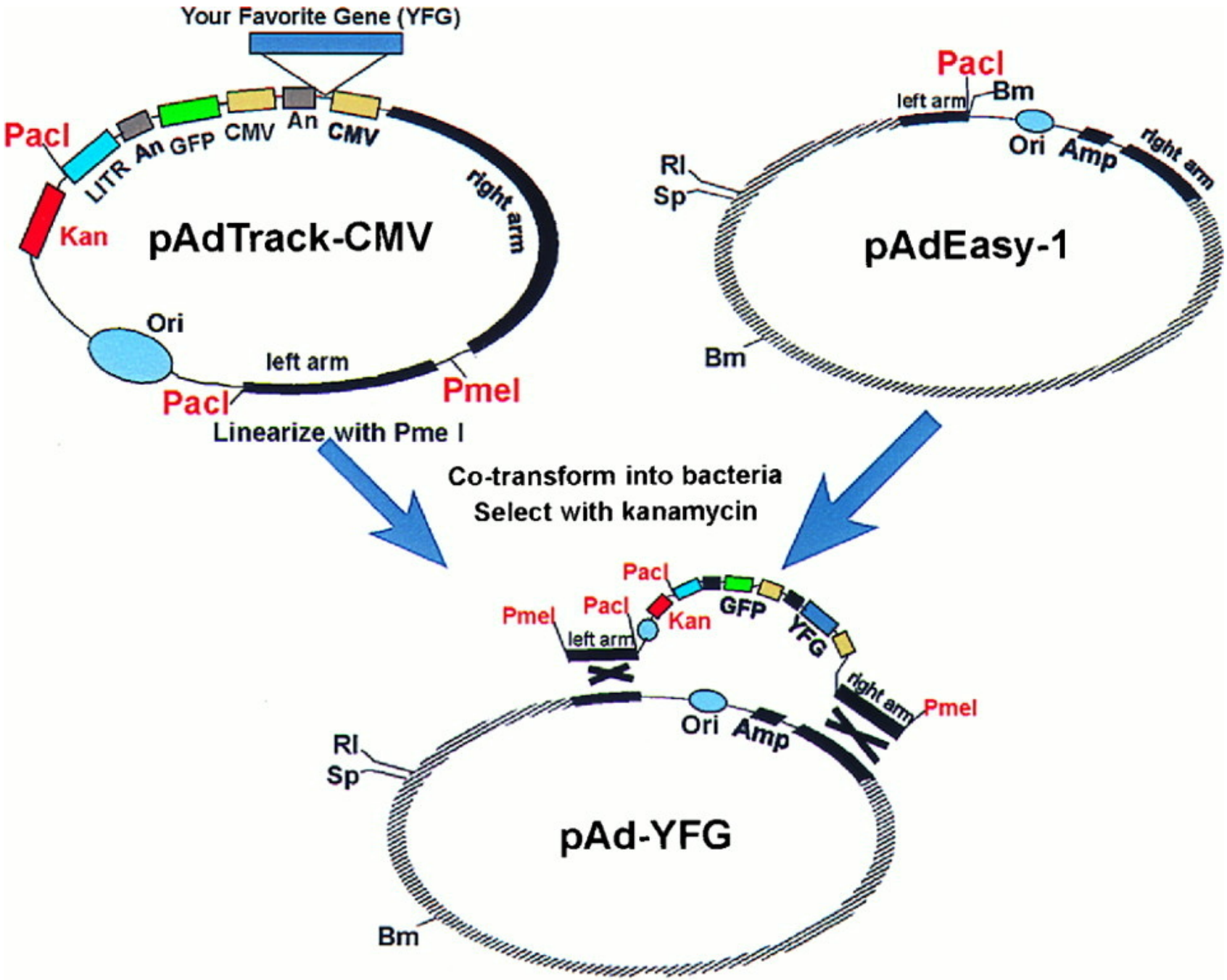
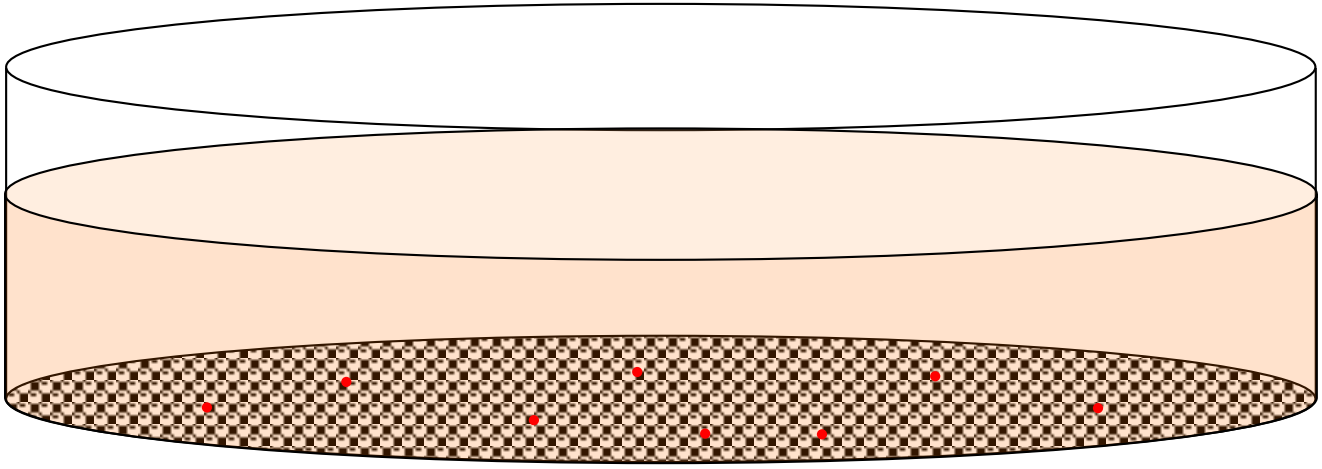
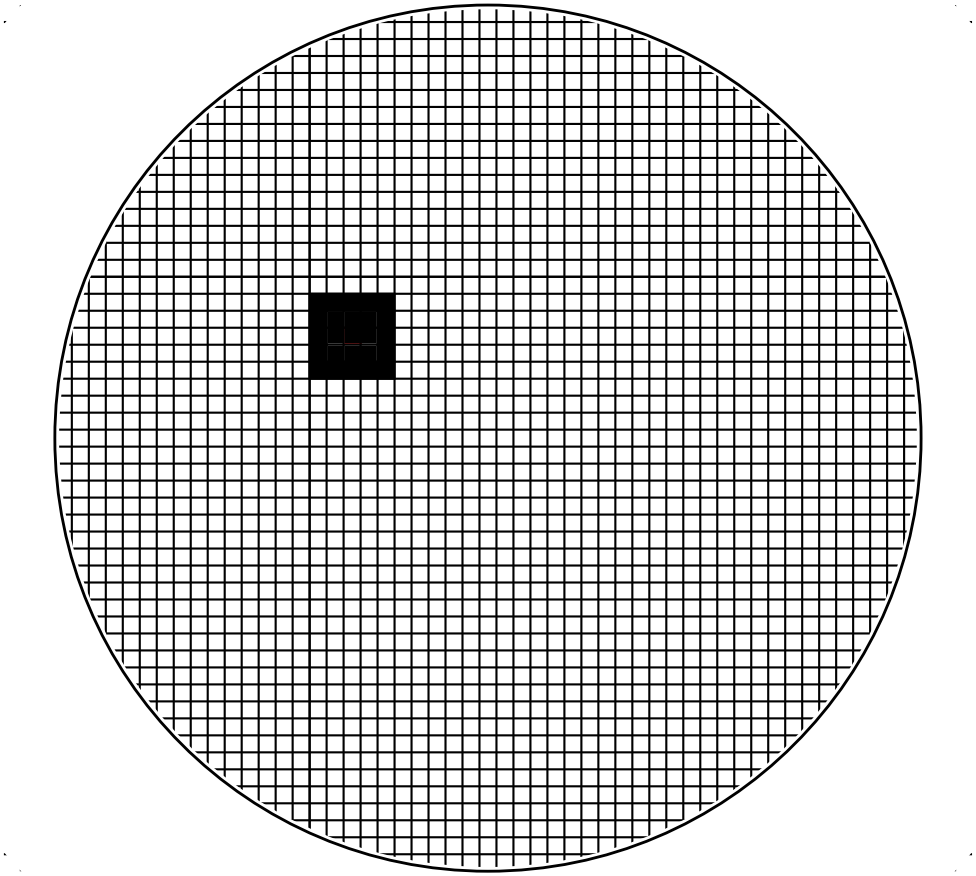
Adenovirus ricombinanti e loro applicazioni

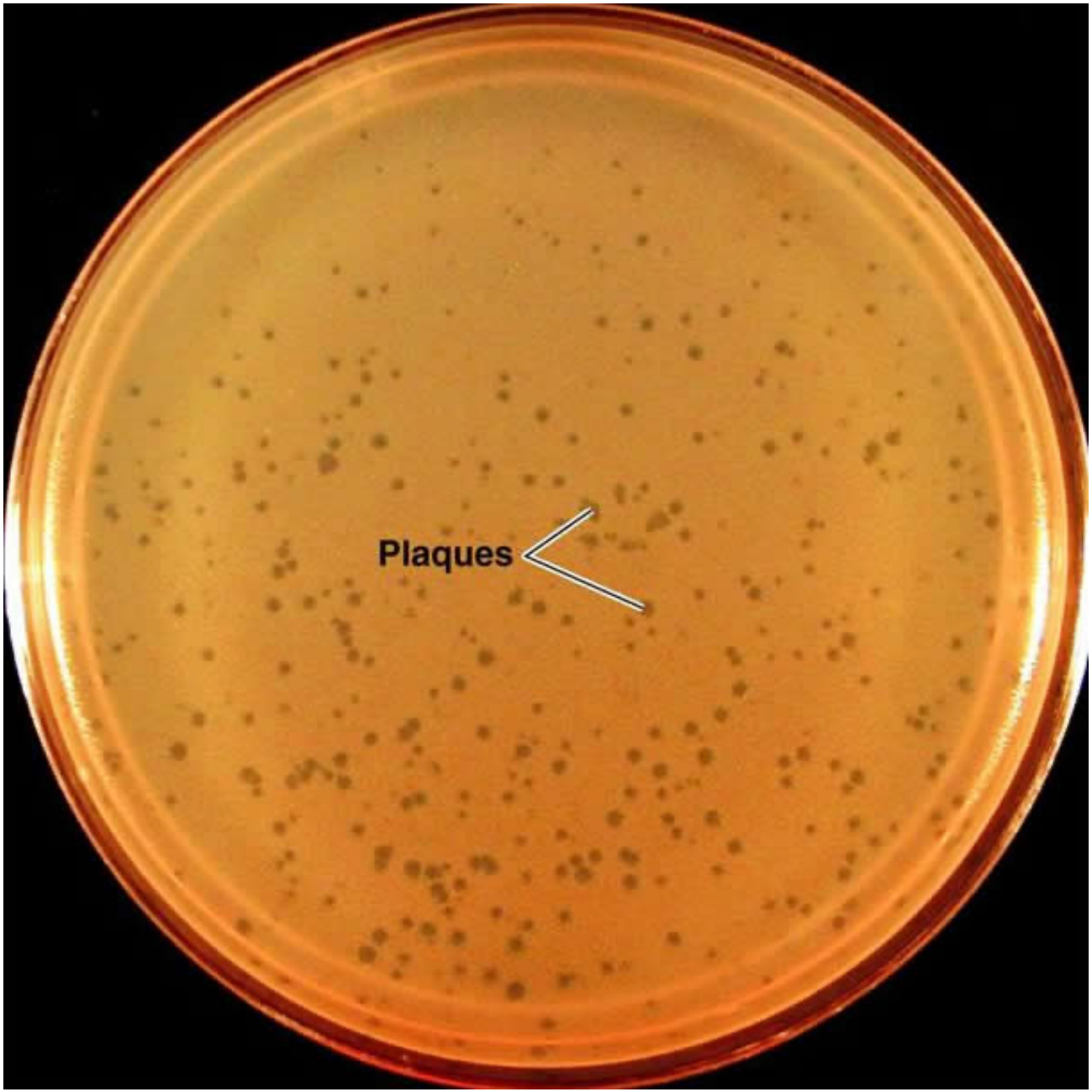
Marco Crescenzi – marco.crescenzi@iss.it

