HIV-1 virüsü için yeni, geniş etkili Bi-spesifik nötralizan antikor oluşturulması ve karakterizasyonu

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XVIII. KLİMİK Kongresi-Antalya

Çıkar çatışması

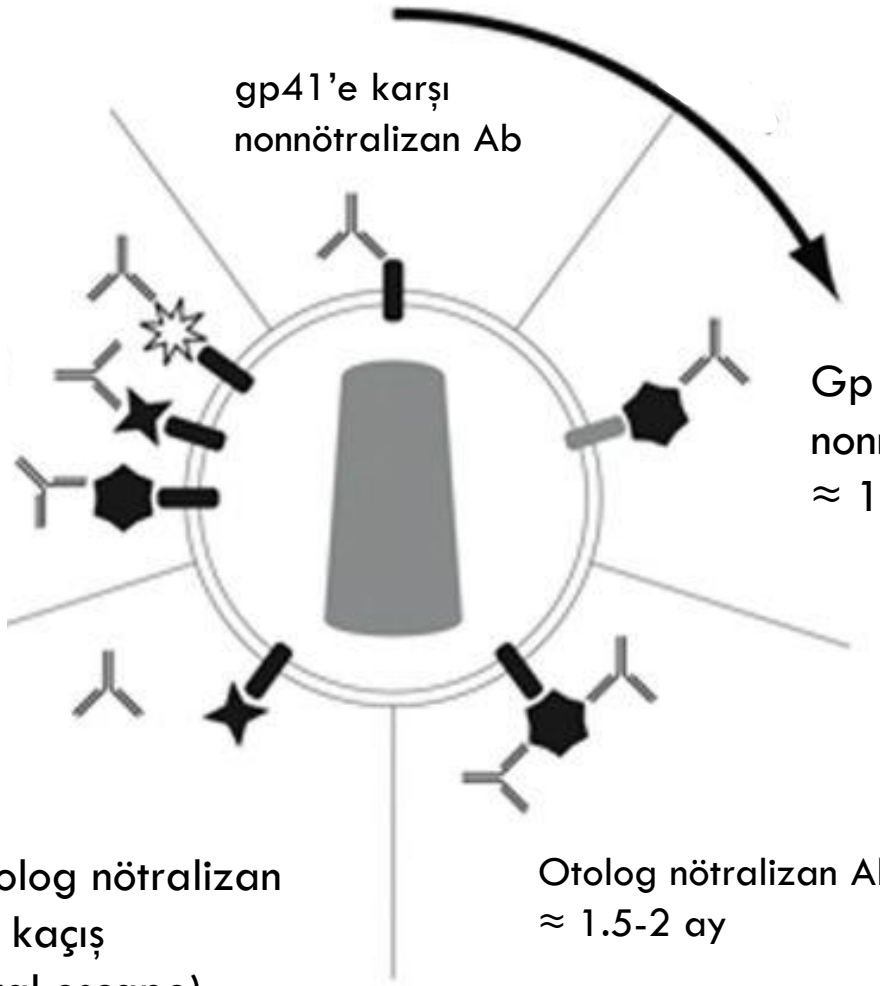
DP1 DA036463

1R21AI122384

Geniş etkili nötralizan antikor (bNAb)

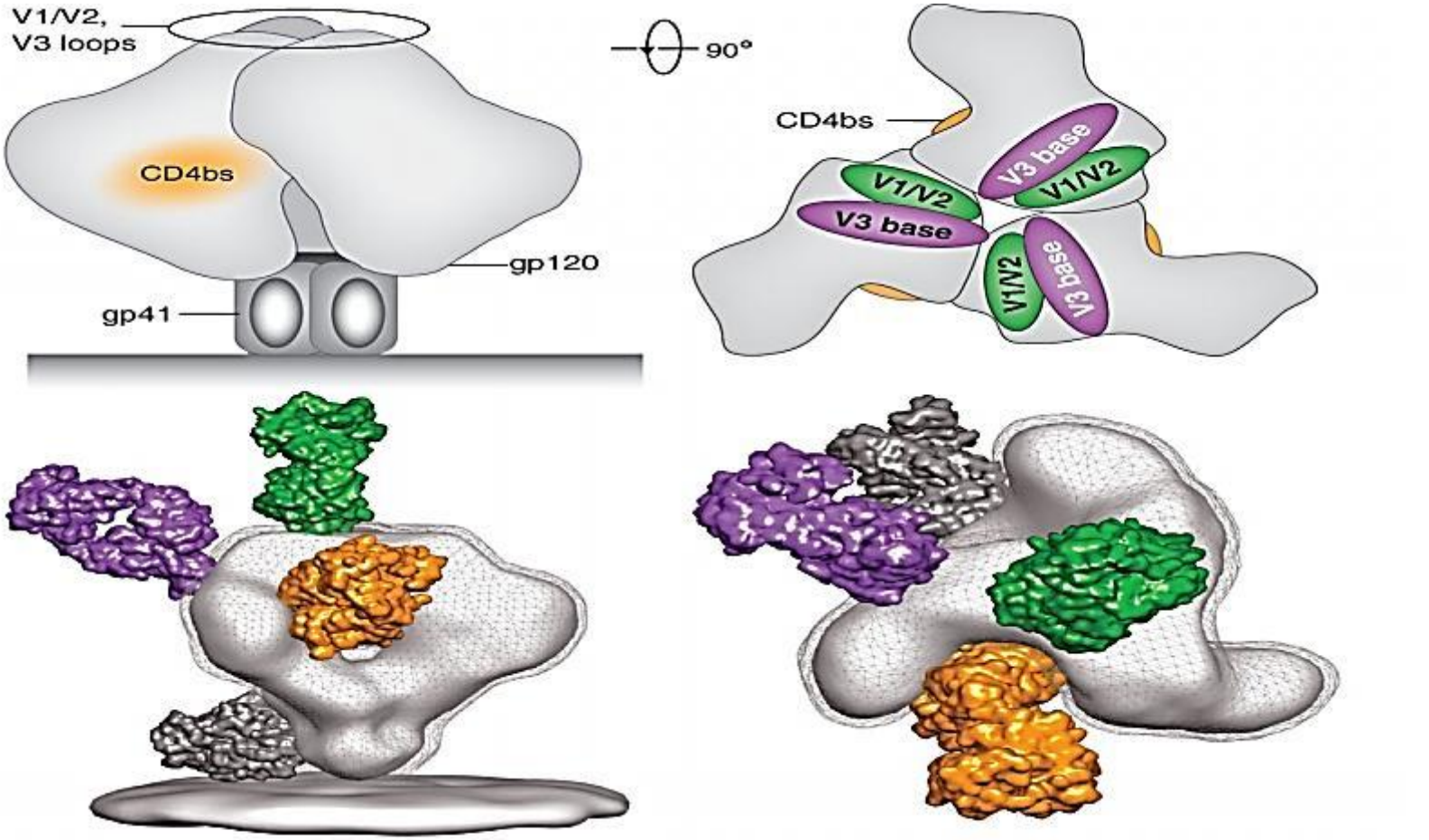
- HIV-1 infeksiyonlu bireylerde zarf glikoproteinleri üzerinde yer alan farklı hedef bölgelere spesifik gelişen antikorlar
- Kronik infekte hastaların %20-30'unda gelişir
- Hafıza B hücrelerden izole edilir

HIV-1 antikor yanıtı



Galit Alter et al J Infect Dis . 2010 October 15; 202(Suppl 2)

