What transfection reagent should I choose?

Promega offers three premium transfection reagents: ViaFect™, FuGENE® 6 and FuGENE® HD. All three offer broad spectrum transfection capability and low toxicity but the same question always comes up: Which one should I choose? First, not every transfection reagent is perfect for every cell line. Second, transfection success can also vary with DNA quality, passage number of cells, cell density, etc.

To get a better understanding of how each reagent performs on a variety of cell lines, a transfection trial was performed with the three Promega reagents and the leading competitor's transfection reagent . Each reagent was tested with a range of transfection reagent: DNA ratios and two concentrations of DNA.

Experiments

See page 2. Cells were transfected with either 50ng or 100ng of luciferase reporter plasmid at various reagent:DNA ratios.

Results

A summary of the results are presented in Table 1 with more information on the exact best conditions in Table 2.

The leading competitor and the Promega reagents suggest trying a 3:1 ratio with 100ng of DNA per well of a 96-well plate if you need a starting place. Table 3 demonstrates how the reagents perform relative to one another when you use the common conditions.

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Conclusion

All four transfection reagents work on a variety of cells. Each reagent clearly works best on certain cell lines but none were perfect for every cell line. Table 2 demonstrates that the common 3:1 ratio is not always the most optimal condition. ViaFect™ Reagent yielded greater luciferase expression over a wider variety of cells in this test whether you optimize or just choose one condition.

This summary is based on data generated by Brad Hook and Alisha Truman, Promega Scientific Applications Services, June 2014.

Table 1. Summary of Transfection Study.

*Transfection was assessed through expression of firefly luciferase (**EXP**). The best condition (highest RLUs) was chosen for each transfection reagent. For each cell line, the maximum RLUs were set at 100% for one reagent and the other 3 were judged relative to that reading. Cells were also assayed for cell viability (**VIA**) and judged versus a no-transfection control for each cell line. Transfection efficiencies lower than 50% are not reported on this table. Greater detail is reported in Table 2.*

Cell Line	FuGENE® 6		FuGENE® HD		ViaFect™ Reagent		Competitor L2K	
	EXP	VIA	EXP	VIA	EXP	VIA	EXP	VIA
A549	++	++	+++	+++	+++	+++		
C2C12					+++	+++		
CHO	++	+++			+++	++	++	++
COS7	+++	+++	+++	+++	+++	+++	+++	+++
H9C2					+++	+++	++	+++
HCT116			+++	++	+++	+++	++	++
HEK 293	++	++			+++	+++		
HeLa			+++	+++	++	+++		
HepG2					+++	++		
HT-29					+++	+++	+++	+++
Huh7					+++	++	++	++
Jurkat					+++	+++	++	+++
K562							+++	+++
LNCaP					+++	+++		
MCF7					+++	+++	+++	+++
NIH 3T3					++	+++	+++	+++
PC-12			++	+++	+++	+++	+++	+++
PC-3			+++	+++	+++	+++		
RAW 264.7	+++	++	+++	++			++	++
U2OS			++	+++	+++	+++		