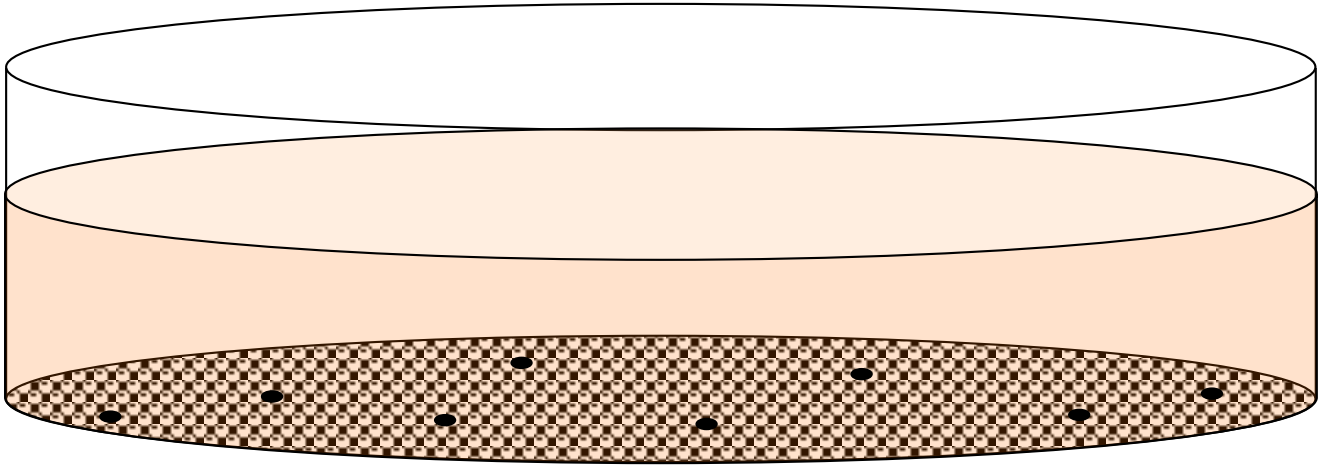
Generazione di adenovirus ricombinanti

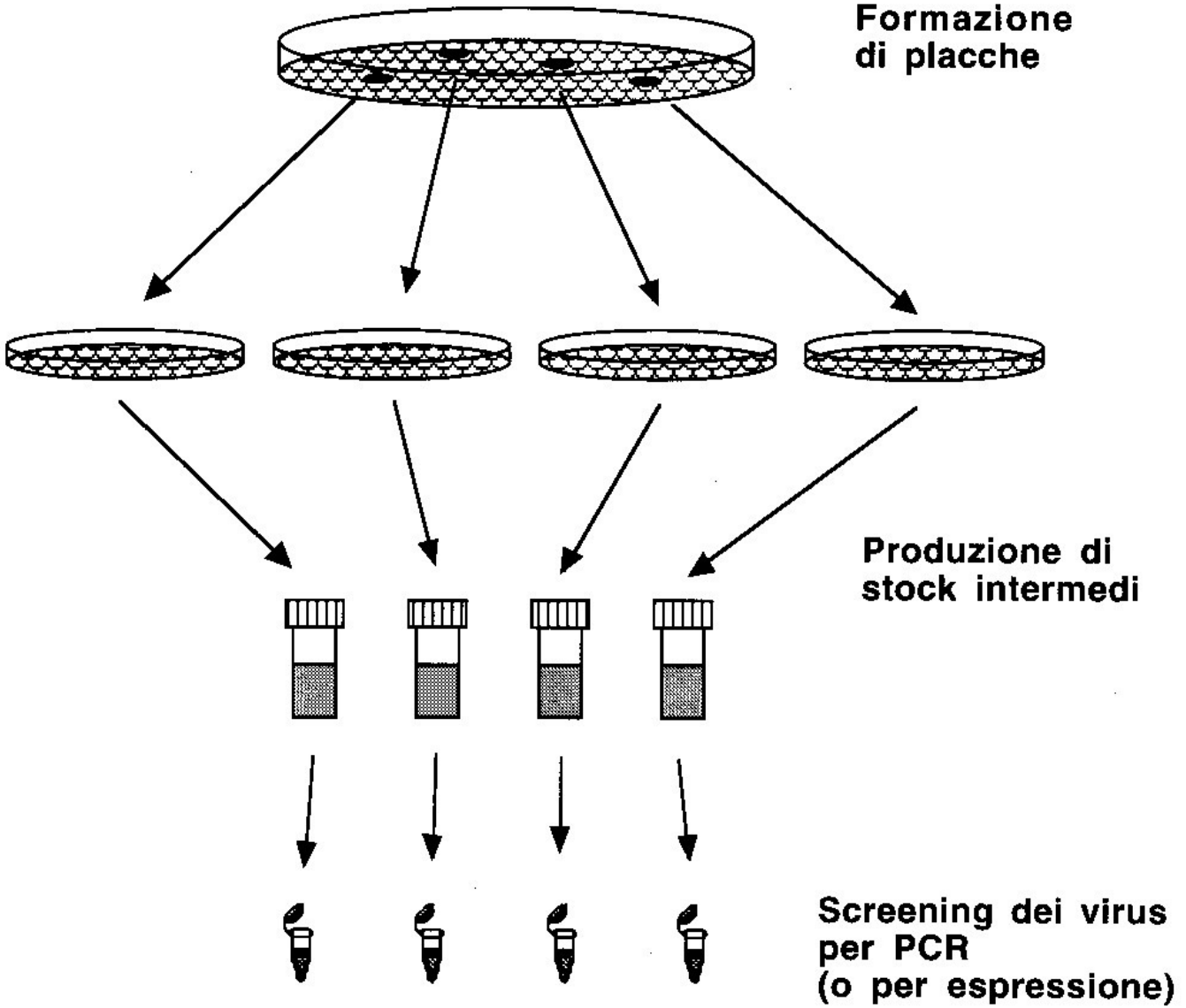

Your Favorite Gene (YFG)
PacI
LITR
An
GFP
CMV
An
CMV
right arm
Kan
pAdTrack-CMV
Ori
left arm
PacI
PmeI
Linearize with Pme I
PacI
left arm
Bm
Ori
Amp
right arm
RI
Sp
pAdEasy-1
Bm
Co-transform into bacteria
Select with kanamycin
PacI
PmeI
PacI
left arm
Kan
GFP
YFG
right arm
PmeI
Ori
Amp
RI
Sp
pAd-YFG
Bm

