ANKARA ÜNİVERSİTESİ
BİYOTEKNOLOJİ
ENSTİTÜSÜ

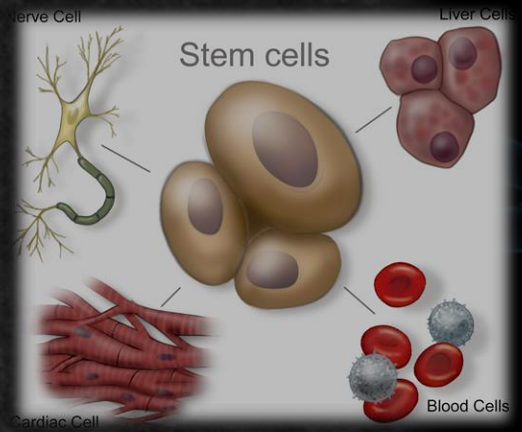
ANKARA ÜNİVERSİTESİ 1946

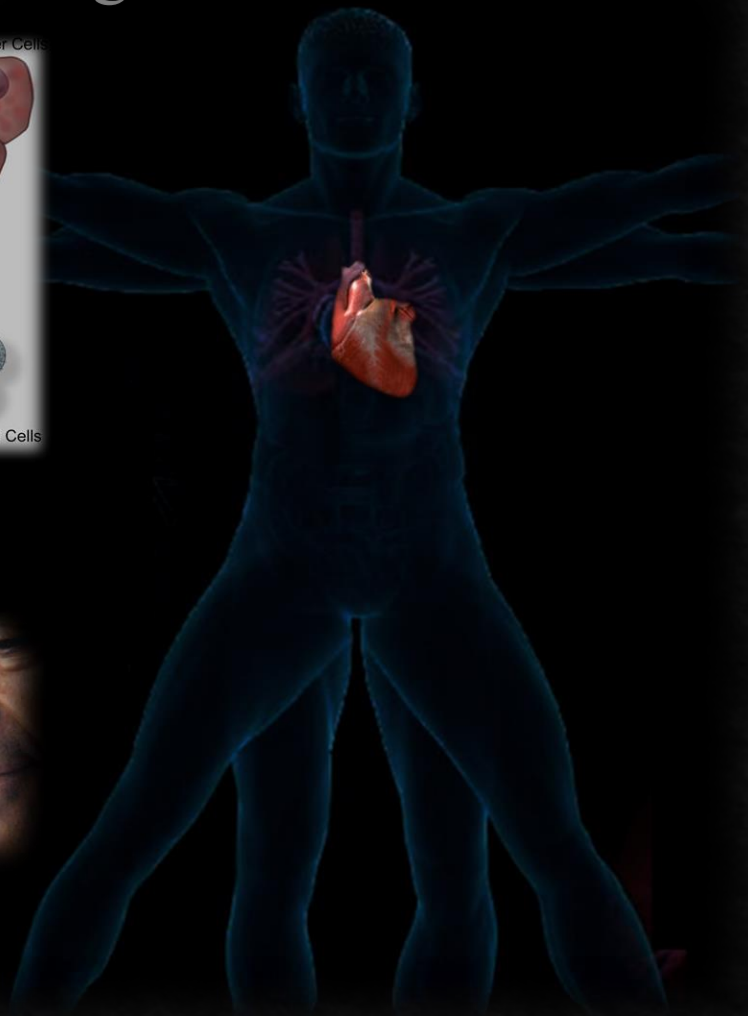
ANKARA UNİVERSİTY
BİOTECHNOLOGY INSTİTUTE

Regenerative Medicine

# Onarımsal Tıp

(Rejeneratif Tıp)



# Delivery Methods for Generating iPSCs

| Delivery Method | Mechanism | Efficiency | Safety | Pros | Cons |
|---|---|---|---|---|---|
| MMLV-derived Retrovirus | Integrating | High | Low | Efficient and Stable | Genome integration<br>Insertional mutagenesis, transgene reactivation, residual expression |
| Lentivirus | Integrating | High | Low | Efficient and Stable | Genome integration<br>Insertional mutagenesis, residual expression |
| piggyBac | Excisible | Medium | Medium | Generates transgene-free and vector-free cells | Genome integration<br>Need to sequence cells to verify excision didn't introduce mutations |
| Adenovirus | Non-integrating | Low | Medium | Generates transgene-free and vector-free cells<br>No genomic integration | Inefficient |