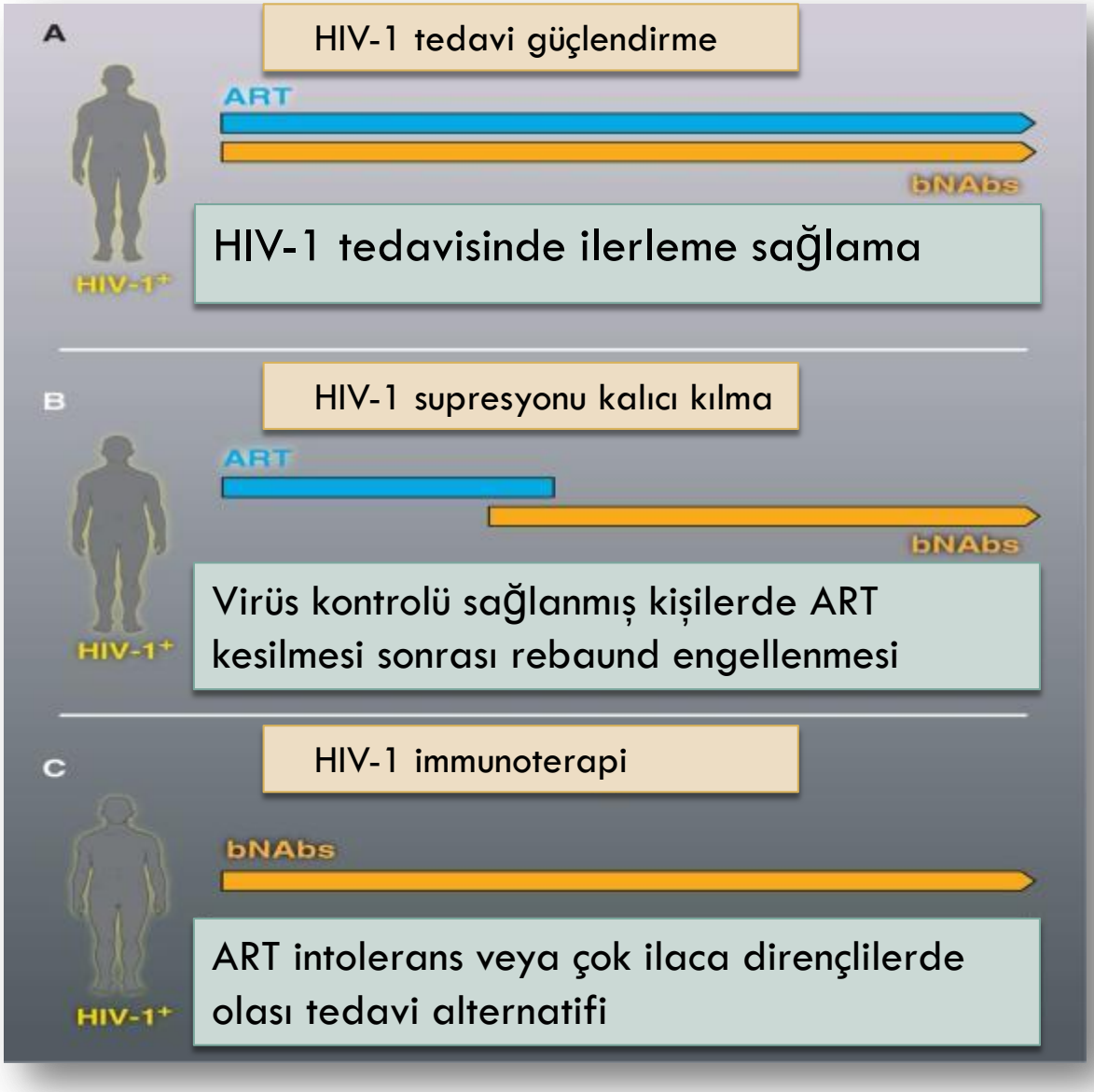
HIV-1zarf (env) üzerinde Antikor hedef bölgeleri



CD4 binding site		Glycan-V3	V1/V2 loop	gp41	Others
b12	1NC9	PGT121-PGT123	PG9/PG16	4E10	8ANC195
HJ16	12A12, 12A21	10-1074	PGT141–145	2F5	2G12
NIH45-46, 45-46^{G54W}	8ANC131, 8ANC134	PGT125-128,130,131	CH01-CH04	10E8	3BC176/
VRC01-03, VRC06	CH30-CH34	PGT135-137		HK20	3BC315
3BNC117, 3BNC60	VRC23	VRC24		Z13	
VRC-PG04	CH103				

Florian Klein et al. Science 2013;341:1199-1204

HIV-1 tedavisinde bNAb'lerin potensiyel kullanımı



Florian Klein et al. Science 2013;341:1199-1204

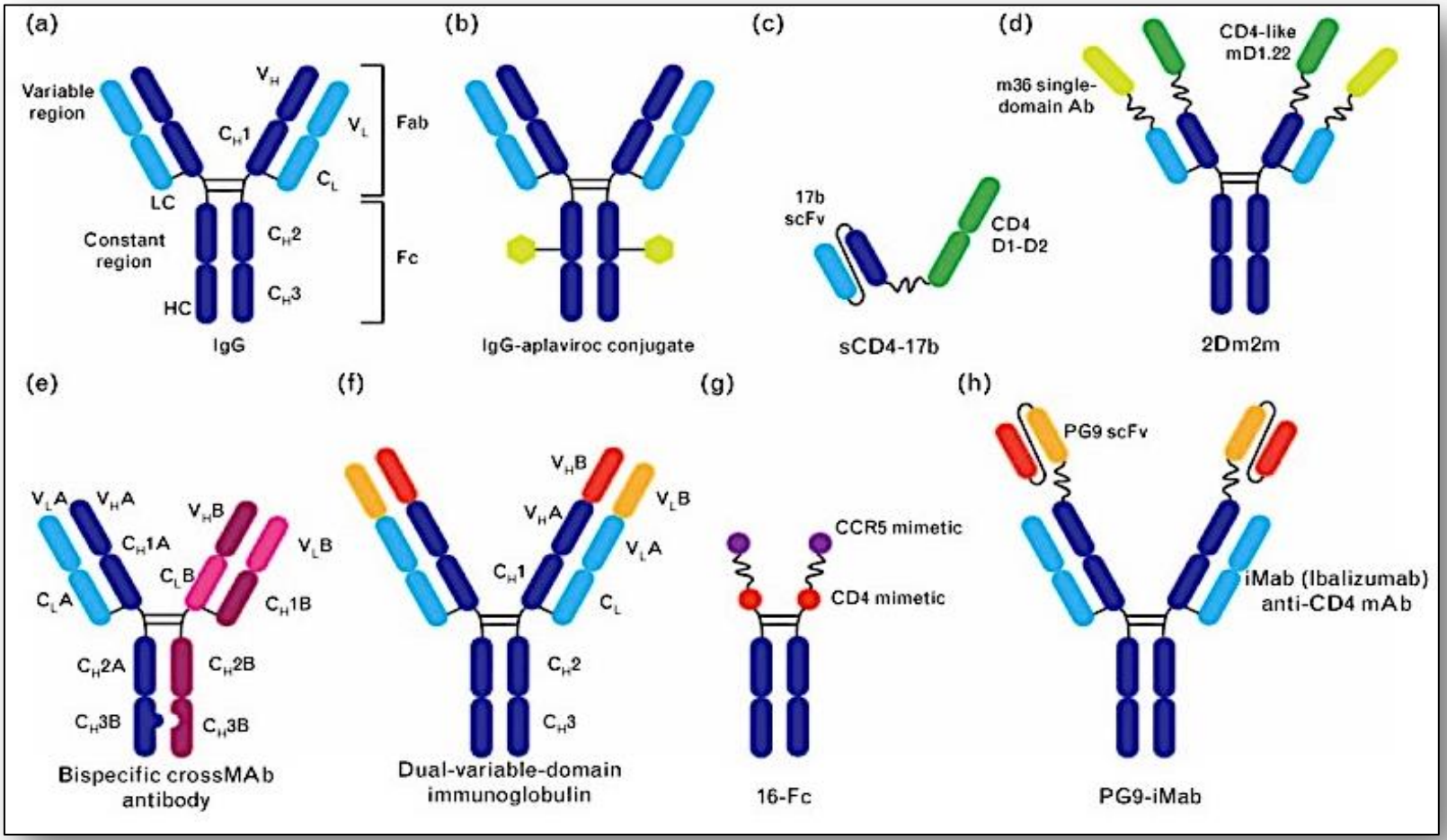
Table 1. Characteristics of broad and potent neutralizing antibodies to HIV-1