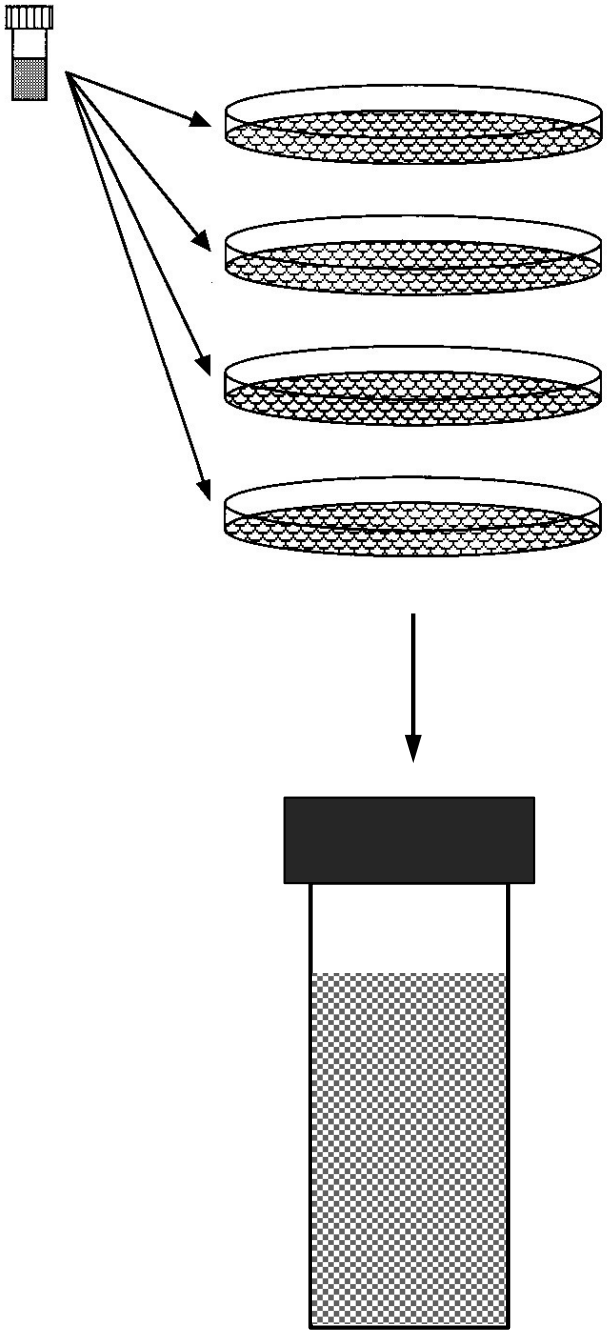




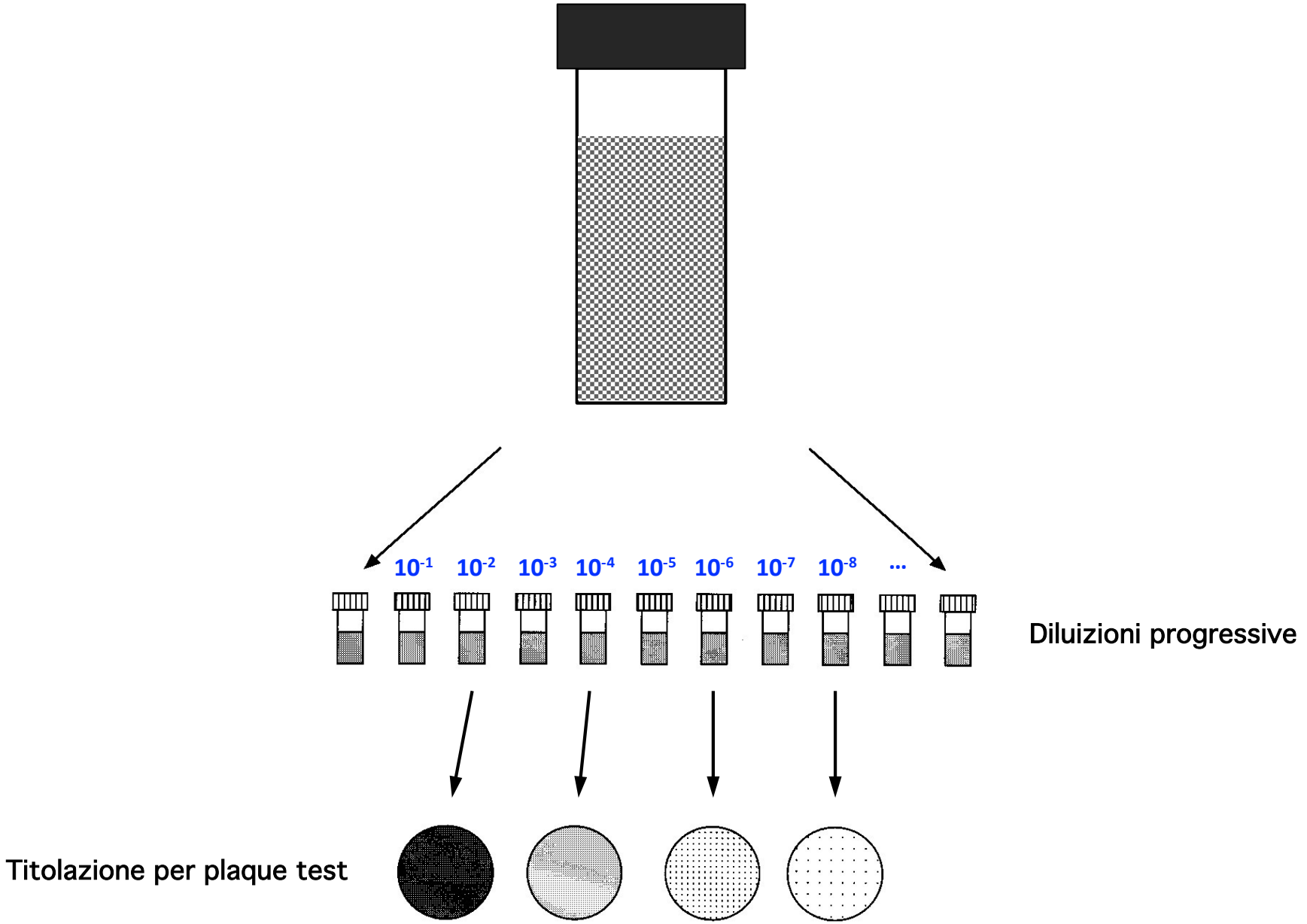

Plaques




Formazione
di placche
Produzione di
stock intermedi
Screening dei virus
per PCR
(o per espressione)



Produzione stock
virale su larga scala


10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} ...
Diluizioni progressive
Titolazione per plaque test

Una applicazione clinica:

virus oncolitici

CANCER

Therapeutic targeting of the RB1 pathway in retinoblastoma with the oncolytic adenovirus VCN-01

Guillem Pascual-Pasto[1,2], Miriam Bazan-Peregrino[3], Nagore G. Olaciregui[1,2], Camilo A. Restrepo-Perdomo[4], Ana Mato-Berciano[3], Daniela Ottaviani[5,6], Klaus Weber[7], Genoveva Correa[1,2], Sonia Paco[1,2], Monica Vila-Ubach[1,2], Maria Cuadrado-Vilanova[1,2], Helena Castillo-Ecija[1,2], Gaia Botteri[1,2], Laura Garcia-Gerique[1,2], Helena Moreno-Gilabert[1,2], Marta Gimenez-Alejandre[3], Patricia Alonso-Lopez[3], Marti Farrera-Sal[3], Silvia Torres-Manjon[8], Dolores Ramos-Lozano[8], Rafael Moreno[8], Isabelle Aerts[5,6], François Doz[5,9], Nathalie Cassoux[5,9,10], Elodie Chapeaublanc[5,6], Montserrat Torrebadell[1,2], Monica Roldan[1,4], Andrés König[11], Mariona Suñol[4], Joana Claverol[1,12], Cinzia Lavarino[1,2], Carmen de Torres[1,2]*, Ligia Fu[13], François Radvanyi[5,6], Francis L. Munier[14], Jaume Catalá-Mora[15], Jaume Mora[1,2], Ramón Alemany[8], Manel Cascalló[3], Guillermo L. Chantada[1,2,16,17], Angel M. Carcaboso[1,2†]

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Retinoblastoma is a pediatric solid tumor of the retina activated upon homozygous inactivation of the tumor suppressor *RB1*. VCN-01 is an oncolytic adenovirus designed to replicate selectively in tumor cells with high abundance of free E2F-1, a consequence of a dysfunctional RB1 pathway. Thus, we reasoned that VCN-01 could provide targeted therapeutic activity against even chemoresistant retinoblastoma. In vitro, VCN-01 effectively killed patient-derived retinoblastoma models. In mice, intravitreous administration of VCN-01 in retinoblastoma xenografts induced tumor necrosis, improved ocular survival compared with standard-of-care chemotherapy, and prevented micrometastatic dissemination into the brain. In juvenile immunocompetent rabbits, VCN-01 did not replicate in retinas, induced minor local side effects, and only leaked slightly and for a short time into the blood. Initial phase 1 data in patients showed the feasibility of the administration of intravitreous VCN-01 and resulted in antitumor activity in retinoblastoma vitreous seeds and evidence of viral replication markers in tumor cells. The treatment caused local vitreous inflammation but no systemic complications. Thus, oncolytic adenoviruses targeting RB1 might provide a tumor-selective and chemotherapy-independent treatment option for retinoblastoma.

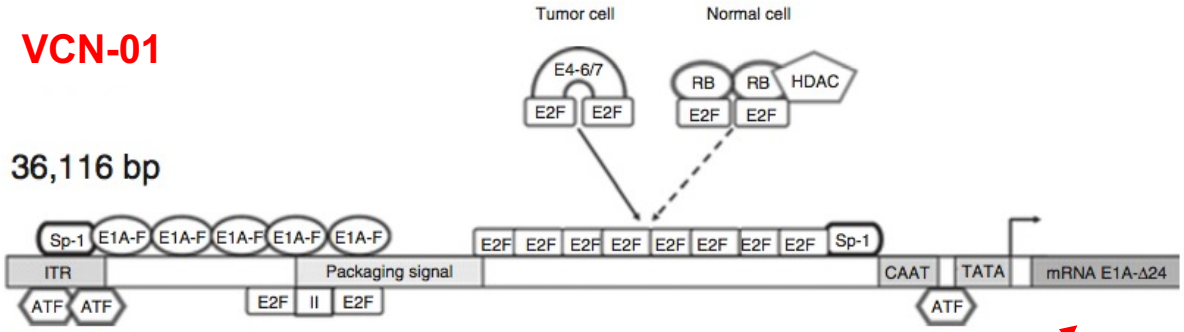
Oncolisi: VCN-01 (Delta-24)

wild type

35,965 bp

VCN-01

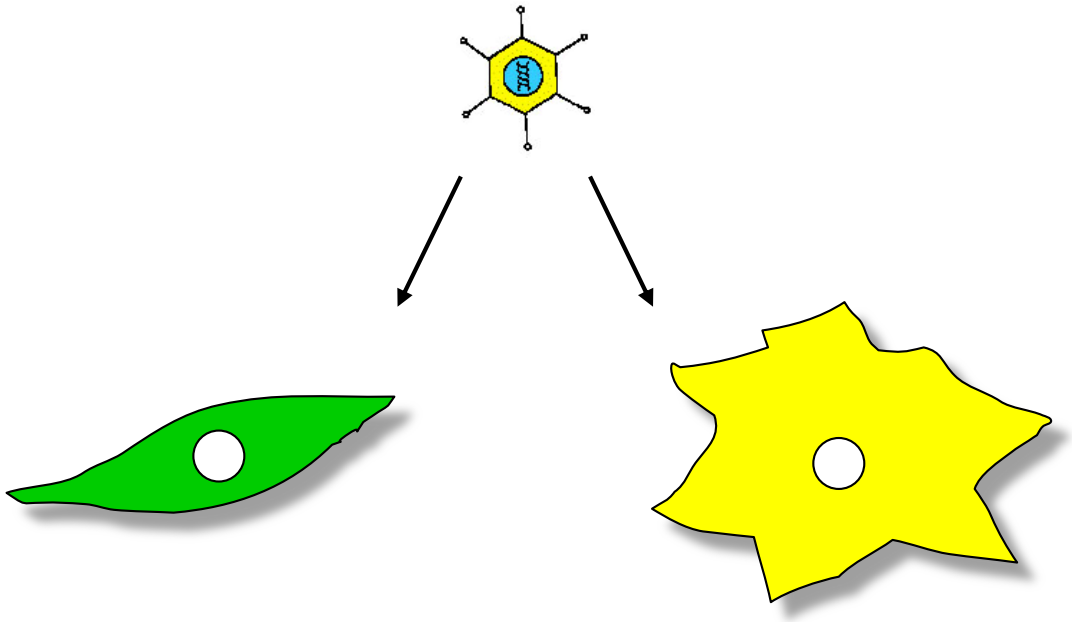
36,116 bp



E1A Δ24

Modificata da: *www.moleculartherapy.org* vol. 18 no. 11, 1960–1971 nov. 2010

Oncolisi: VCN-01 (Delta-24)