KEY

+++ • >80% of maxRLUs; • >80% of cells were viable

++ • >50% to <80% max RLUs; • >50% to <80% of cells were viable

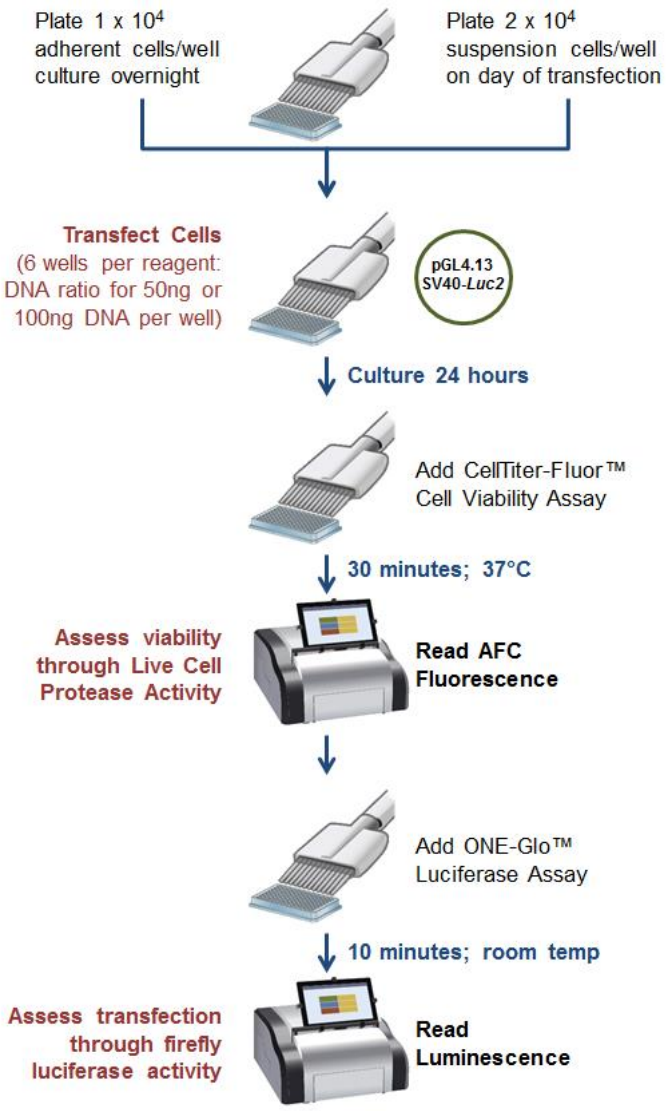
Experimental Overview

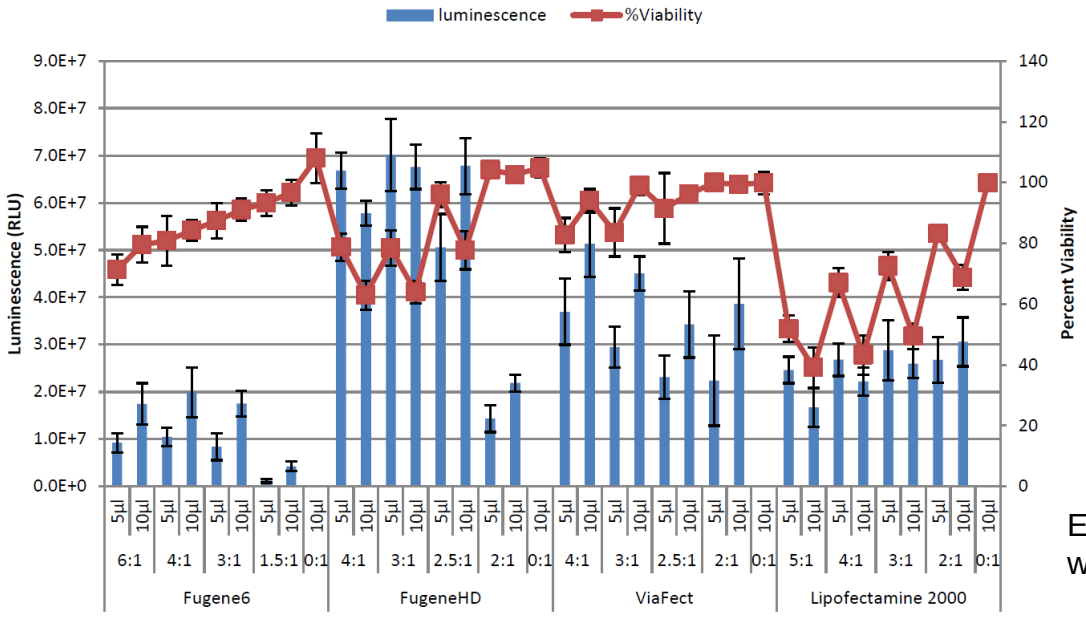
1. Transfection reagents were prepared at room temperature following the directions for each transfection reagents. The following reagent to DNA ratios were tested:

FuGENE® 6 Reagent	6:1	4:1	3:1	1.5:1
FuGENE® HD Reagent	4:1	3:1	2.5:1	2:1
ViaFect™ Reagent	4:1	3:1	2.5:1	2:1
Competitor L2K	5:1	4:1	3:1	2:1

2. Each reagent:DNA ratio was pipetted onto 6 wells of cells in either 5µl or 10µl volumes. One 96 well plate/transfection reagent was used per cell line.
3. Plates were mixed for 15 seconds on a plate shaker for 10-15 seconds
4. Plates were transferred to a 37°C/5% CO_2 incubator for 24 hours prior to assay for cell viability and luciferase reporter activity. Wells with cells only were 100% viability controls.