Antibody Name	Site of Contact	Study	Antibody Type	Glycan dependence	Quaternary Structure dependence	Neutralization Breadth (%)	Infecting Subtype	Therapeutic Potential
IgG1b12	CD4bs	Burton *et al.* (1991) Proc. Natl. Acad. Sci. USA; 88: 10134-10137	Whole Antibody	No	No	35 (n=190)	B	Effective against SHIV vaginal challenge and protects macaques.
VRC01,02,03	CD4bs	Wu *et al.* (2010) Science 329: 856-861	Whole Antibody	No	No	91, 91, 57 (n=190)	B	Effective against SHIV mucosal challenge and protects macaques.
NIH45-46	CD4bs	Scheid *et al.* (2011) Science, 333: 1633-1637	Whole Antibody	No	No	96 (n=118)	B	Not tested
VRC-PG04	CD4bs	Wu *et al.* (2011) Science; 333:1593-602	Whole Antibody	No	No	76 (n=178)	A-D recombinant	Not tested
3BNC117	CD4bs	Scheid *et al.* (2011) Science, 333: 1633-1637	Whole Antibody	No	No	96 (n=118)	B	Not tested
VHH J3	CoRbs	McCoy, L. and Weiss, R. (2013). J Exp Med 210: 209-223.	Domain Antibody	NA	NA	96 (n=100)	-	Not tested
m36	CoRbs	Chen (2008) Proc Natl Acad Sci USA 2008; 105: 17121-17126	Domain Antibody	No	No	91 (n=11)	-	Not tested
2G12	Glycan	Trkola *et al.* (1996) J. Virol. 70: 1100-1108)	Whole Antibody	Yes	No	32 (n=162)	B	Reduces viral load and increases CD4 T cell count in combination with 2F5, 4E10
PGT121-123	Glycan	Walker *et al.* (2011) Nature; 477:466-470)	Whole Antibody	Yes	No	65-70 (n=162)	A	Not tested
PGT125-128,130-131	Glycan	Walker *et al.* (2011) Nature; 477:466-470)	Whole Antibody	Yes	No	40-72 (n=162)	CRF02_AG	Not tested
PGT135	Glycan	Walker *et al.* (2011) Nature; 477:466-470)	Whole Antibody	Yes	No	33 (n=162)	C	Not tested
PGT141-145	Glycan	Walker *et al.* (2011) Nature; 477:466-470)	Whole Antibody		Yes	38-78 (n=162)	A or D	Not tested
PG9, PG16	Quaternary structure including V1V2,V3	Walker *et al.* (2009) Science 326: 285-289	Whole Antibody	Yes	Yes	79 and 73 (n=190)	A	Not tested
2F5	MPER	Purtscher *et al.* (1994) AIDS Res Human Retroviruses 10: 1651-1658	Whole Antibody	No	No	57 (n=177)	B	Tested in combination with 2G12. Help reduce viral load and increase CD4+ T cell count in HIV infected individuals.
4E10	MPER	Stiegler *et al.* (2001) AIDS Res. Hum. Retroviruses 17:1757-1765	Whole Antibody	No	No	98 (n=180)	B	Moderately suppresses viral load in combination with 2F5
10E8	MPER	Huang *et al* (2012) Nature, 491: 406-412)	Whole Antibody	No	No	98 (n=180)	B	Not tested

Proje amacı

- Bispesifik BNAb dizayn etmek ve paternal antikorlardan (PG16 ve NIH45-46 ^{G54W}) daha fazla etkinlik
- Bispesifik BNAb ile Paternal Antikorların etki kapsamını genişletmek
- Multipl HIV-1 epitoplarını hedefleyen alternatif strateji oluşturmak ve dirençli suşlarda etkinlik sağlamak