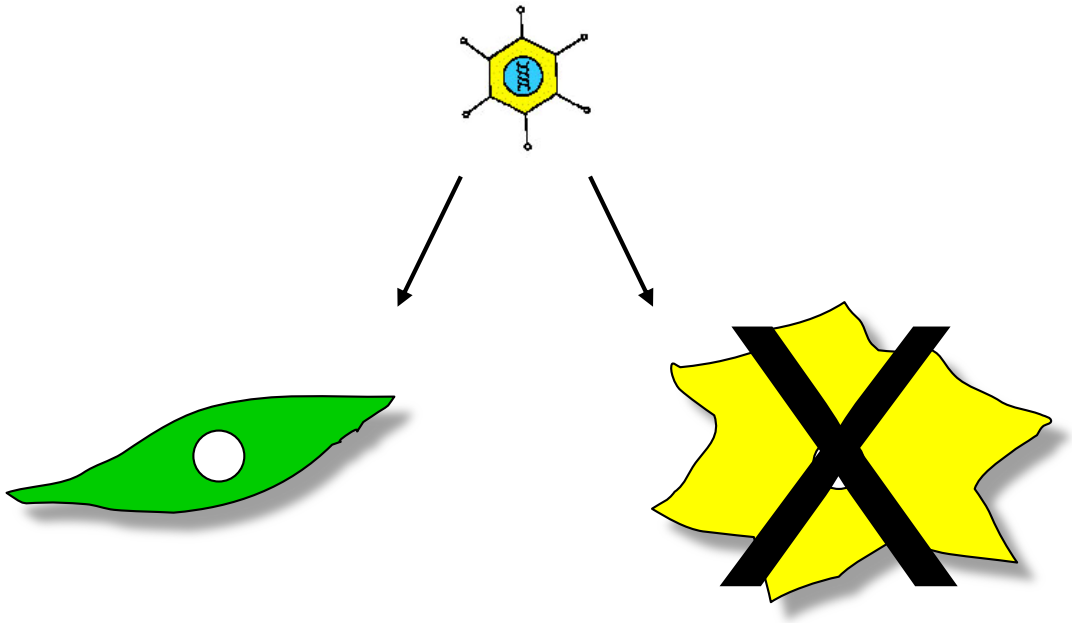
Cellula normale

Rb$^{+/+}$

Cellula di retinoblastoma

Rb$^{-/-}$

Oncolisi: VCN-01 (Delta-24)



Cellula normale

Cellula di retinoblastoma

$Rb^{+/+}$

$Rb^{-/-}$

clinicaltrials.gov

National Library of Medicine
National Center for Biotechnology Information

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Search Results

Viewing 1-6 out of 6 studies

Sort studies by: Relevance

Showing results for: **Other terms: VCN-01**

NCT03799744 Unknown status *

Safety,Tolerability,and Efficacy of **VCN-01** With Durvalumab in R/M Head and Neck Squamous Cell Carcinoma

Conditions

Carcinoma, Squamous Cell of Head and Neck
Head and Neck Neoplasms | Metastasis | Recurrence

Locations

Barcelona, Spain
Hospitalet de Llobregat, Barcelona, S...

NCT03284268 Completed

Evaluate Safety and the Oncolitic Adenovirus **VCN-01** Activity in Patients With Refractory Retinoblastoma

Conditions

Retinoblastoma, Recurrent

Locations

Barcelona, Spain

NCT02045589 Completed

A Phase I Dose Escalation Study of Intratumoral **VCN-01** Injections With Gemcitabine and Abraxane® in Patients With Advanced Pancreatic Cancer

Conditions

Metastatic Pancreatic Adenocarcinoma | Pancreatic Adenocarcinoma

Locations

Madrid, Spain (2)
L'Hospitalet De Llobregat, Barcelona, ...

NCT02045602 Completed

Phase I Dose Escalation Study of Intravenous **VCN-01** With or Without Gemcitabine and Abraxane® in Patients With Advanced Solid Tumors

Conditions

Locally Advanced Solid Tumors | Metastatic Solid Tumors
Pancreatic Adenocarcinoma

Locations

Barcelona, Spain
Hospitalet De Llobregat, Spain
Madrid, Spain (3)

NCT05673811 Active, not recruiting

Study of Nab-Paclitaxel and Gemcitabine and Plus/minus **VCN-01** in Patients with Metastatic Pancreatic Cancer

Conditions

Metastatic | Pancreatic Adenocarcinoma

Locations

Newport Beach, California, United Sta...
Sacramento, California, United States
Louisville, Kentucky, United States
New York, New York, United States

Show all 17 locations

NCT05057715 Active, not recruiting

huCART-meso + **VCN-01** in Pancreatic and Ovarian Cancer

Conditions

Pancreatic Cancer | Serous Ovarian Cancer

Locations

Philadelphia, Pennsylvania, United St...

* Study has passed its completion date and status has not been verified in more than two years.

Sito consultato il 22/3/25

Review > Neurosurg Focus. 2021 Feb;50(2):E6. doi: 10.3171/2020.11.FOCUS20853.

Delta-24 adenoviral therapy for glioblastoma: evolution from the bench to bedside and future considerations

Chibawanye I Ene [1], Juan Fueyo [1 2], Frederick F Lang [1]

Affiliations + expand

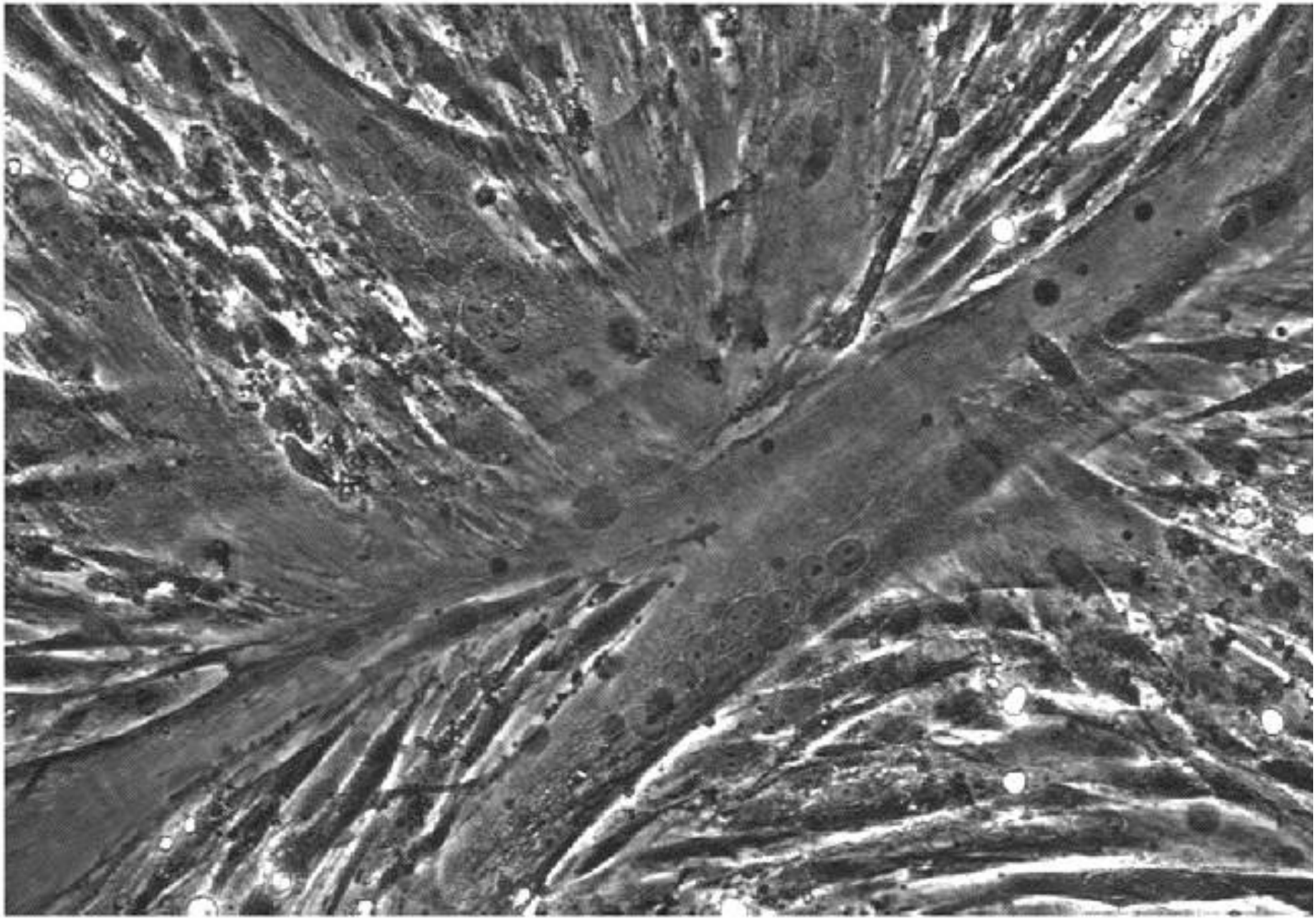
PMID: 33524949 DOI: 10.3171/2020.11.FOCUS20853