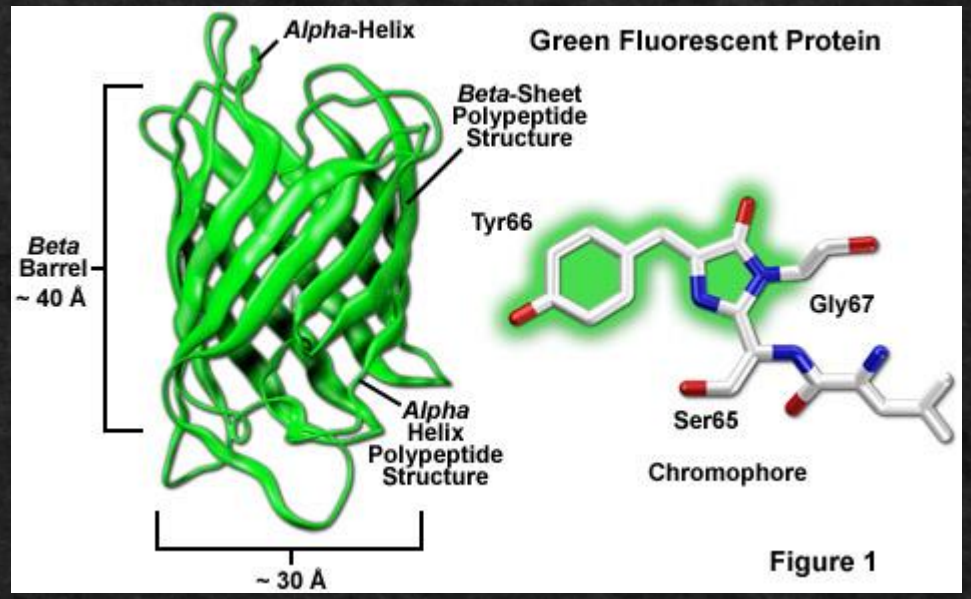
| Delivery Method | Mechanism | Efficiency | Safety | Pros | Cons |
|---|---|---|---|---|---|
| sendai Viru | Non-integrating | Medium | Medium | Generates transgene-free and vector-free cells<br>No genomic integration | Can be difficult to fully remove virus from cells |
| Plasmid | Non-integrating, usually | Medium | Medium | Generates transgene-free and vector-free cells<br>Limited genomic integration | Multiple transfections required<br>Need to sequence to verify there's no genomic integration |
| Replicating EBNA1 episome | Non-integrating | Medium | Medium | Generates transgene-free and vector-free cells<br>No genomic integration | Inefficient<br>Need to verify loss of episome |
| Minicircle | Non-integrating | Medium | Medium | Generates transgene-free and vector-free cells<br>No genomic integration | Inefficient<br>Need to verify loss of minicircle |
| RNA Delivery | DNA-Free | High | High | Transgene-free and vector-free | Multiple transfections required |
| Protein Delivery | DNA-Free | Low | High | Transgene-free and vector-free. | Slow and inefficient<br>Difficult to purify reprogramming proteins. |

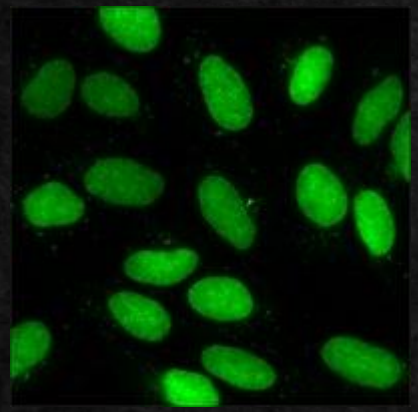
# Gene aktarmada takıp ve izolasyon metotları

