3 ul complex into wells C2-F4
For 62.5 ng/ul plasmid:
        Add 74.4 ul 1x FM to the 74.4 ul 62.5 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 20-30 min
        Then pipette 8.3 ul complex into wells C5-F7
For 125 ng/ul plasmid:
        Add 74.4 ul 1x FM to the 74.4 ul 125 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 20-30 min
        Then pipette 8.3 ul complex into wells C8-F10
Then put them into the incubator. Done
```

PP protocol

```
Detailed steps for PP
```

PP Prepare DNA

```
stock_dna_concentration = 1 * (u.ug/u.uL)
plasmid_extra_factor = 1.5
```

```
DNA:
We have 3 levels of DNA
        For 10 ng/ul
                Take 1.8 ul from stock and add 174.2 ul serum free medium
        For 20 ng/ul
                Take 3.5 ul from stock and add 172.4 ul serum free medium
        For 40 ng/ul
                Take 7.0 ul from stock and add 168.9 ul serum free medium
```

PP Prepare Cells and add them to wells

```
cell_extra_factor = 1.5
```

```
Cells:
We have 2 levels of cell densities and 2 medias
Repeat for each media!
We assume that on T=0 the cell densitity for both medias are 4e+06 cells/ml
        For 2e+06 cells/ml
                Take ~2.6 ml from stock and add ~2.6 ml media
        For 4e+06 cells/ml
                Take ~5.3 ml from stock and add ~909.5 zl media



Add 195.5 ul 2e+06 cells/ml CD_CHO to C2-C11
Add 195.5 ul 4e+06 cells/ml CD_CHO to D2-D11
Add 195.5 ul 2e+06 cells/ml Freestyle_EM_CHO to E2-E11
Add 195.5 ul 4e+06 cells/ml Freestyle_EM_CHO to F2-F11
```

PP Make complexes

```
reagent_extra_factor = 1.1
```

```
Prepare 1x working solution by:
        Mix 34.8 ul PP with 580.5 ul OptiPRO SFM (total = 615.3 ul 1x solution)
Vortex immidiatly and wait 15 min
For 10 ng/ul plasmid:
        Add 175.9 ul 1x PP to the 175.9 ul 10 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 20-30 min
        Then pipette 19.5 ul complex into wells C2-F4
For 20 ng/ul plasmid:
        Add 175.9 ul 1x PP to the 175.9 ul 20 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 20-30 min
        Then pipette 19.5 ul complex into wells C5-F7
For 40 ng/ul plasmid:
        Add 175.9 ul 1x PP to the 175.9 ul 40 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 20-30 min
        Then pipette 19.5 ul complex into wells C8-F10
Then put them into the incubator. Done
```

FP protocol

```
Detailed steps for FP
```

FP Prepare DNA

```
stock_dna_concentration = 1 * (u.ug/u.uL)
plasmid_extra_factor = 1.5
```

```
DNA:
We have 3 levels of DNA
        For 2.5 ng/ul
                Take 2.9 ul from stock and add 1.2 ml serum free medium
        For 5 ng/ul
                Take 5.9 ul from stock and add 1.2 ml serum free medium
        For 10 ng/ul
                Take 11.7 ul from stock and add 1.2 ml serum free medium
```

FP Prepare Cells and add them to wells

```
cell_extra_factor = 1.5
```

```
Cells:
We have 2 levels of cell densities and 2 medias
Repeat for each media!
We assume that on T=0 the cell densitity for both medias are 4e+06 cells/ml
        For 2e+06 cells/ml
                Take ~2.6 ml from stock and add ~2.6 ml media
        For 4e+06 cells/ml
                Take ~5.2 ml from stock and add ~0.0 ul media



Add 193.5 ul 2e+06 cells/ml CD_CHO to C2-C11
Add 193.5 ul 4e+06 cells/ml CD_CHO to D2-D11
Add 193.5 ul 2e+06 cells/ml Freestyle_EM_CHO to E2-E11
Add 193.5 ul 4e+06 cells/ml Freestyle_EM_CHO to F2-F11
```

FP Make complexes

```
reagent_extra_factor = 1.1
```

Vortex FectoPRO for 5 sec and spin down. Then add 38.7 ul FP to an empty tube
For 2.5 ng/ul plasmid:
        Add 11.7 ul FP to the 1172.7 ul 2.5 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 10 min
        Then pipette 65.8 ul complex into wells C2-F4
For 5 ng/ul plasmid:
        Add 11.7 ul FP to the 1172.7 ul 5 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 10 min
        Then pipette 65.8 ul complex into wells C5-F7
For 10 ng/ul plasmid:
        Add 11.7 ul FP to the 1172.7 ul 10 ng/ul plasmid
        Mix gently by pipette up/down 5 times (no vortex) and wait 10 min
        Then pipette 65.8 ul complex into wells C8-F10
Then put them into the incubator. Done