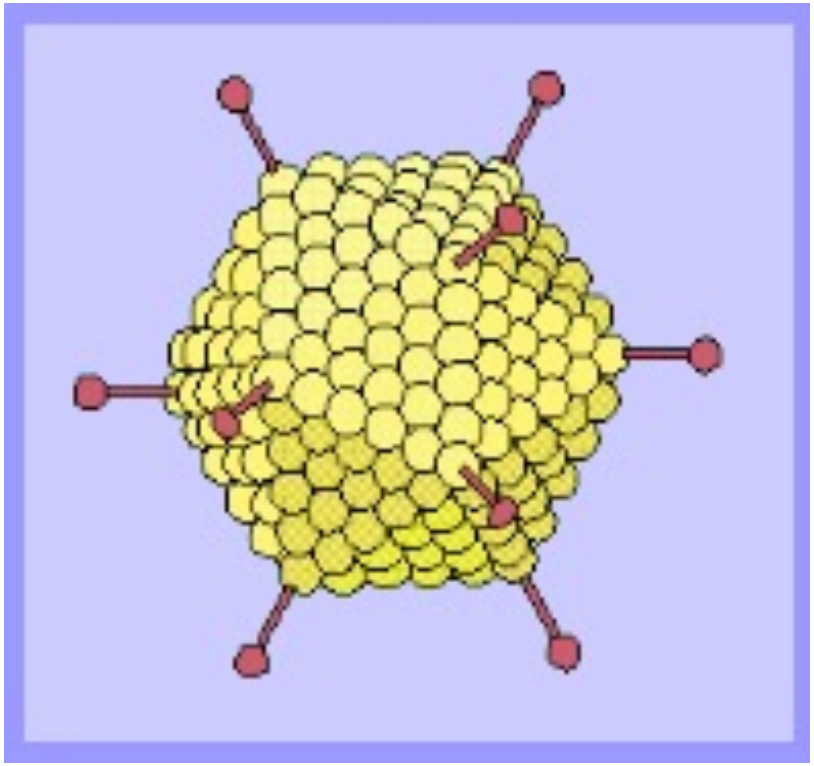
Abstract

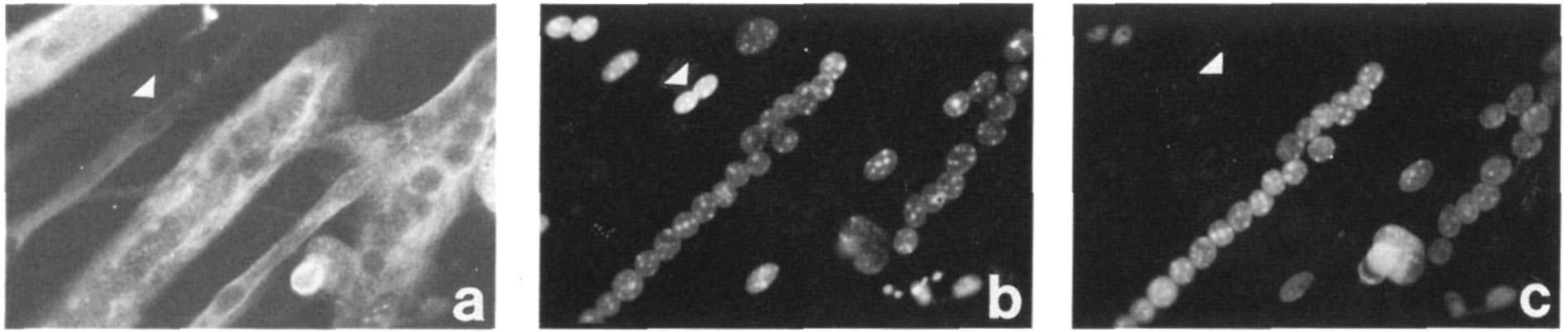
Delta-24-based oncolytic viruses are conditional replication adenoviruses developed to selectively infect and replicate in retinoblastoma 1 (Rb)-deficient cancer cells but not normal cell with intact Rb1 pathways. Over the years, there has been a significant evolution in the design of Delta-24 based on a better understanding of the underlying basis for infection, replication, and spread within cancer. One example is the development of Delta-24-RGD (DNX-2401), where the arginine-glycine-aspartate (RGD) domain enhances the infectivity of Delta-24 for cancer cells. DNX-2401 demonstrated objective biological and clinical responses during a phase I window of opportunity clinical trial for recurrent human glioblastoma. In long-term responders (> 3 years), there was evidence of immune infiltration (T cells and macrophages) into the tumor microenvironment with minimal toxicity. Although more in-depth analysis and phase III studies are pending, these results indicate that Delta-24-based adenovirus therapy may induce an antitumor response in glioblastoma, resulting in long-term antitumor immune response. In this review, the authors discuss the preclinical and clinical development of Delta-24 oncolytic adenoviral therapy for glioblastoma and describe structural improvements to Delta-24 that have enhanced its efficacy in vivo. They also highlight ongoing research that attempts to address the remaining obstacles limiting efficacy of Delta-24 adenovirus therapy for glioblastoma.

Keywords: Delta-24; adenovirus; glioblastoma; oncolytic virus; retinoblastoma gene mutation.

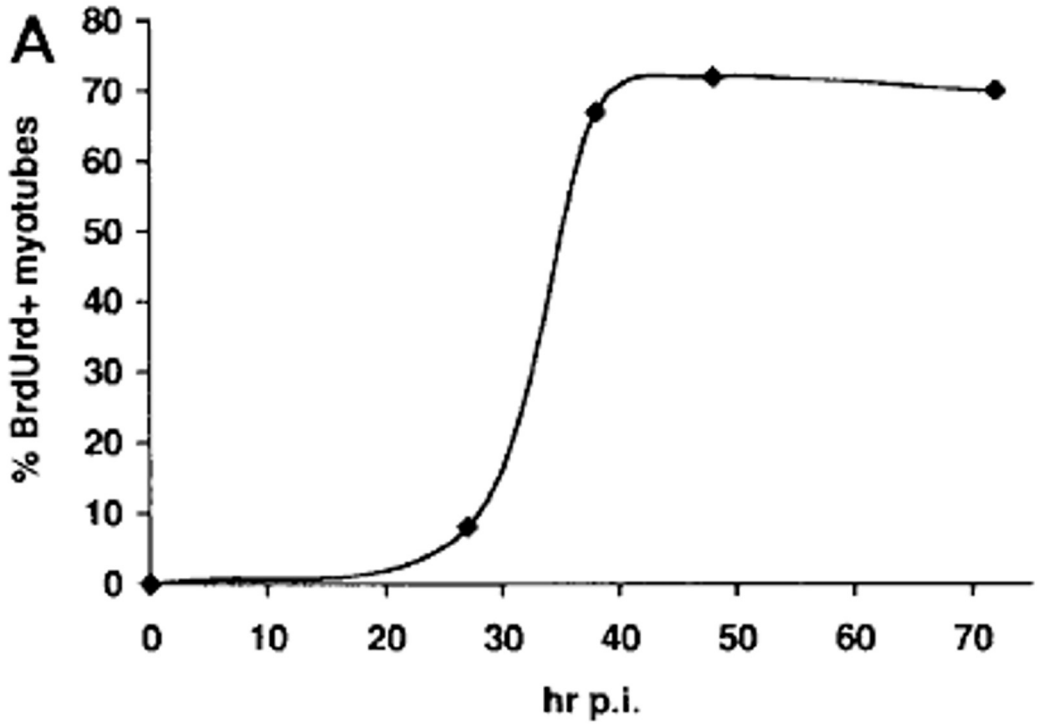
Applicazioni nella ricerca

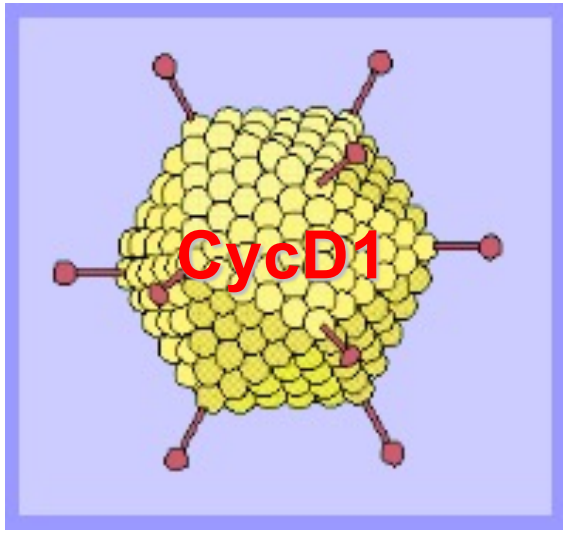


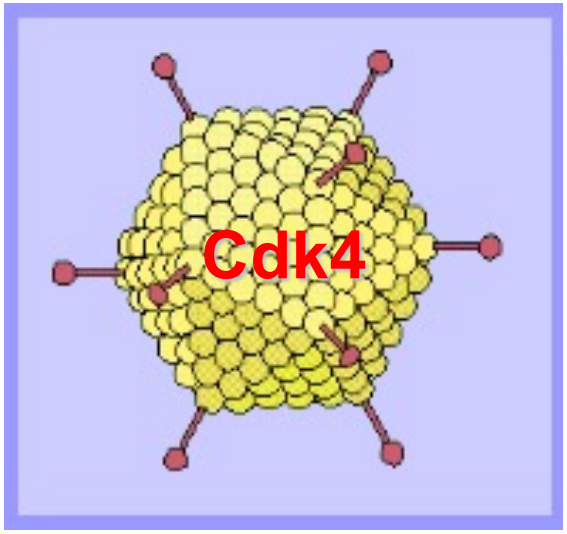


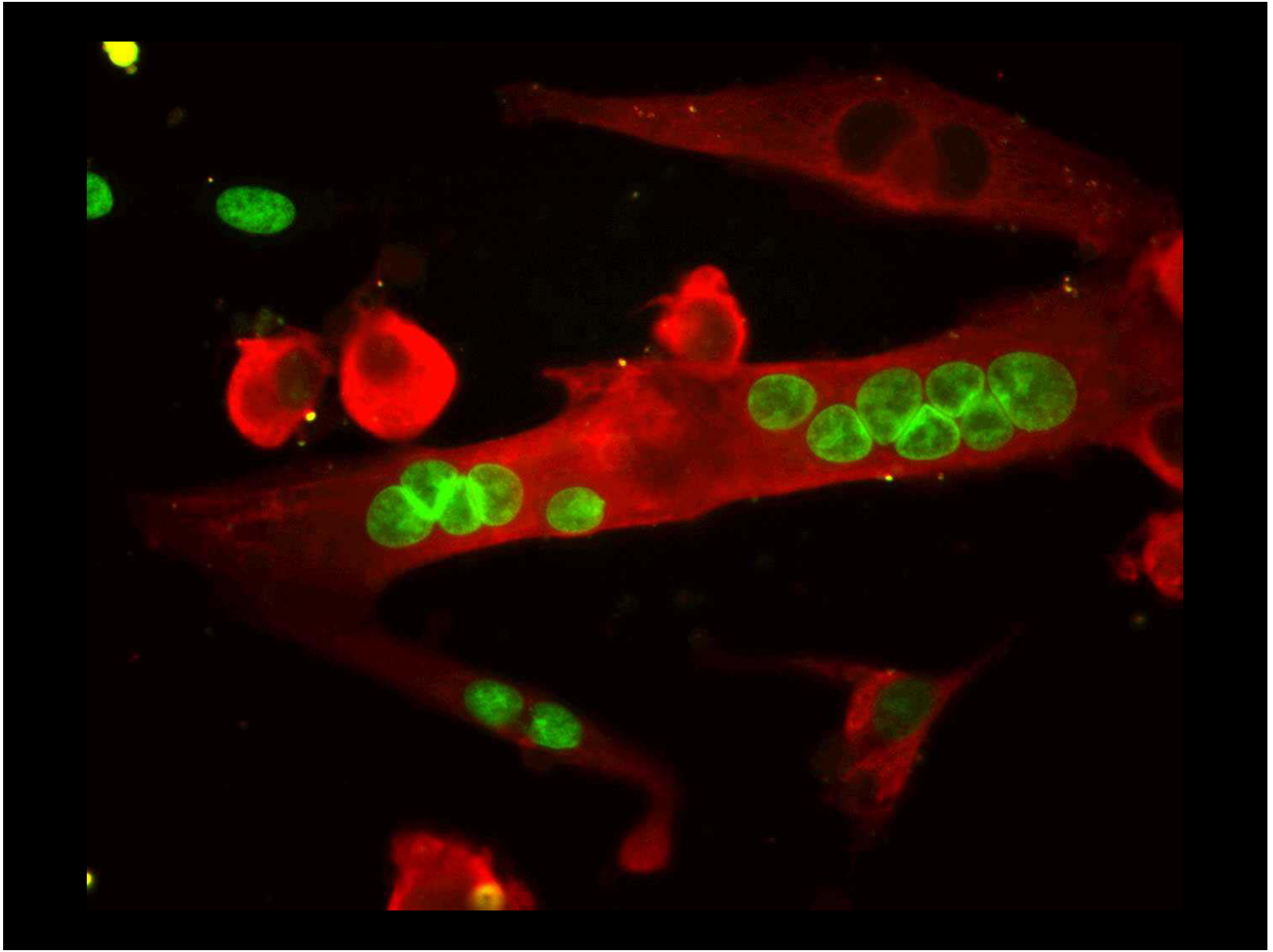

a
b
c

Virus	E1A 13S	E1A 12S	E1B 19 kDa	E1B 55 kDa	% miotubi BrdU+
wt Ad5	+	+	+	+	1
dl520	-	+	±	±	80
pm975	+	-	+	+	35
dl338	+	+	+	-	30
dl313	tronca	tronca	-	-	30
dl312	-	-	±	±	0