More complete experimental details are available upon request.





Example data with HeLa cells

Table 2. Optimal 96-well transfection conditions for each cell line at 24 hours post-transfection.

Transfection was judged through expression of firefly luciferase. The conditions that produced the highest RLUs for each transfection reagent were compared relative to the reagent that produced the highest overall RLUs (MAX). The percent of RLU Max is reported along with the optimal reagent:DNA ratio and whether 50ng (5µl) or 100ng (10µl) produced the highest RLUs for that reagent. Viability was judged relative to untransfected control cells. Details for luciferase expression below 50% of MAX RLUs are not reported in this table.

Cell Line	Measure	FuGENE® 6	FuGENE® HD	ViaFect™	L2K
A549	% RLU Max (ratio; vol.)	60% (4:1; 10µl)	90% (3:1; 5µl)	**MAX (4:1; 10µl)**	30%
	% Viable @ 24hr	>90%	>90%	>90%	60%
C2C12	% RLU Max (ratio; vol.)	<10%	<10%	**MAX (4:1; 10µl)**	25%
	% Viable @ 24hr	>90%	>90%	>90%	>90%
CHO	% RLU Max (ratio; vol.)	60% (6:1; 5µl)	30%	**MAX (4:1; 10µl)**	65% (2:1; 10µl)
	% Viable @ 24hr	>90%	>90%	65%	60%
COS7	% RLU Max (ratio; vol.)	**MAX (3:1; 10µl)**	90%(3:1; 5µl)	80% (4:1; 10µl)	90% (4:1; 10µl)
	% Viable @ 24hr	>90%	>90%	>90%	85%
H9C2	% RLU Max (ratio; vol.)	10%	<10%	**MAX (4:1; 10µl)**	50% (3:1; 10µl)
	% Viable @ 24hr	>90%	>90%	>90%	85%
HCT116	% RLU Max (ratio; vol.)	15%	**MAX (2.5:1; 5µl)**	95% (2:1; 5µl)	60% (2:1; 5µl)
	% Viable @ 24hr	80->90%	70%	80%	70%
HEK 293	% RLU Max (ratio; vol.)	60% (4:1; 10µl)	20%	**MAX (4:1; 10µl)**	30%
	% Viable @ 24hr	75%	60%	>90%	75%
HeLa	% RLU Max (ratio; vol.)	30%	**MAX (3:1; 5µl)**	60% (4:1; 10µl)	45%
	% Viable @ 24hr	80%	80%	>85%	70%
HepG2	% RLU Max (ratio; vol.)	30%	30%	**MAX (4:1; 10µl)**	30%
	% Viable @ 24hr	75%	55%	55%	65%
HT-29	% RLU Max (ratio; vol.)	<10%	40%	**MAX (3:1; 10µl)**	90% (3:1; 10µl)
	% Viable @ 24hr	>90%	>90%	>90%	>90%
Huh7	% RLU Max (ratio; vol.)	40%	10%	**MAX (2.5:1; 5µl)**	60% (2:1; 5µl)
	% Viable @ 24hr	80%	75%	75%	70%
Jurkat	% RLU Max (ratio; vol.)	<10%	<10%	**MAX (2:1; 5µl)**	65% (2:1; 10µl)
	% Viable @ 24hr	>90%	>90%	>90%	80%
K562	% RLU Max (ratio; vol.)	<10%	10%	30%	**MAX (2:1; 10µl)**
	% Viable @ 24hr	>90%	>90%	>90%	>90%
LNCaP	% RLU Max (ratio; vol.)	<10%	40%	**MAX (4:1; 10µl)**	20%
	% Viable @ 24hr	>90%	75%	>90%	50%
MCF7	% RLU Max (ratio; vol.)	10%	15%	80% (4:1; 10µl)	**MAX (2:1; 10µl)**
	% Viable @ 24hr	>90%	>90%	>90%	80%
NIH 3T3	% RLU Max (ratio; vol.)	<10%	15%	60% (4:1; 10µl)	**MAX (4:1; 10µl)**
	% Viable @ 24hr	>90%	>90%	>90%	>90%
PC-12	% RLU Max (ratio; vol.)	10%	70% (2.5: 1; 10µl)	**MAX (4:1; 10µl)**	90% (2:1; 10µl)
	% Viable @ 24hr	>90%	>90%	>90%	>90%
PC-3	% RLU Max (ratio; vol.)	15%	90% (4:1; 10µl)	**MAX (4:1; 10µl)**	15%
	% Viable @ 24hr	>90%	>90%	80%	40-70%
RAW 264.7	% RLU Max (ratio; vol.)	**MAX (3:1; 10µl)**	85% (2.5:1; 5µl)	<10%	50% (2:1; 5µl)
	% Viable @ 24hr	75%	65%	>90%	70%
U2OS	% RLU Max (ratio; vol.)	40%	60% (3:1; 5µl)	**MAX (4:1; 10µl)**	20%
	% Viable @ 24hr	>90%	>90%	>90%	>90%
	≥80% of RLU Max Viability ≥80% @ 24hr		50 to <80% of RLU Max >50% to <80% Viability @ 24hr		