Bispesifik Anti-HIV-1 dizayn örnekler



Stuart A. Sievers, Curr Opin HIV AIDS 2015

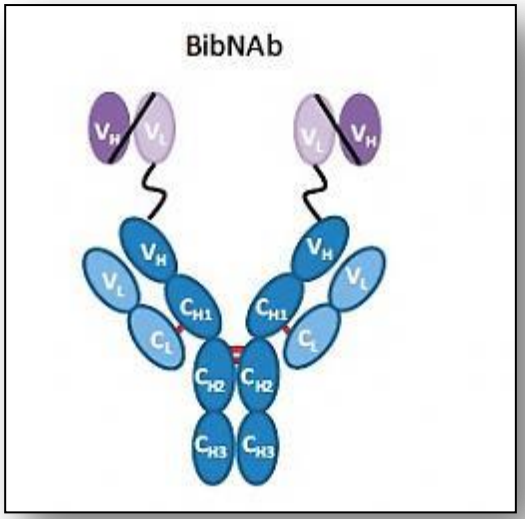
PG16 VH-VL

Overlap PCR

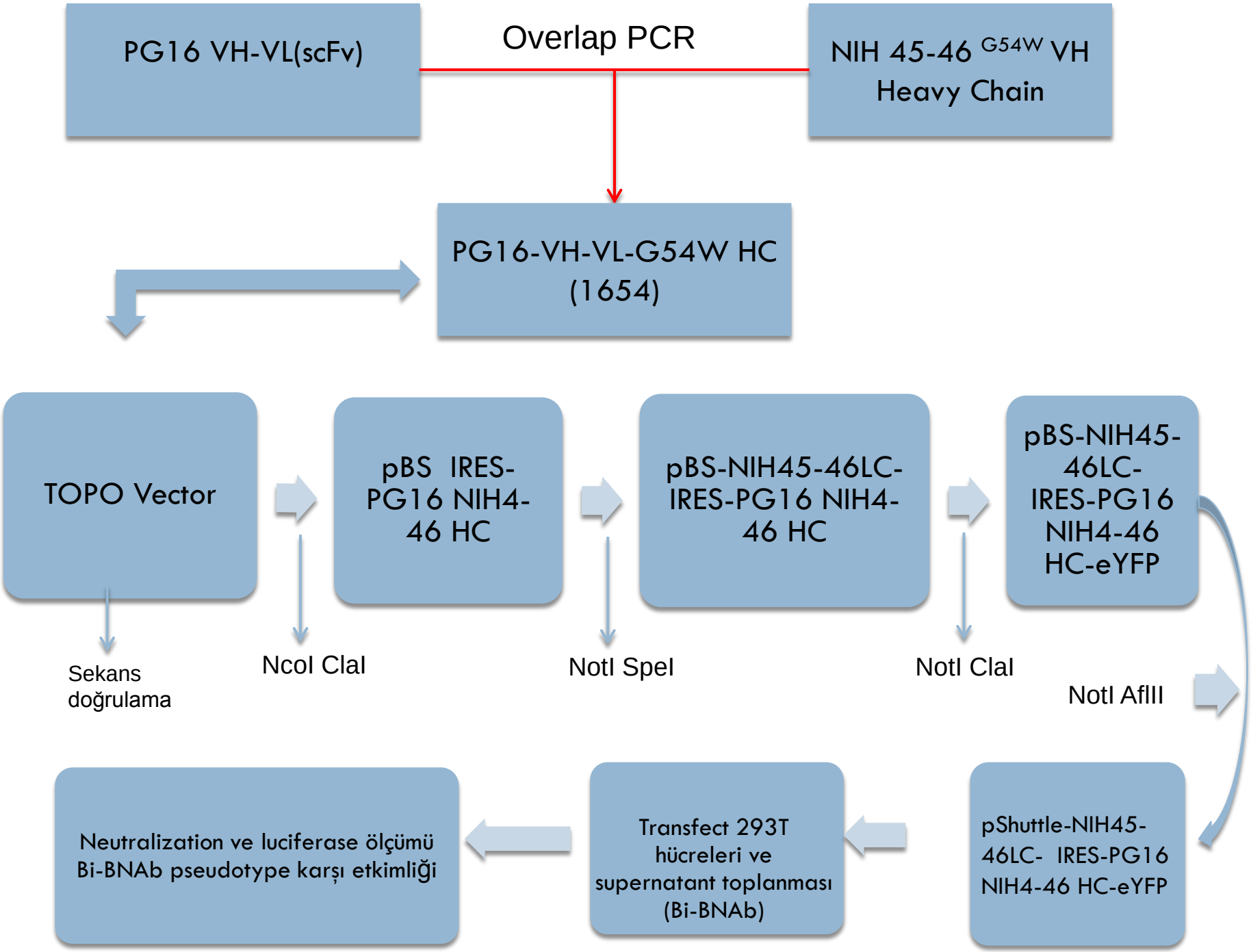
NIH 45-46 ^{G54W} Heavy Chain

PG16 VH-VL-NIH 45-46 ^{G54W} HC

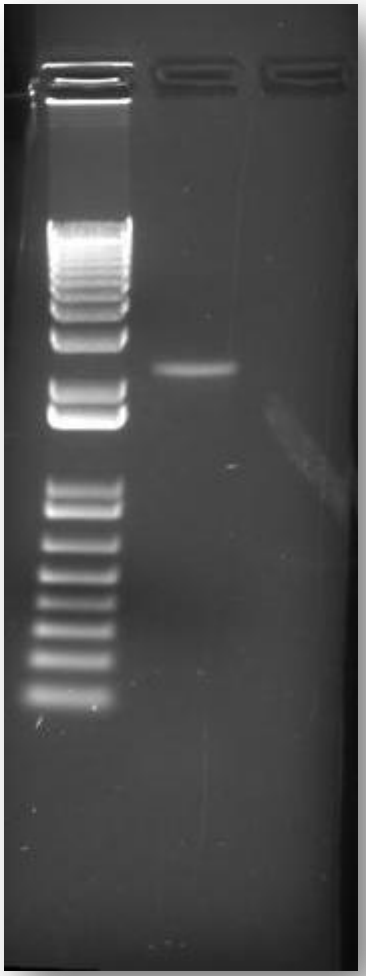
PG16 VH	Linker	PG16 VL	Linker	NIH 45-46 ^{G54W} VH	CH1	Hinge	CH2	CH3



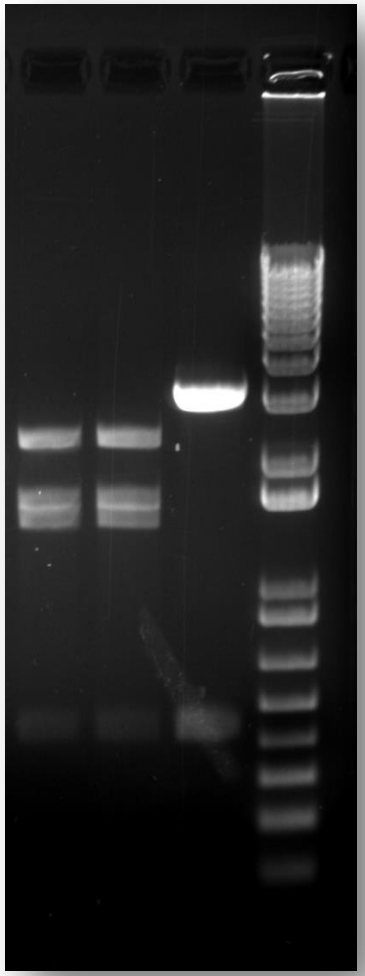
Craig S. Pace.PNAS August 2013. Vol:10 No:33


PG16 VH-VL(scFv)
Overlap PCR
NIH 45-46 G54W VH Heavy Chain
PG16-VH-VL-G54W HC (1654)
TOPO Vector
Sekans doğrulama
pBS IRES-PG16 NIH4-46 HC
NcoI ClaI
pBS-NIH45-46LC-IRES-PG16 NIH4-46 HC
NotI SpeI
pBS-NIH45-46LC-IRES-PG16 NIH4-46 HC-eYFP
NotI ClaI
NotI AflII
pShuttle-NIH45-46LC- IRES-PG16 NIH4-46 HC-eYFP
Transfect 293T hücreleri ve supernatant toplanması (Bi-BNAb)
Neutralization ve luciferase ölçümü Bi-BNAb pseudotype karşı etkimliği