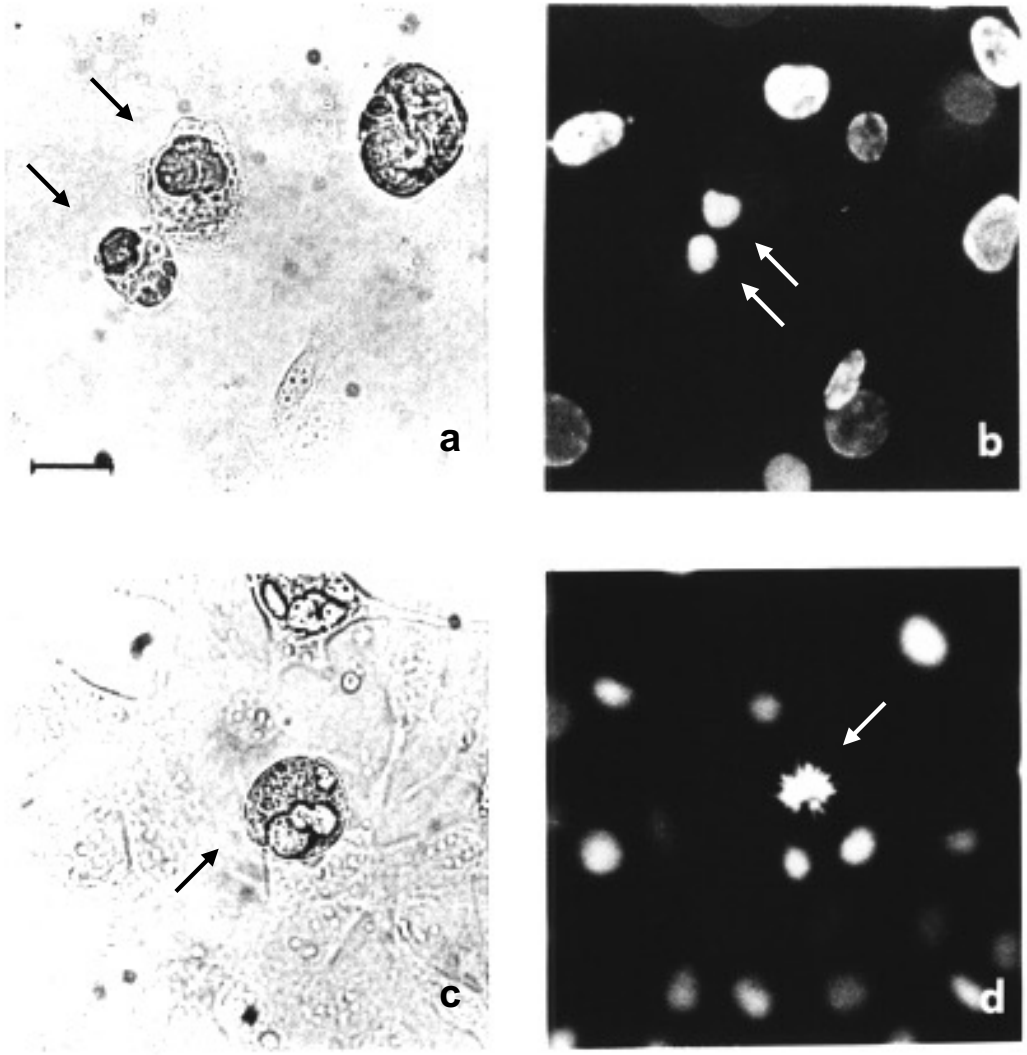

A
% BrdUrd+ myotubes
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
hr p.i.


CycD1

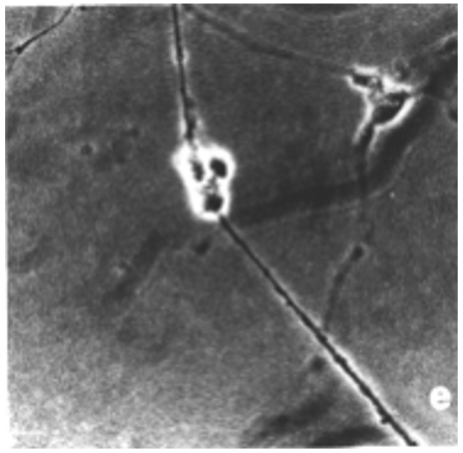

Cdk4

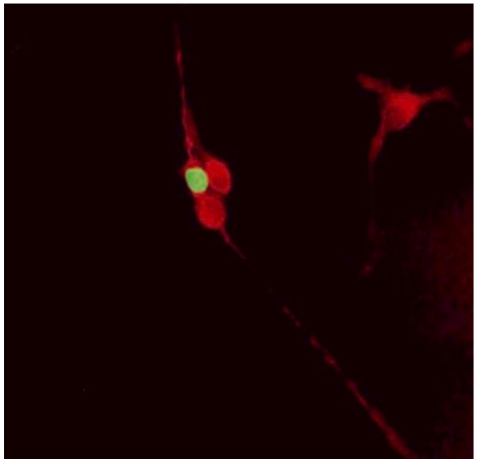


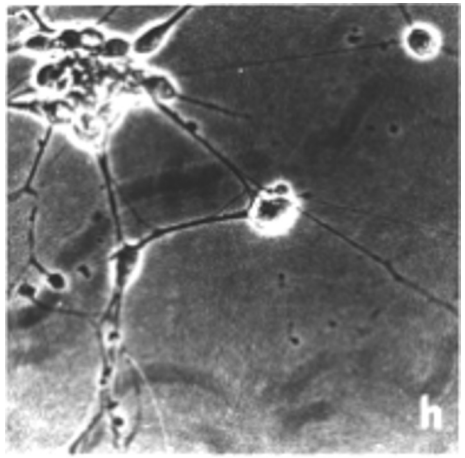
Cyclin D1/cdk4 induce DNA synthesis in 3T3-L1-derived adipocytes….