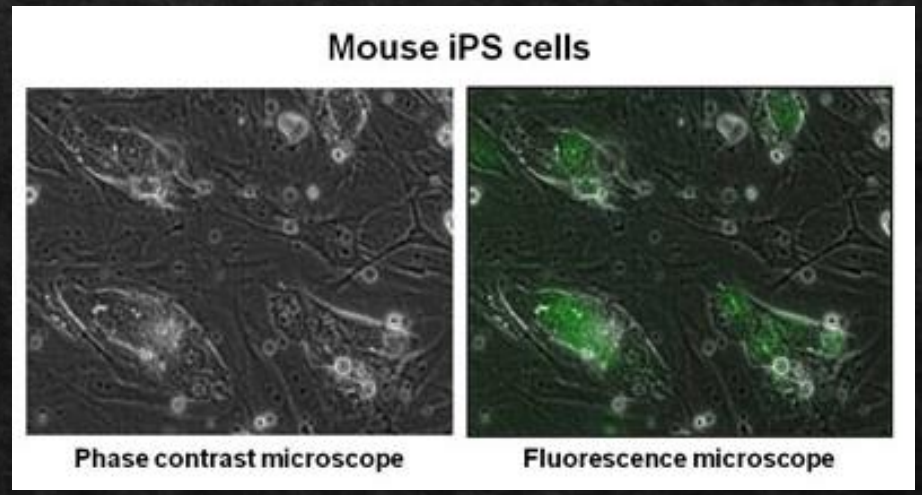
- GFP (Jelly fish Aequoria Victoria) yeşil floresanı
- Antibiyotik direnci
- Gen deney analizi
- Protein analizi……

