Date: 27.06.21

Name: Zviki/Hodaya

Exp no. 13

A. Experiment type: (Partial reprogramming, Comparative expression etc.)

Comparative expression

B. Experiment goal: to compare the effect of cleaning out dsRNA with cellulose on a different protocols for IVT using GFP.

C. Experimental variables:

1. GFP (jvr) Cellulose/no Cellulose
2. ModGFP (jvr) Cellulose/no Cellulose
3. GFP AG (txk) Cellulose/no Cellulose
4. ModGFP AG (txk) Cellulose/no Cellulose
5. GFP (jvr)+EKB Cellulose/no Cellulose
6. GFP (jvr)+ModEKB Cellulose/no Cellulose
7. ModGFP (jvr)+EKB Cellulose/no Cellulose
8. ModGFP (jvr)+ModEKB Cellulose/no Cellulose
9. GFP AG (txk)+EKB Cellulose/no Cellulose
10. ModGFP AG (txk)+ModEKB Cellulose/no Cellulose
11. NC

IVT preparation:

	GFP (jvr)	GFP AG (txk)	ModGFP (jvr)	ModGFP AG (txk)	EKB	ModEKB	Lipofectamin (ul)
GFP (jvr)	1200						24 (6ul x 4)

ModGFP (jvr)		1200	1200				24 (6ul x 4)
GFP AG (txk)							24 (6ul x 4)
ModGFP AG (txk)				1200			24 (6ul x 4)
GFP (jvr)+EKB	1200				200		24 (6ul x 4)
GFP (jvr)+ModEKB	1200					200	24 (6ul x 4)
ModGFP (jvr)+EKB			1200		200		24 (6ul x 4)
ModGFP (jvr)+ModEKB			1200			200	24 (6ul x 4)
GFP AG (txk)+EKB		1200			200		24 (6ul x 4)
ModGFP AG (txk)+ModEKB				1200		200	24 (6ul x 4)
~~ModGFP (jvr) +EKB SG~~			~~1200~~				~~24 (6ul x 4)~~
	3600	2400	3600	2400	600	600	240
x2 duplicate	7200	4800	7200	4800	1200	1200	
x3 transfections	21600	14400	21600	14400	3600	3600	720
for cellulose cleaning	54000	36000	54000	36000	9000	9000	
all together	**75600**	**50400**	**75600**	**50400**	**12600**	**12600**	**720**

D. Starting conditions

1. Cells type: (type, age, gender) NHDF73M
2. Cells passage: p6
3. No. of replications: 2
4. Size of plate: 6 Well
5. No. of wells/plates: 42 wells/7 plates
6. No. of cells per well: 150,000
7. No. of cells total: 6,300,000
8. Confluency et starting exp: 60%

9. Medium(s): rpmi

10. Medium volume (per well): 2.5

11. Medium replacement: (every other day etc.)

- Before the first transfection
- 3hr after each transfection.

12. Splitting during the experiment? (when, how many?) no

13. Incubator – general/low oxygen

E. The methods of the experiment (transfections, staining, sort, qPCR etc.)

Method	**Link for detailed protocol**	**No. of cells**	**Step in the experiment**	**Done yes/no**	**Link for results**
Seeding 27.06.21		150,000cells per well (42 wells)		yes	
Mixes preparation 27.06.21	Transfection calculation exp.13 27.6.21			yes	
1st transfection (morning) 28.06.21	Transfection calculation exp.13 27.6.21			yes	
Medium replacement			3hr post transfection	yes	

28.06.21					
Pictures 1st transfection 29.06.21				yes	06.07.21 new
2nd transfection (morning) 29.06.21	Transfection calculation exp.13 27.6.21			yes	
Medium replacement 29.06.21			3hr post transfection	yes	
Pictures 2nd transfection 30.06.21				yes	06.07.21 new
3rd transfection (morning) 30.06.21	Transfection calculation exp.13 27.6.21			yes	
Medium replacement 30.06.21			3hr post transfection	yes	
Pictures 3rd transfection 01.07.21				yes	06.07.21 new
Cells counting 01.07.21				yes	cells counting & FACS results 01.07.21
GFP intensity (FACS) 01.07.21				yes	cells counting & FACS results 01.07.21
RNA extraction				yes	06.07.21 new

for qPCR 01.07.21					

F. Experiment end date: 01.07.21

G. Conclusion: (With reference to experiment goal)

Cells viability (cells counting):

- Cellulose cleaning improves the cells viability in the most samples.
- The best cells viability improvement is in non-modified samples (mostly).

GFP expression (FACS analysis):

- ModGFP with cellulose cleaning presents the highest expression of GFP.
- ModGFP / modGFP+EKB / modGFP+modEKB with cellulose cleaning present the highest improvement (GFP expression) in compared to the same samples W/O cellulose cleaning.
- Addition of EKB/modEKB to modGFP did not improve the cells viability and GFP expression of modGFP.