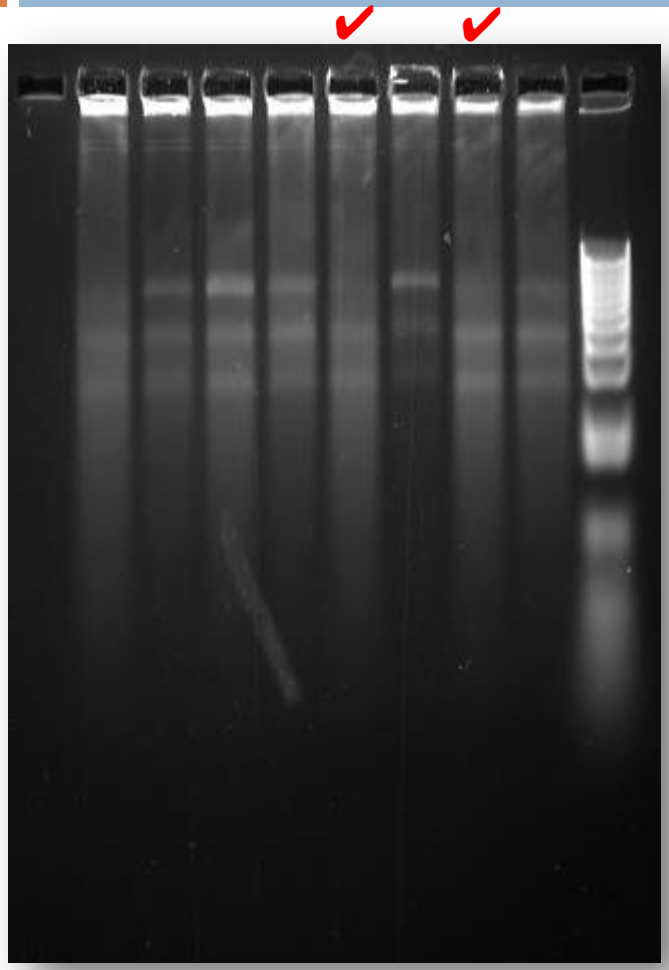
Overlap PCR



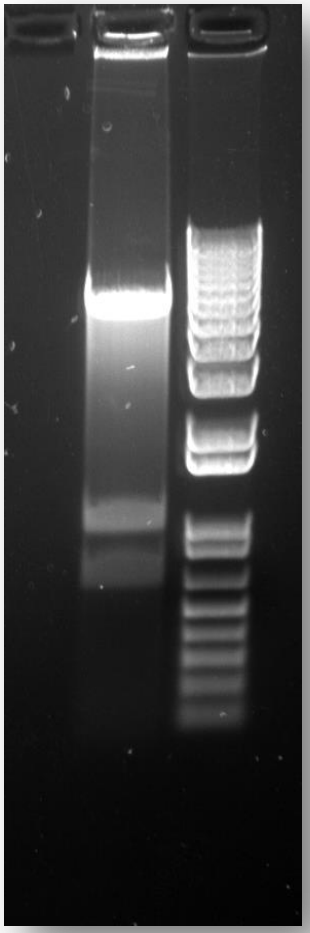
TOPO PG16 NIH45-46^{G54W} HC
Nco1+Cla1



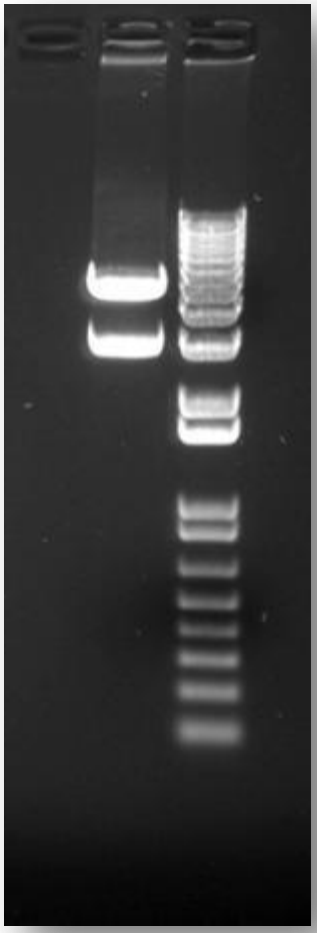
pBS-IRES-PG16-G54W HC Nco1+Cla1



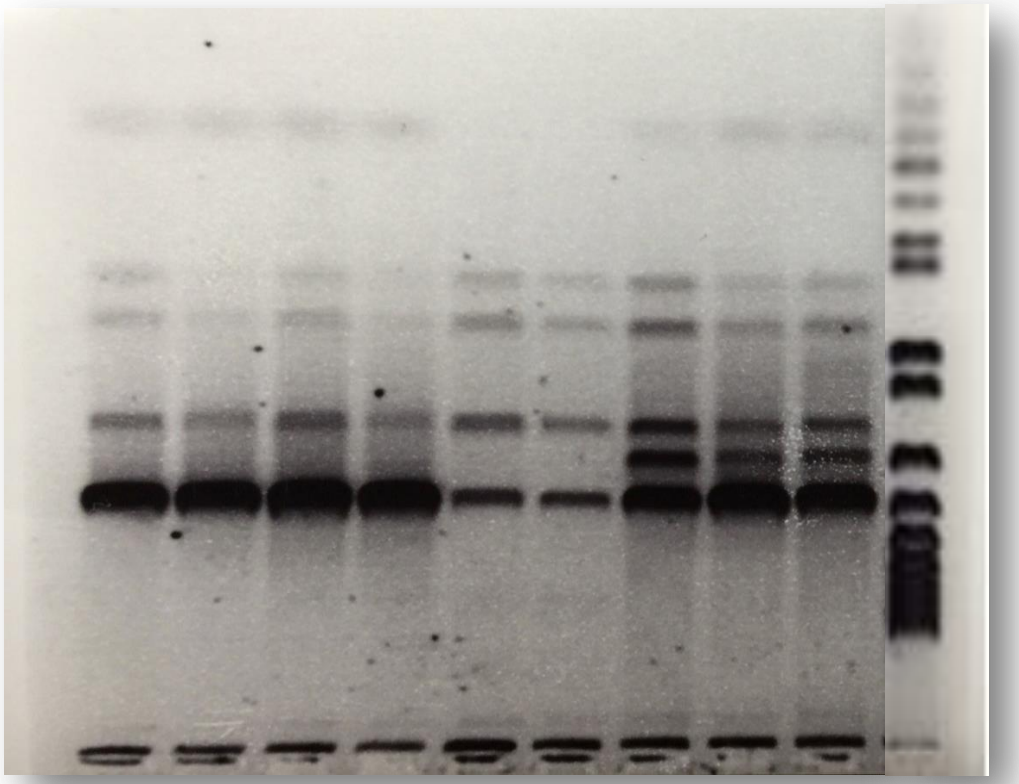
pBS-eYFP+PG16-G54W HC LC Not1+EcoR1



pBS-eYFP+PG16-G54W HC LC

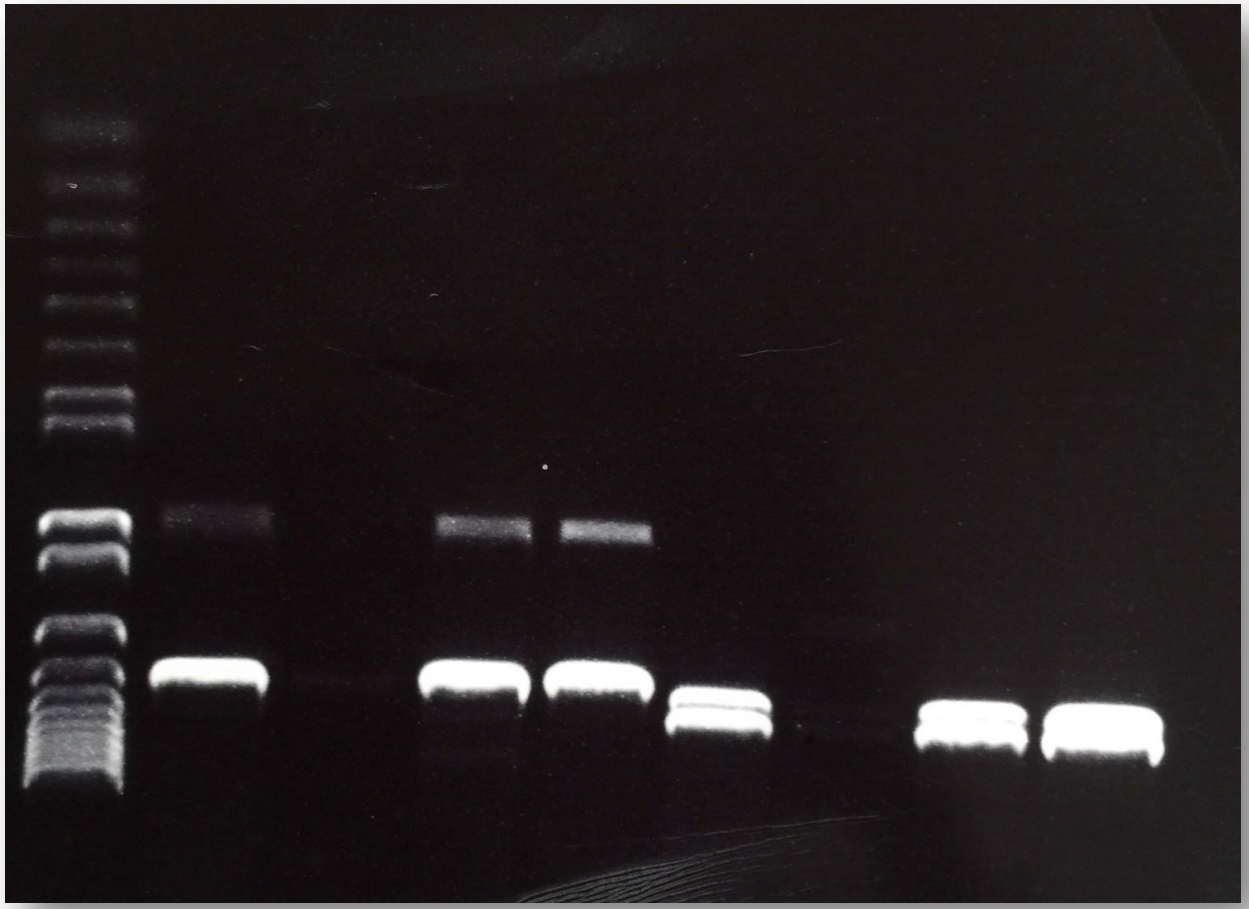


Miniprep



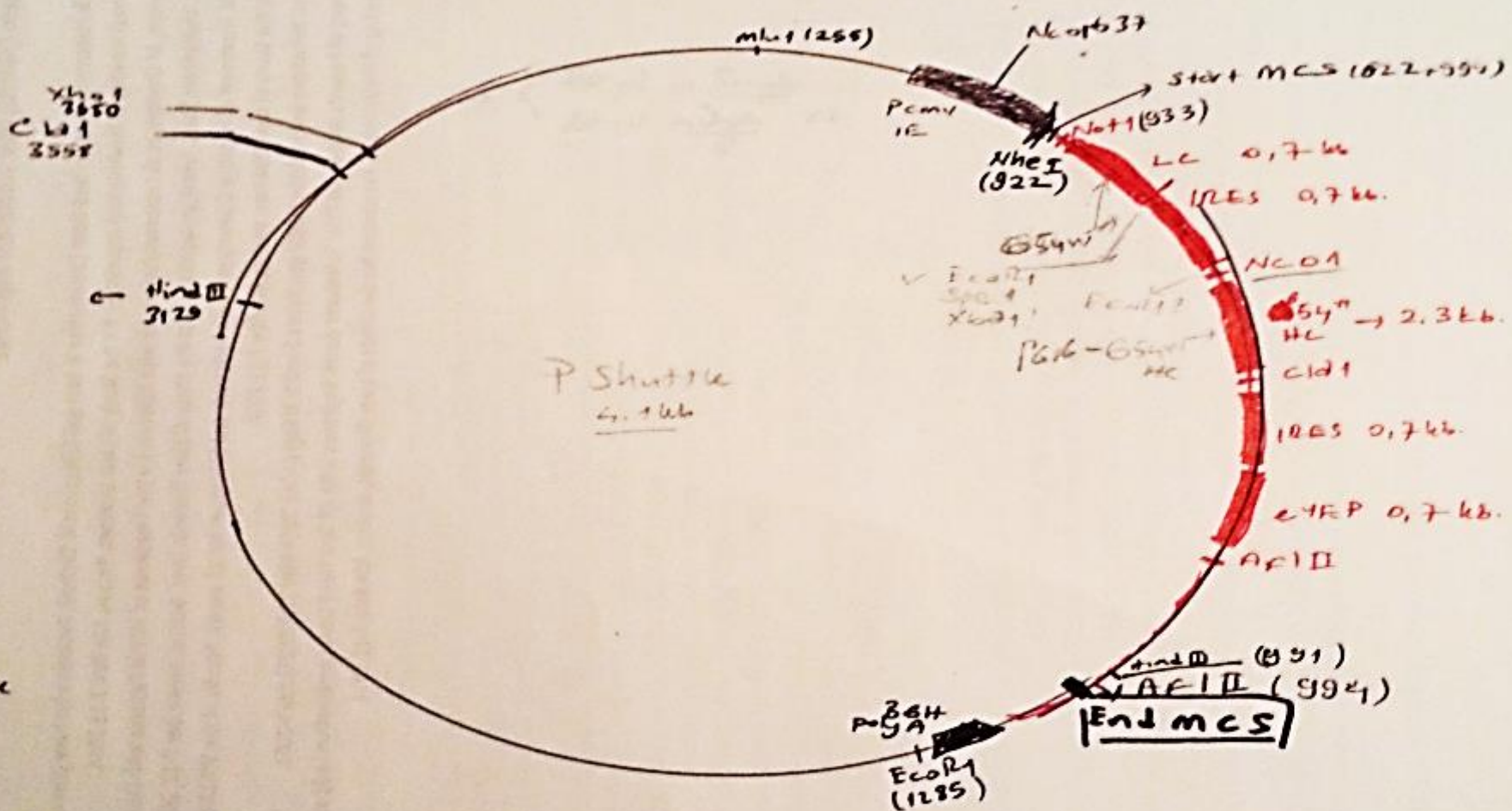
Nco I+ Afl II

Maxiprep



Not I+ Cla I

Not I+ Afl II



	Plasmid	bp	
654wt HC →	1792	142566	EcoR1 + HindIII
Pc16 LC →	1790	0,70 kb. →	EcoR1 + HindIII
654wt HC →	1833	14666bp →	NcoI + ClaI (/AsiSI)