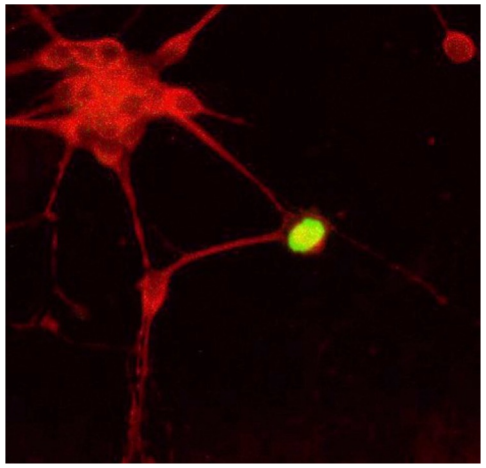


….and in P19-derived neurons





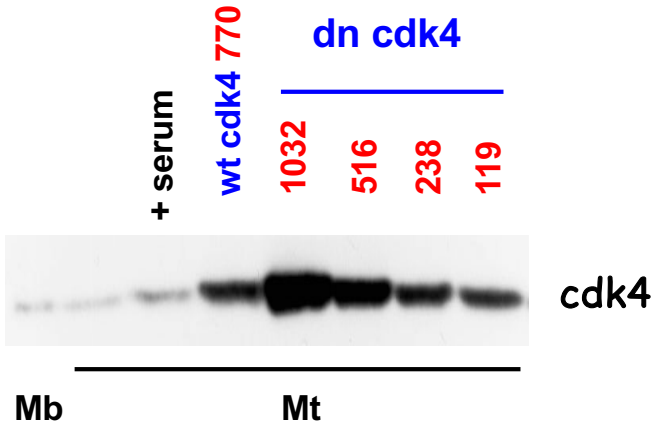


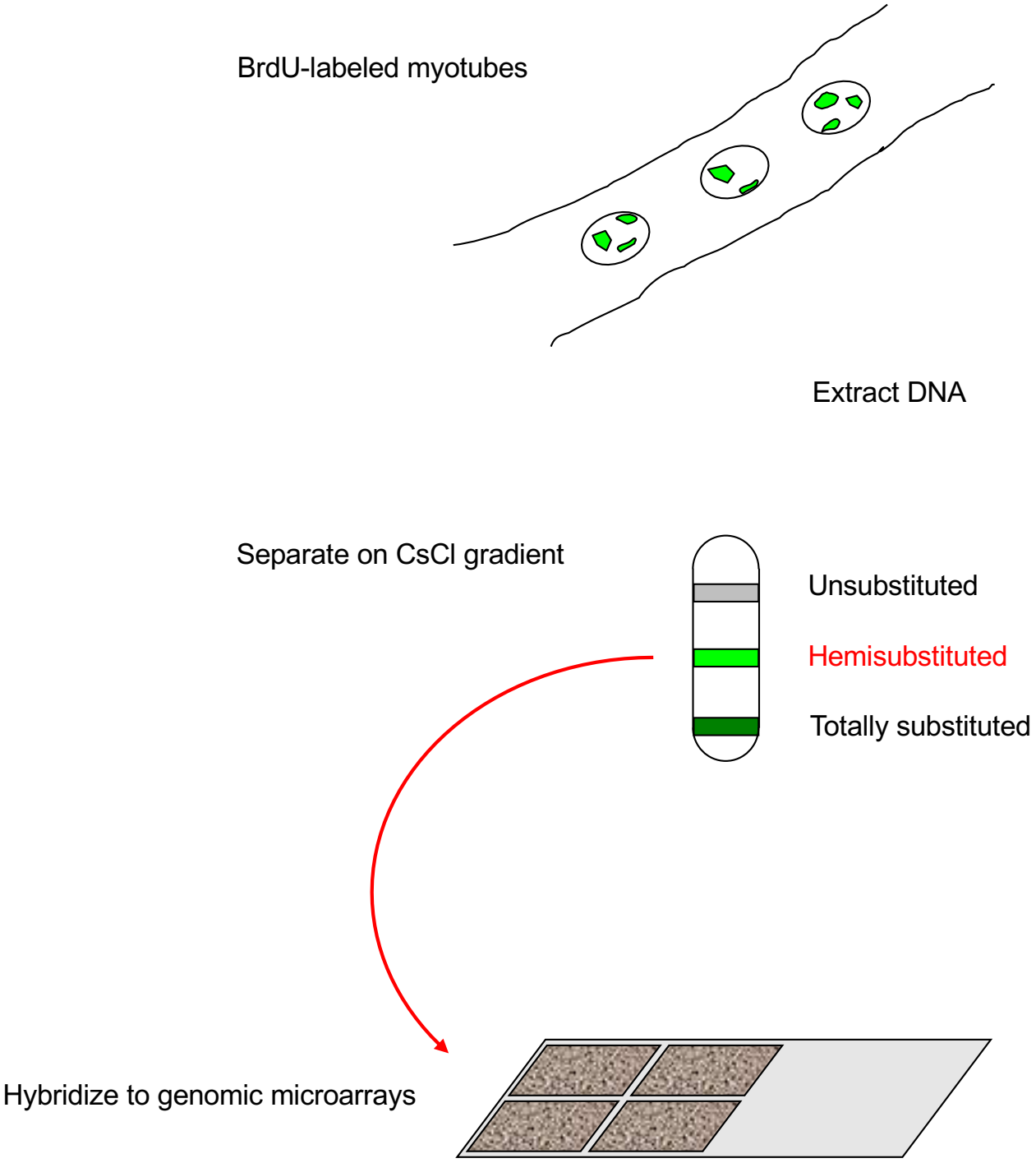


Ad-cycD1 - moi

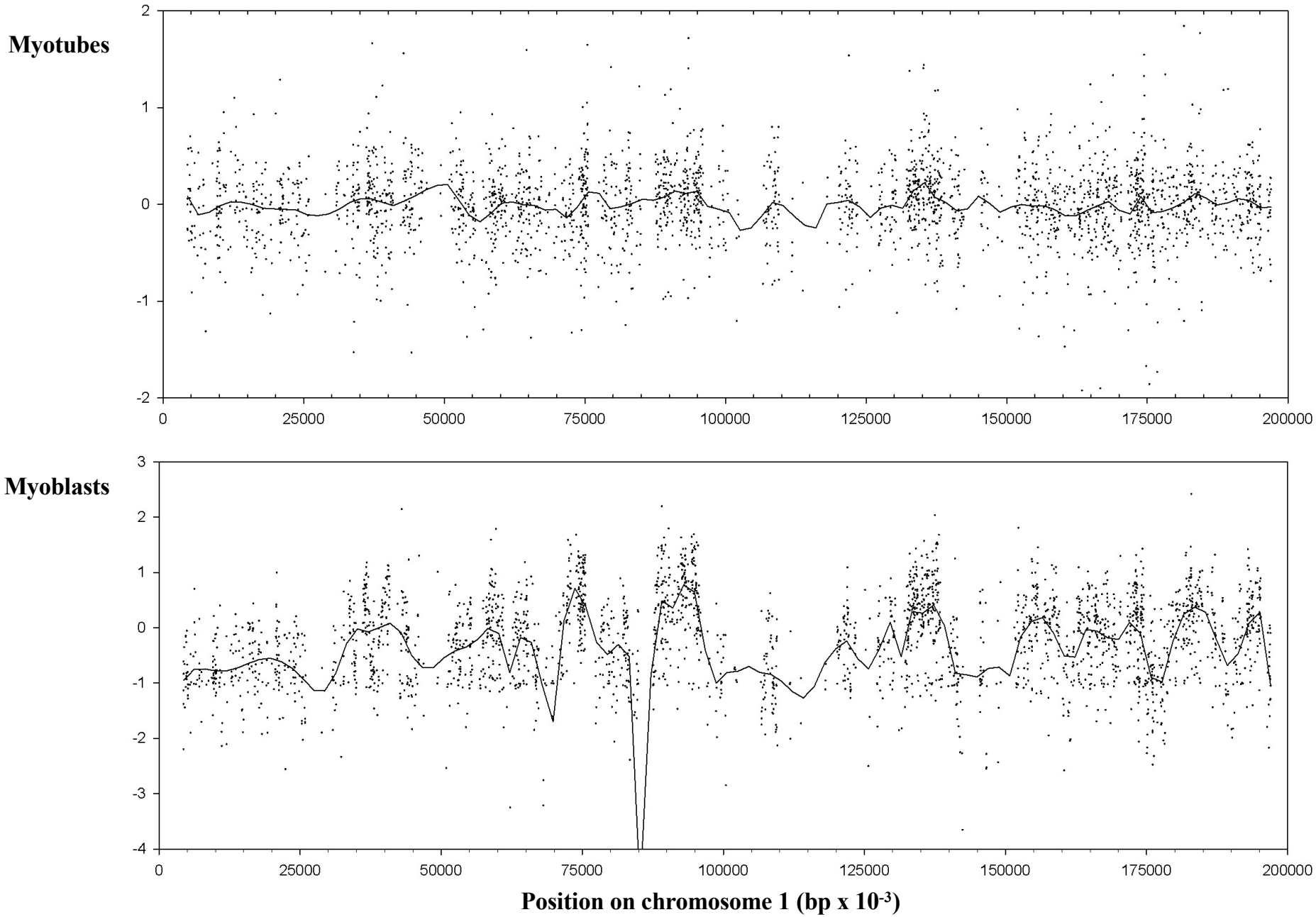
Ad-cdk4 moi	60	30	15	7,5	0
770	72	44	10	21	0
255	30	8	12	1	0
85	1	0	1	1	0
28	1	0	0	0	0
0	0	0	0	0	0

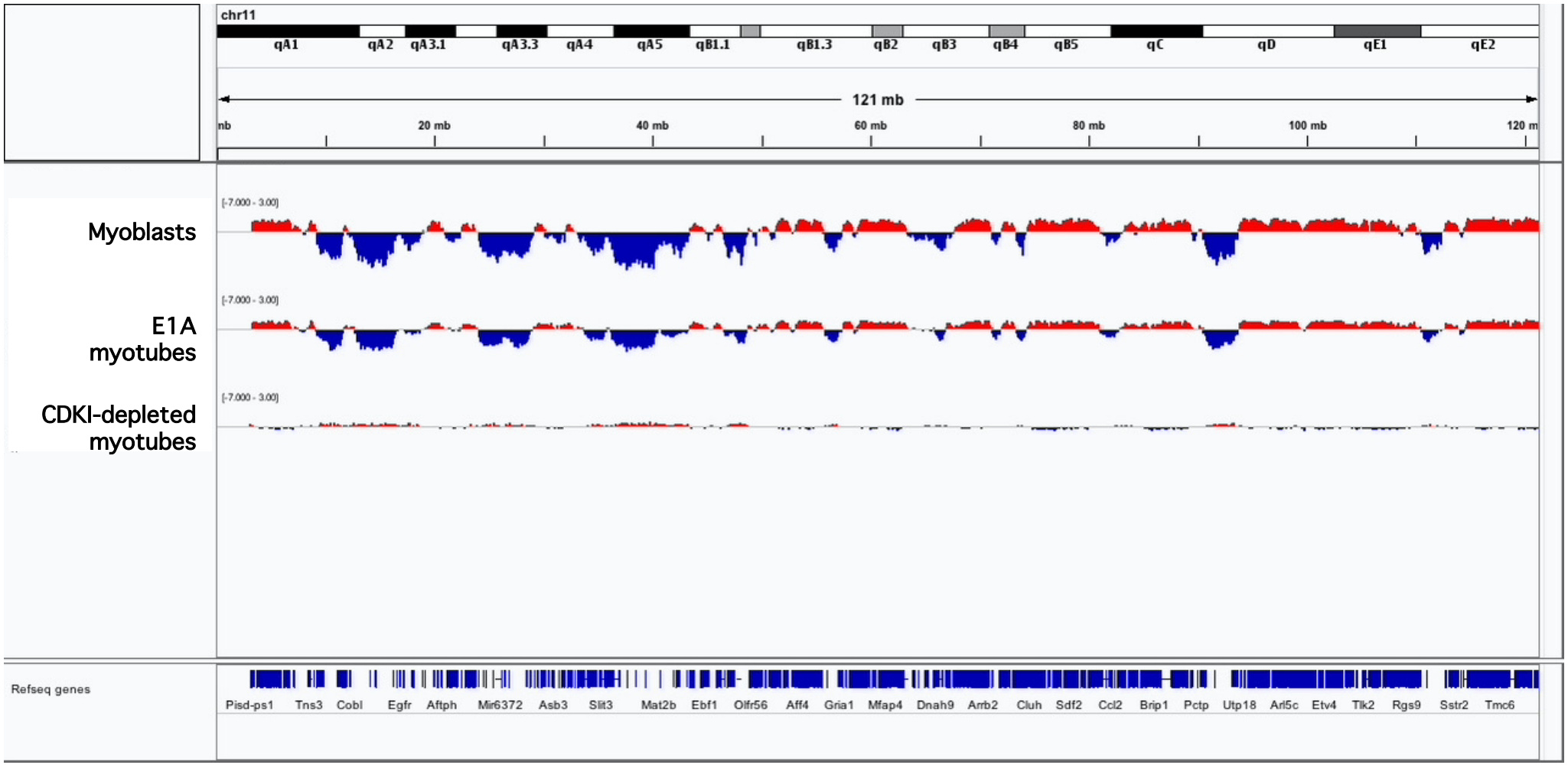
Percentuale di miotubi BrdU+




BrdU-labeled myotubes
Extract DNA
Separate on CsCl gradient
Unsubstituted
Hemisubstituted
Totally substituted
Hybridize to genomic microarrays

Reactivated myotubes lack a replication timing program




chr11
qA1
qA2
qA3.1
qA3.3
qA4
qA5
qB1.1
qB1.3
qB2
qB3
qB4
qB5
qC
qD
qE1
qE2
121 mb
nb
20 mb
40 mb
60 mb
80 mb
100 mb
120 m
Myoblasts
E1A myotubes
CDKI-depleted myotubes
[-7.000 - 3.00]
Refseq genes
Pisd-ps1
Tns3
Cobl
Egfr
Aftph
Mir6372
Asb3
Slit3
Mat2b
Ebf1
Olfr56
Aff4
Gria1
Mfap4
Dnah9
Arrb2
Cluh
Sdf2
Ccl2
Brip1
Pctp
Utp18
Arl5c
Etv4
Tlk2
Rgs9
Sstr2
Tmc6

I vettori adenovirali nella ricerca e nella clinica

Sono dotati di ampio tropismo; l'infezione non dipende dalla replicazione cellulare