Table 3: Comparison of all four transfection reagents at the commonly recommended conditions of 3:1 Reagent:DNA ratio and 100ng DNA per well of a 96-well plate.

This data was extracted from the overall transfection study. Luciferase expression (**EXP**) is judged for each cell line relative to the maximum RLUs observed (**MAX**). Viability (**VIA**; % of untransfected cells) is listed for each cell line under these conditions.

Cell Line	FuGENE® 6		FuGENE® HD		ViaFect™ Reagent		Competitor L2K	
	EXP	VIA	EXP	VIA	EXP	VIA	EXP	VIA
A549	60%	**>90%**	60%	60%	**MAX**	**>90%**	<10%	30%
C2C12	15%	>90%	<10%	>90%	**MAX**	**>90%**	15%	>90%
CHO	**55%**	**>90%**	40%	>90%	**MAX**	**85%**	50%	45%
COS7	**MAX**	**85%**	**90%**	>90%	60%	**>90%**	**85%**	>90%
H9C2	15%	>90%	<10%	>90%	**MAX**	**>90%**	65%	**>90%**
HCT116	20%	80%	**80%**	65%	**MAX**	**80%**	40%	45%
HEK 293	**70%**	75%	20%	50%	**MAX**	**>90%**	30%	40%
HeLa	25%	>90%	**MAX**	65%	65	**>90%**	40%	50%
HepG2	35%	85%	20%	50%	**MAX**	55%	25%	55%
HT-29	<10%	>90%	45%	>90%	**MAX**	**>90%**	**90%**	**85%**
Huh7	40%	70%	<10%	40%	**MAX**	70%	25%	45%
Jurkat	35%	>90%	<10%	>90%	**MAX**	**80%**	**95%**	**80%**
K562	<10%	>90%	<10%	>90%	20%	>90%	**MAX**	**>90%**
LNCaP	<10%	>90%	60%	75%	**MAX**	**>90%**	25%	60%
MCF7	<10%	>90%	20%	>90%	**95%**	**>90%**	**MAX**	70%
NIH 3T3	<10%	>90%	25%	>90%	65%	**>90%**	**MAX**	**>90%**
PC-12	15%	>90%	70%	**>90%**	**MAX**	**>90%**	**95%**	**85%**
PC-3	20%	>90%	55%	**80%**	**MAX**	**>90%**	15%	65%
RAW 264.7	**MAX**	75%	45%	55%	<10%	>90%	35%	45%
U2OS	40%	>90%	70%	**>90%**	**MAX**	**>90%**	25%	>90%

KEY ≥80% MAX RLUs >50% to <80% MAX RLUs

Product	Size	Cat. #
ViaFect™ Transfection Reagent	0.75ml	E4981
	2 x 0.75ml	E4982
FuGENE® HD Transfection Reagent	1ml	E2311
	5 x 1ml	E2312
FuGENE® 6 Transfection Reagent	1ml	E2691
	5 x 1ml	E2692