654wt LC →	1834	0,7 kb. →	NotI + EcoR1

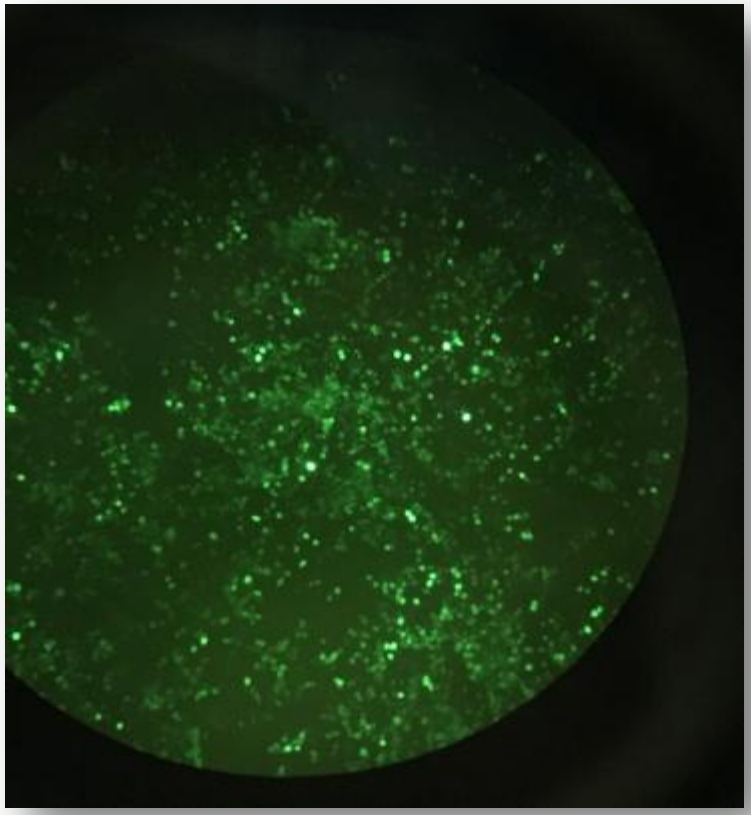
→ Vector PCR Blunt II Topo.
→

Transfection

Bispesific-BNAb

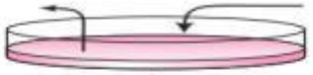
PG16

NIH45-46^{G54W}



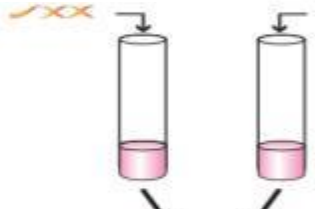
Transfection öncesi media değiştirilir

293 T Hücre



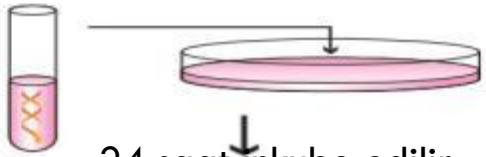
5µg Plasmid DNA
100 µL 0.25mM CCl_2

100 µL HeBS Buffer

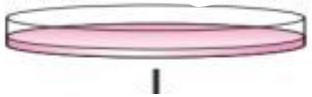


Karıştırılır ve inkube edilir

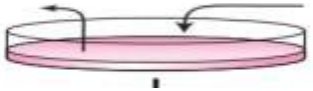
Karışım 293 T hücre ve media içeren plate eklenir