# GFP

- (Jelly fish Aequoria Victoria)
- green fluoresent  27 kDal

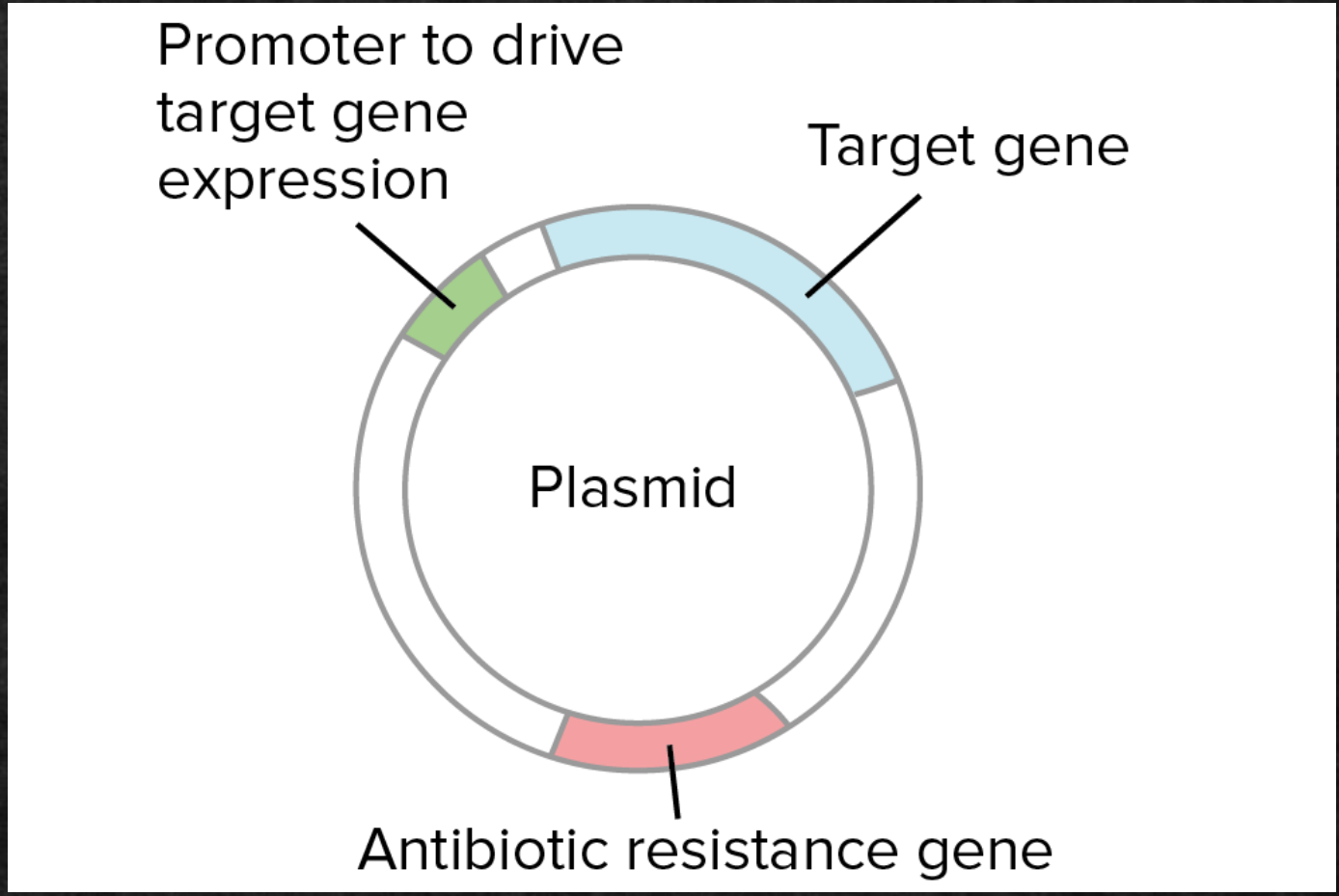


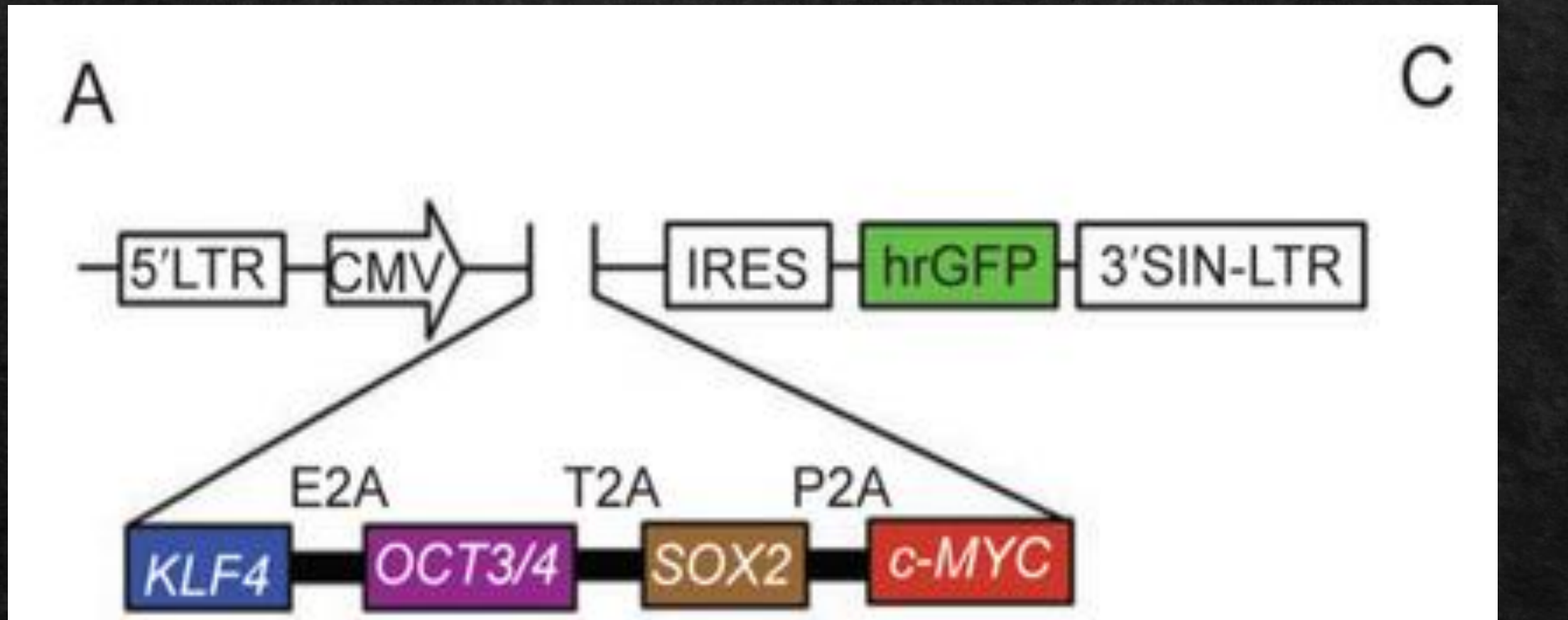


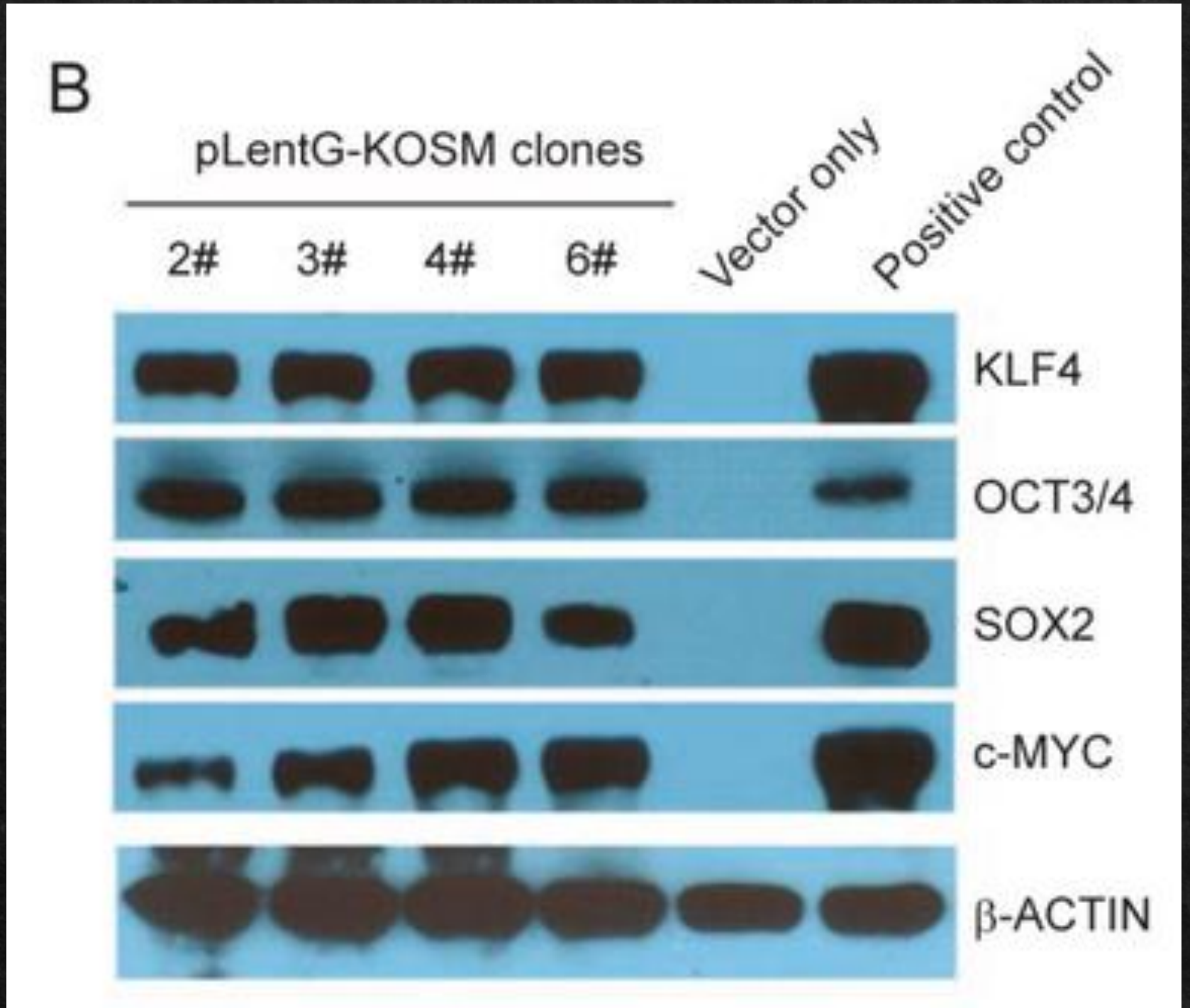
# Antibiyotik direnci

Gene aktarmada takıp ve izolasyon metotlarından biri olan antibiotik direnç geninin aktarılmasından sonra ortamda aynı antibiotik kullanıldığında gen aktarılan hücrelerin yaşadığı ve gen aktarılmayan hücrelerin öldüğü için izolasyon yapılmaktadır.