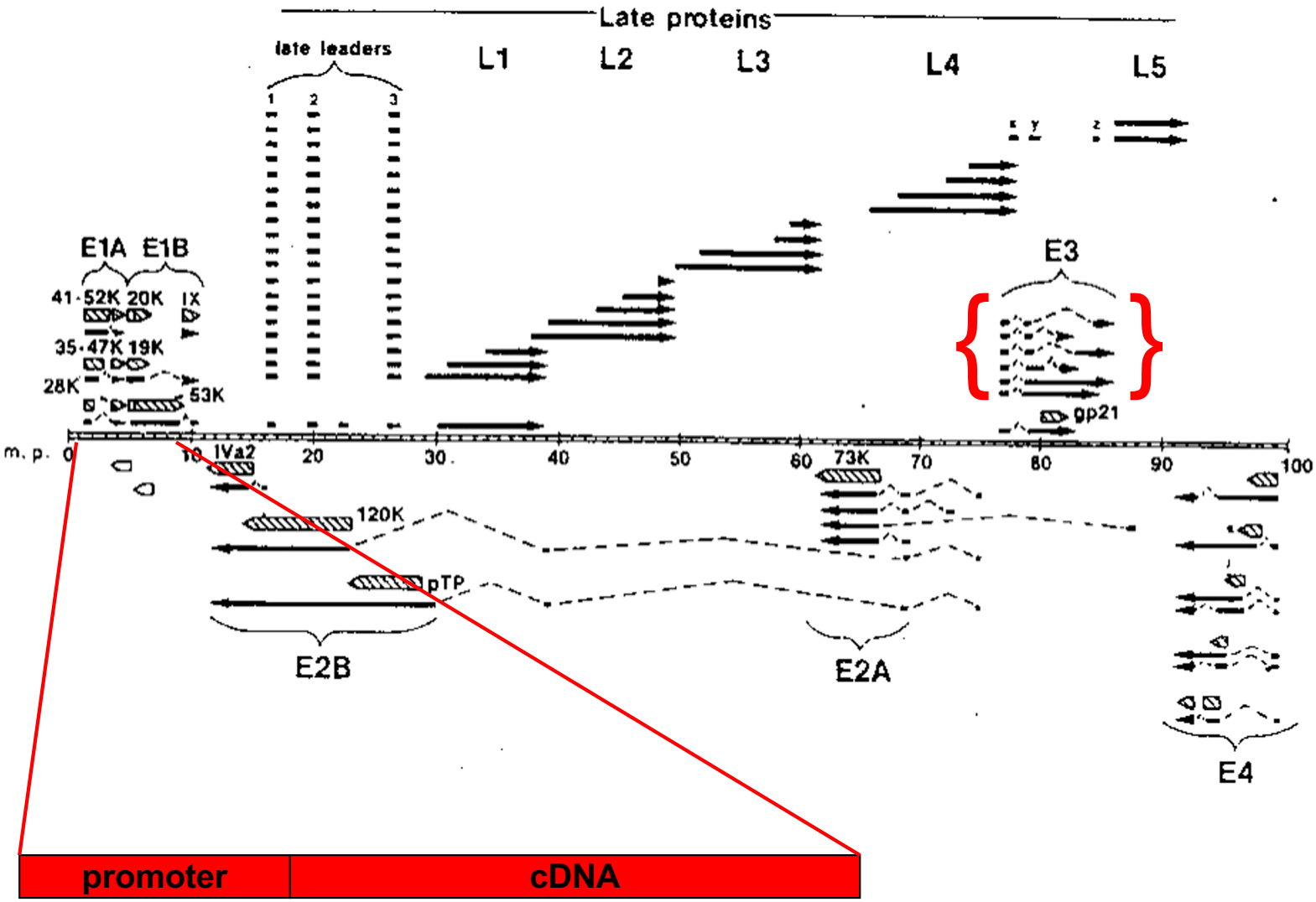
L'espressione genica può essere modulata variando la molteplicità di infezione

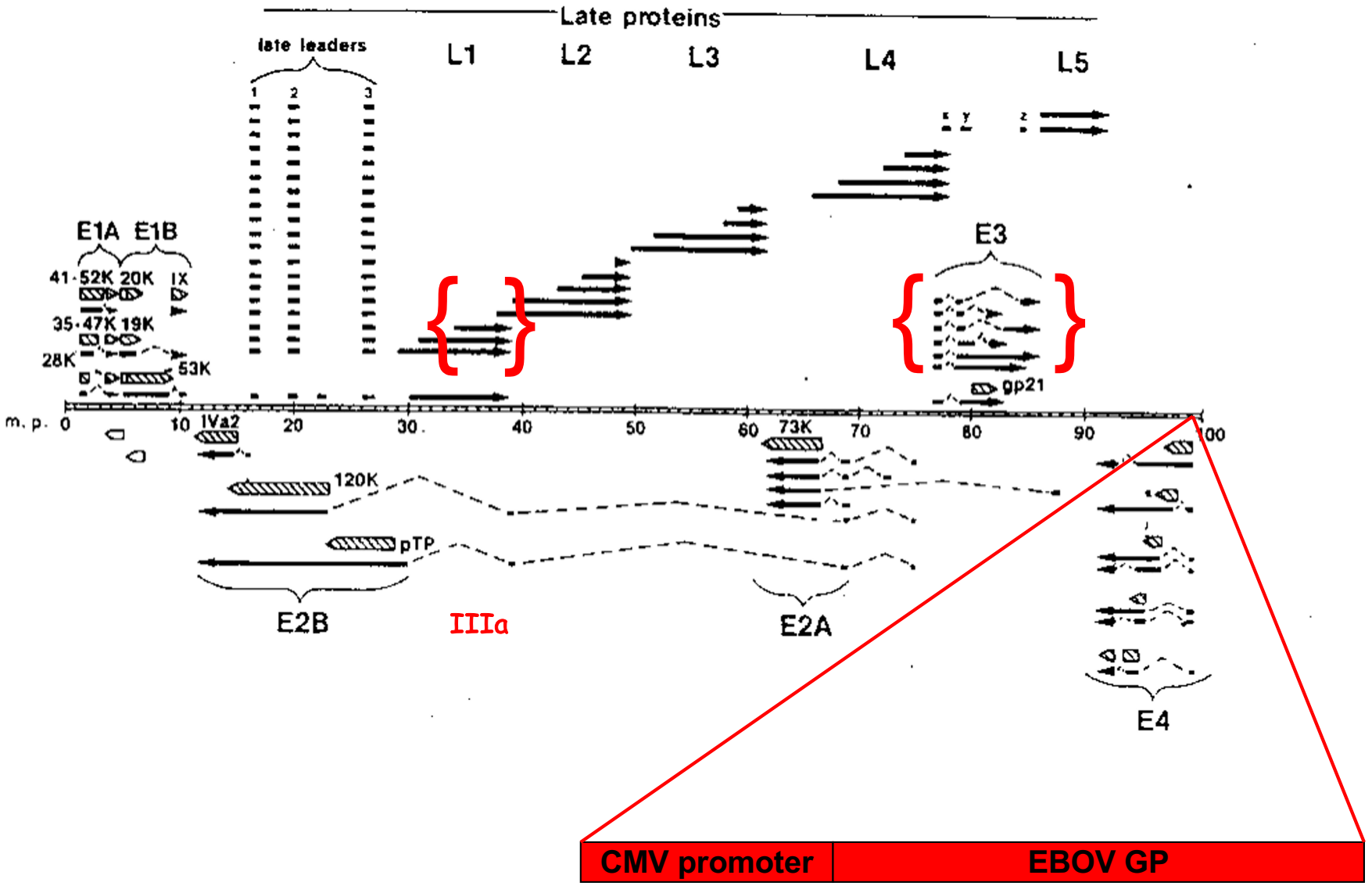
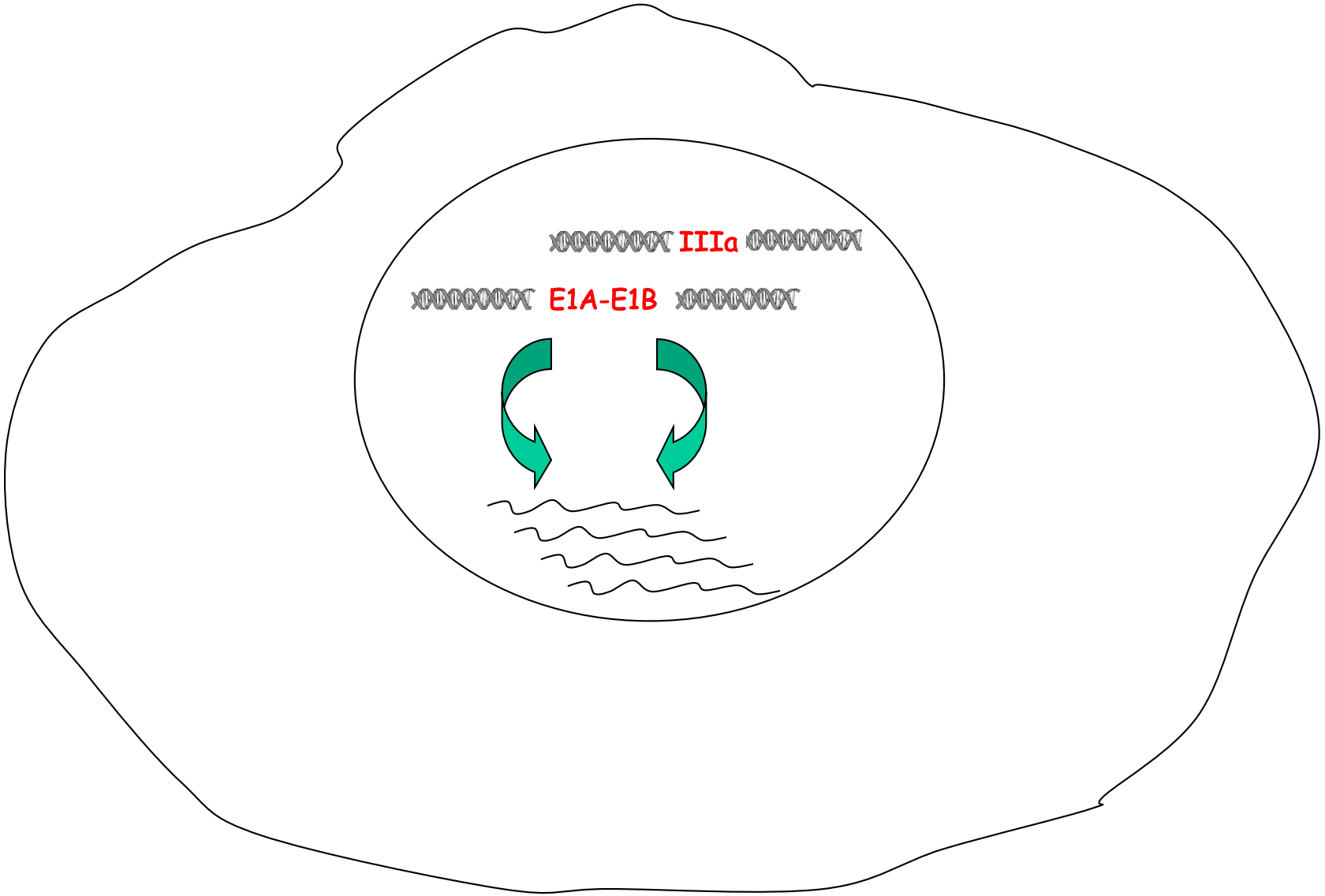
L'infezione avviene in maniera sincrona, facilitando le procedure e le analisi successive

Gli adenovirus sono utilizzabili per studi in vivo

In vivo, gli adenovirus sono fortemente immunogenici e possono esserlo anche i prodotti dei geni veicolati

Single-cycle adenovirus (SC-Ad)


Late proteins
late leaders
L1
L2
L3
L4
L5
E1A
E1B
41-52K
20K
IX
35-47K
19K
28K
53K
E3
gp21
m.p. 0
10
IVa2
20
30
40
50
60
73K
70
80
90
100
120K
pTP
E2B
E2A
E4
promoter
cDNA


Late proteins
late leaders
L1
L2
L3
L4
L5
E1A E1B
41·52K 20K IX
35·47K 19K
28K
53K
E3
gp21
m.p. 0 10 IVa2 20 30 40 50 60 73K 70 80 90 100
120K
pTP
E2B
IIIa
E2A
E4
CMV promoter
EBOV GP

293-III