24 saat inkube edilir

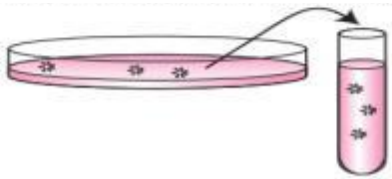


Transfeksiyon sonrası media değiştirilir



48 saat süre ile inkube edilir

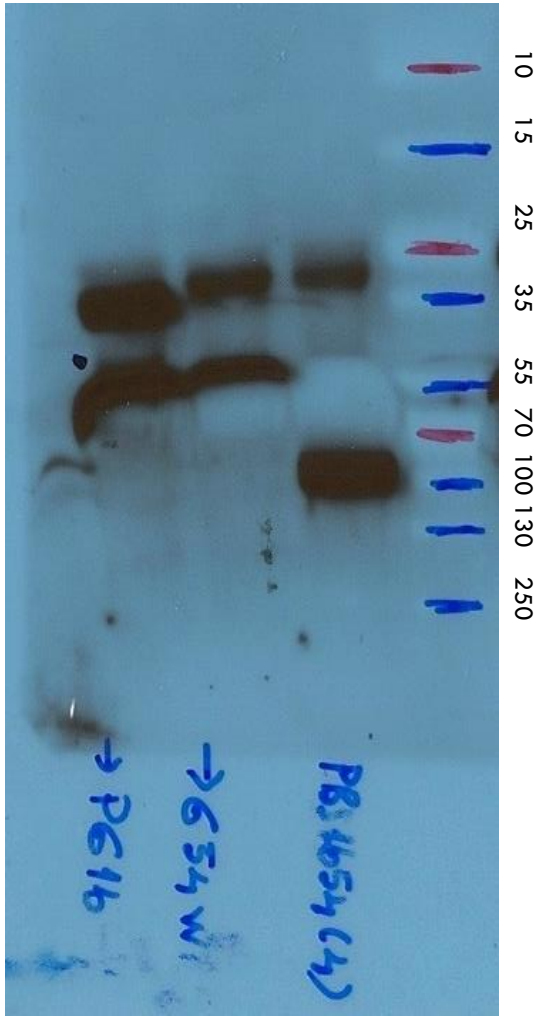
Bi-BNAb içeren media toplanır



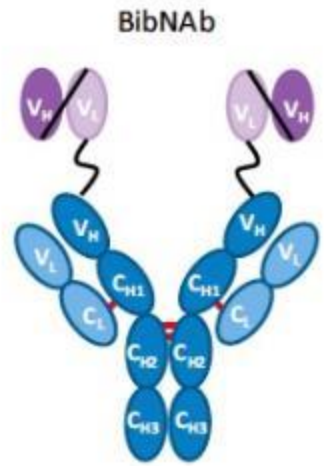
Western Blot

~25 k.Da LC

~50 k.Da HC

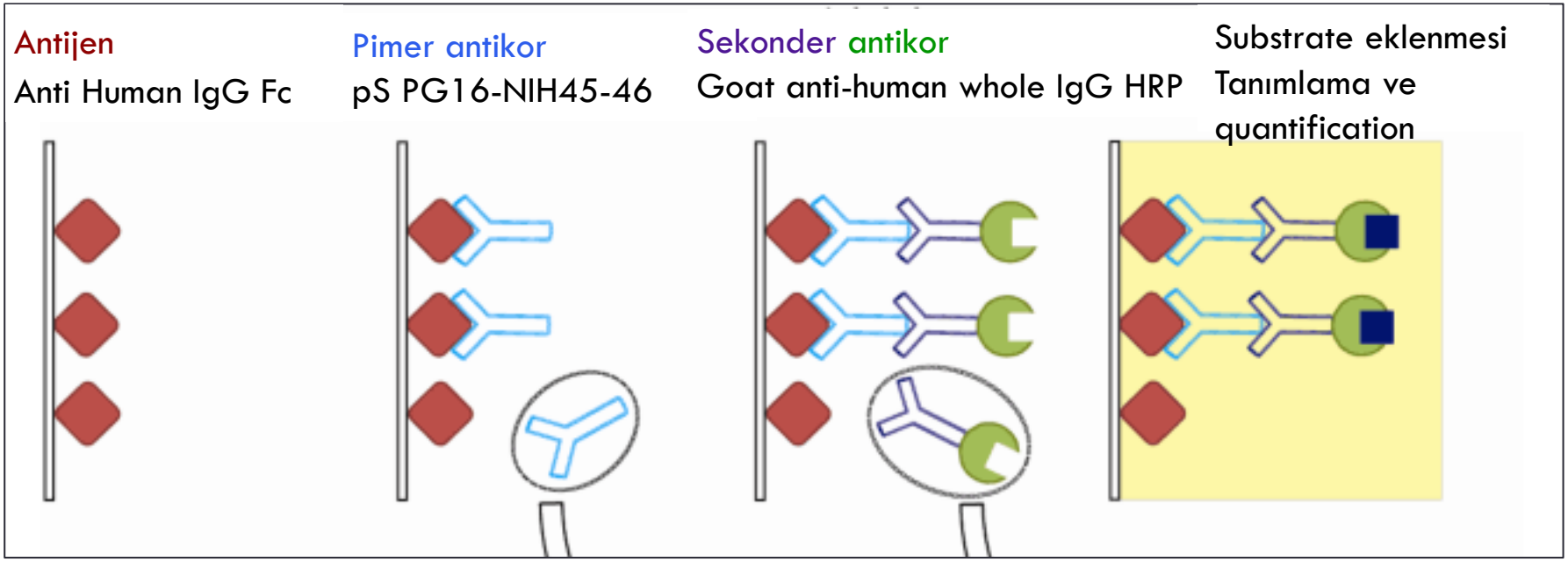


~85 k.Da HC



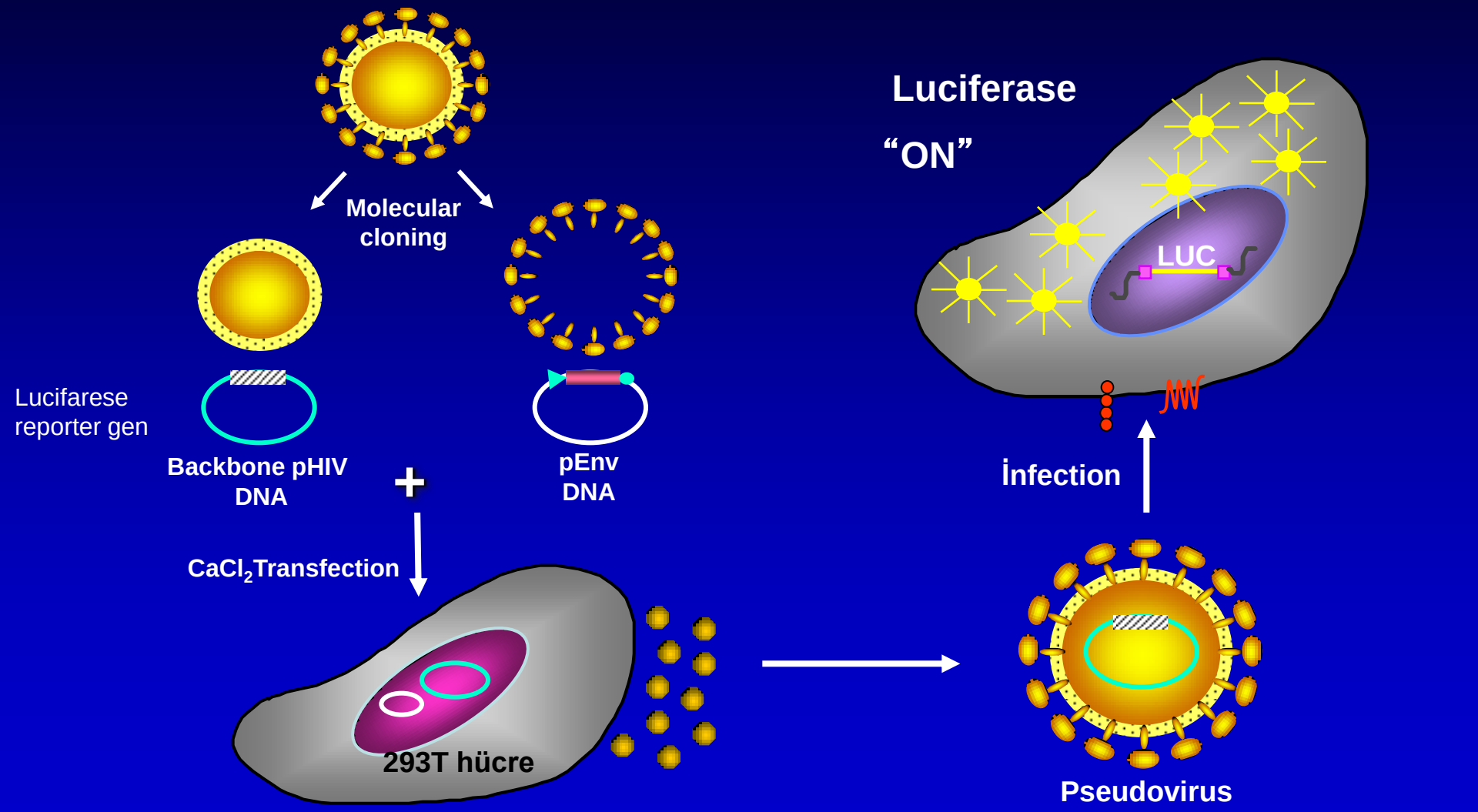
- 96 well plate Anti Human IgG Fc (kırmızı) ile kaplanır
- Örnekler eklenir pShuttle NIH45-46LC- IRES-PG16 NIH4-46 HC-eYFP (mavi)
- Bi-BNAb'e bağlanması için sekonder antikor eklenir (goat anti-human whole IgG HRP) (Mor ve yeşil)
- Normal IgG ise standart olarak kullanıldı