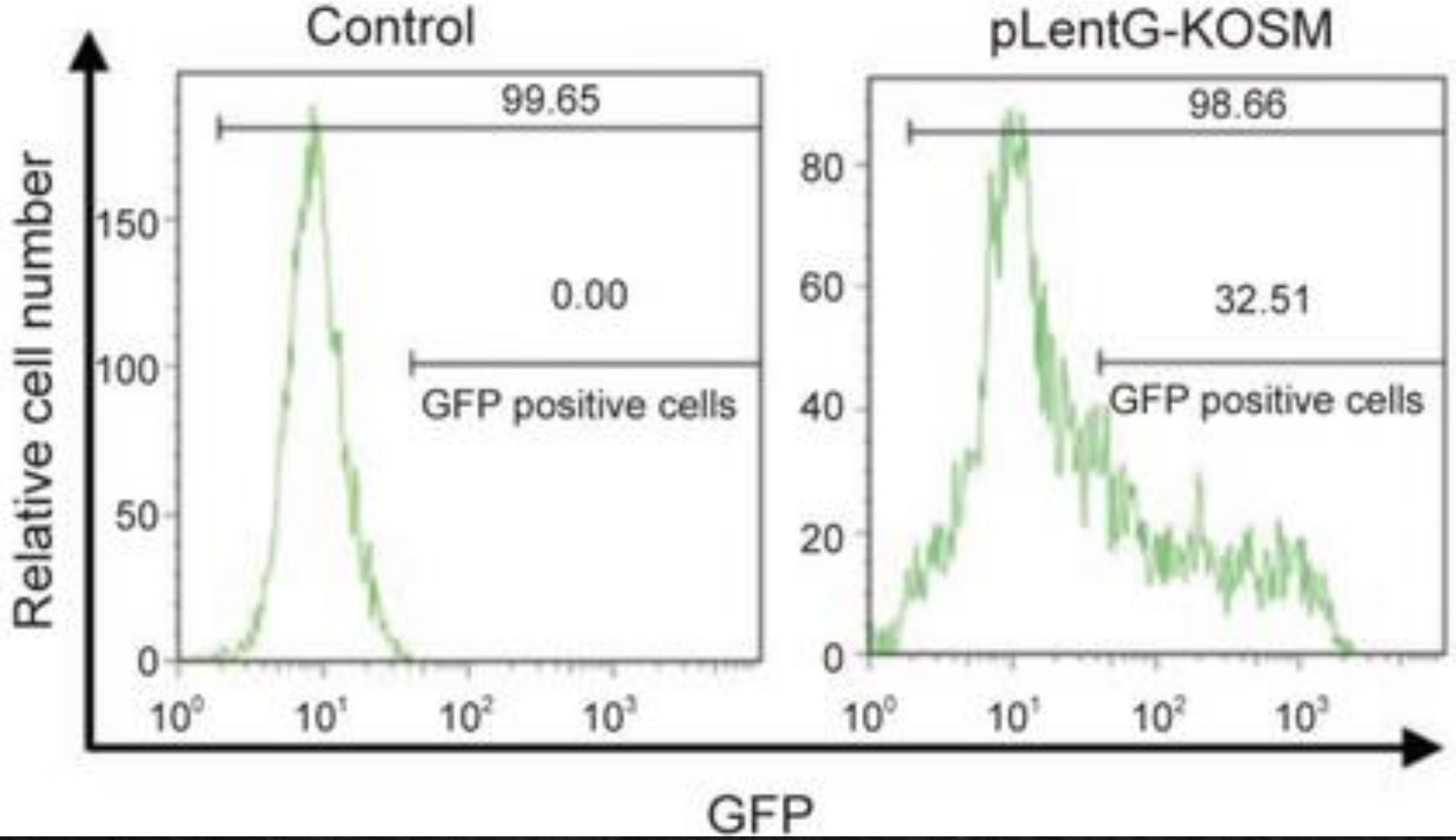


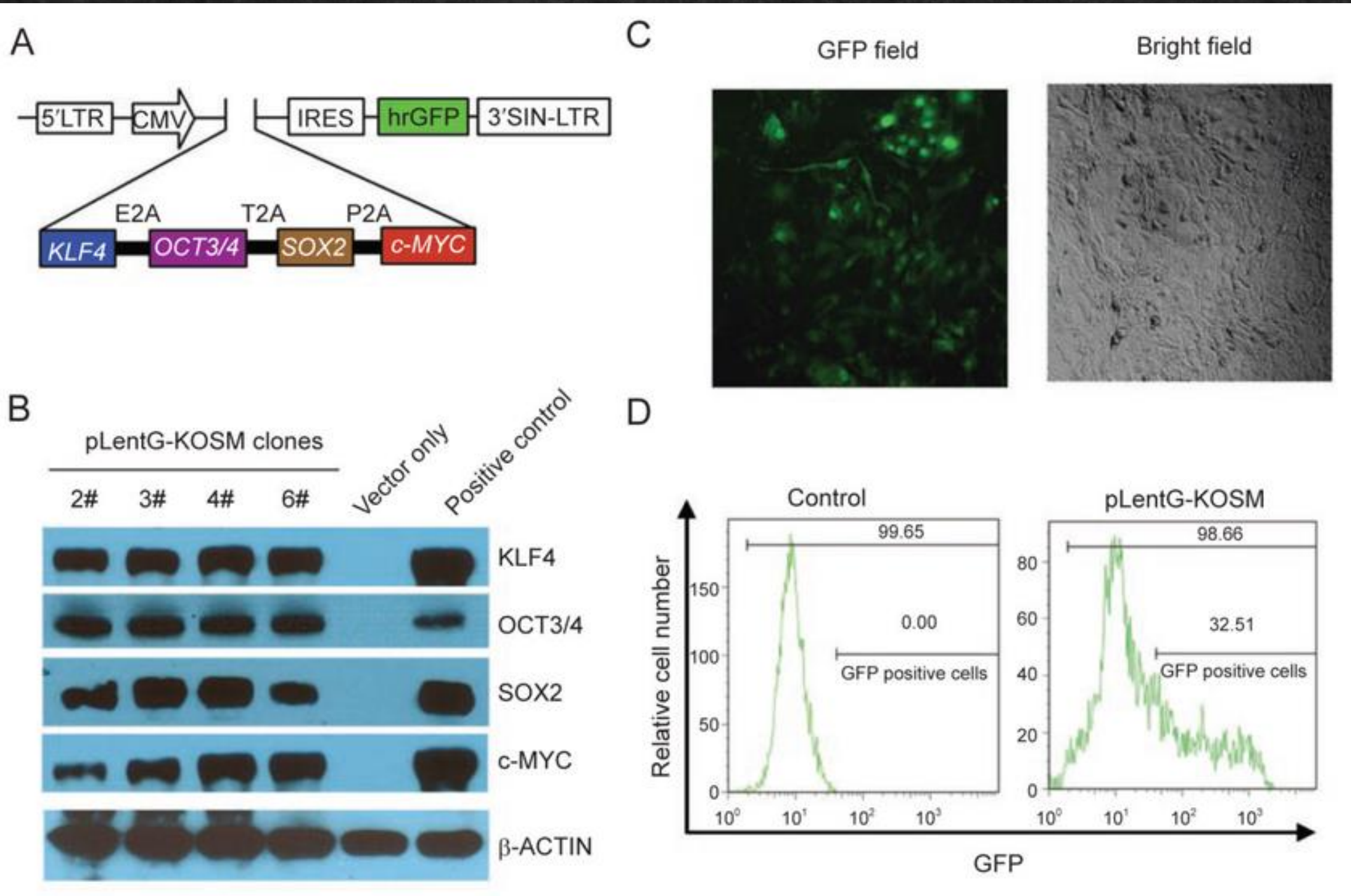
# Polisistronik gen aktarımı




B
pLentG-KOSM clones
2#
3#
4#
6#
Vector only
Positive control
KLF4
OCT3/4
SOX2
c-MYC
β-ACTIN

D


Control
99.65
0.00
GFP positive cells
pLentG-KOSM
98.66
32.51
GFP positive cells
Relative cell number
0
50
100
150
0
20
40
60
80
$10^0$
$10^1$
$10^2$
$10^3$
GFP


A
5'LTR
CMV
IRES
hrGFP
3'SIN-LTR
E2A
T2A
P2A
KLF4
OCT3/4
SOX2
c-MYC
B
pLentG-KOSM clones
2#
3#
4#
6#
Vector only
Positive control
KLF4
OCT3/4
SOX2
c-MYC
β-ACTIN
C
GFP field
Bright field
D
Control
pLentG-KOSM
99.65
0.00
GFP positive cells
98.66
32.51
GFP positive cells
Relative cell number
GFP