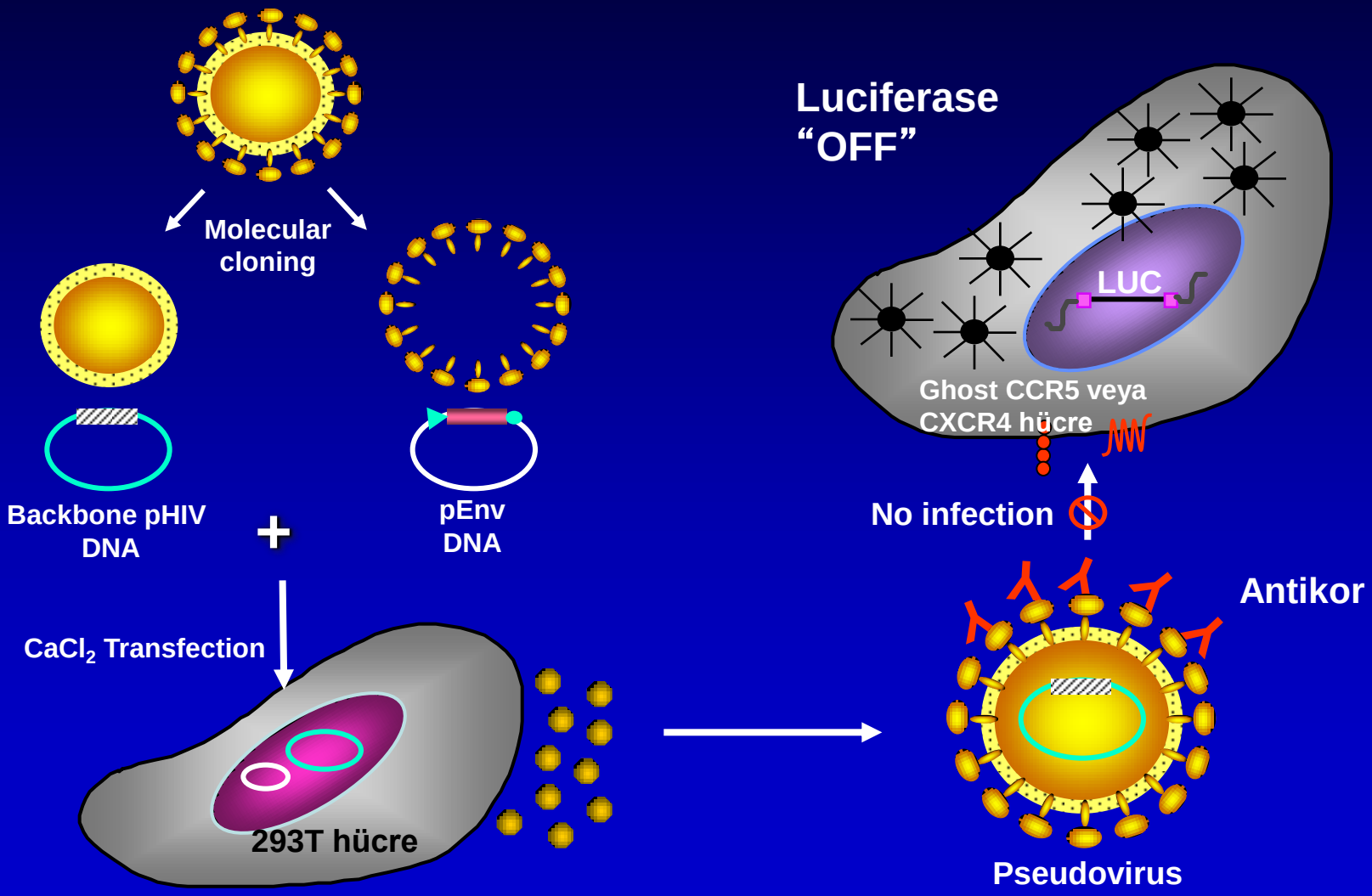
Sandwich ELISA



Antikor	PBS 1654 (4)	PBS 1654 (5)	PBS1654 (5.2)	G54W	PG16
Konsantrasyon (ng/mL)	2301.28	2144.05	1630.22	1341.47	4577.17

Nötralizasyon


Molecular cloning
Lucifarese reporter gen
Backbone pHIV DNA
pEnv DNA
+
CaCl2Transfection
293T hücre
Pseudovirus
İnfection
Luciferase
"ON"
LUC


Molecular cloning
Backbone pHIV DNA
pEnv DNA
+
CaCl2 Transfection
293T hücre
Pseudovirus
Antikor
No infection
Luciferase "OFF"
LUC
Ghost CCR5 veya CXCR4 hücre

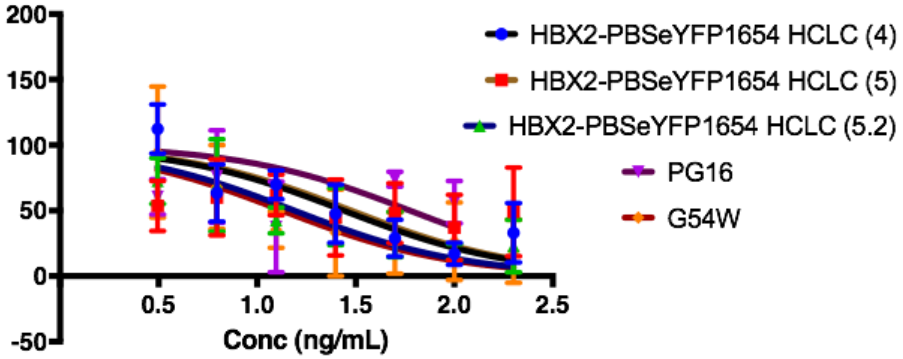
HBX2

Concentration	PBSeYFP1654 HCLC (4)	PBSeYFP1654 HCLC (5)	PBSeYFP1654 HCLC (5.2)	PG16	G54W
200	127	489	82	2812	32
100	199	216	233	819	76
50	354	422	404	1435	198
25	440	340	500	2282	266
12,5	1460	1307	821	1139	387
6,25	2449	553	669	938	881
3,125	2369	731	2391	1286	2251
0	2826	1541	1544	1804	1457

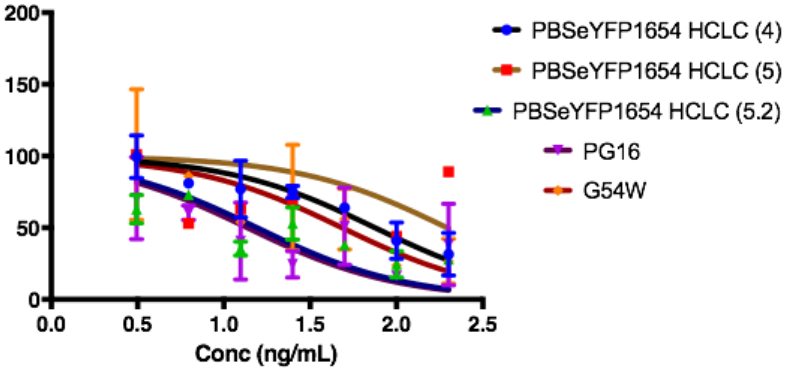
Average	1834,4				
	7%	27%	4%		2%
	11%	12%	13%	45%	4%
	19%	23%	22%	78%	11%
	24%	19%	27%		15%
	80%	71%	45%	62%	21%
	134%	30%	36%	51%	48%
	129%	40%		70%	123%
	154%	84%	84%	98%	79%

	PBSeYFP1654 HCLC (4)	PBSeYFP1654 HCLC (5)	PBSeYFP1654 HCLC (5.2)	PG16	G54W
log(inhibitor) vs. normalized response					
Best-fit values					
LogIC50	1,449	0,7798	0,8527	1,781	0,9294
IC50	28,11	6,022	7,123	60,35	8,5
Std. Error					
LogIC50	0,2764	0,228	0,1109	0,3229	0,2132
95% CI (profile likelihood)					
LogIC50	0,8923 to 2,06	-0,1097 to 1,424	0,5016 to 1,142	0,4931 to +infinity	0,4382 to 1,387
IC50	7,804 to 114,9	0,7768 to 26,54	3,174 to 13,88	3,112 to ???	2,743 to 24,38
Goodness of Fit					
Degrees of Freedom	6	6	5	4	6
R square	0,7001	-0,4502	0,5271	-3,444	0,7201
Absolute Sum of Squares	5635	3282	528,5	3230	3099
Sy.x	30,65	23,39	10,28	28,42	22,73
Number of points					
# of X values	7	7	7	7	7
# Y values analyzed	7	7	6	5	7

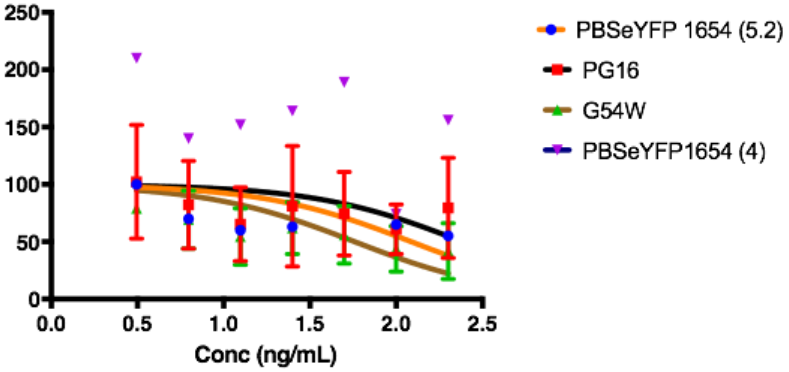
HXB2-CXCR4

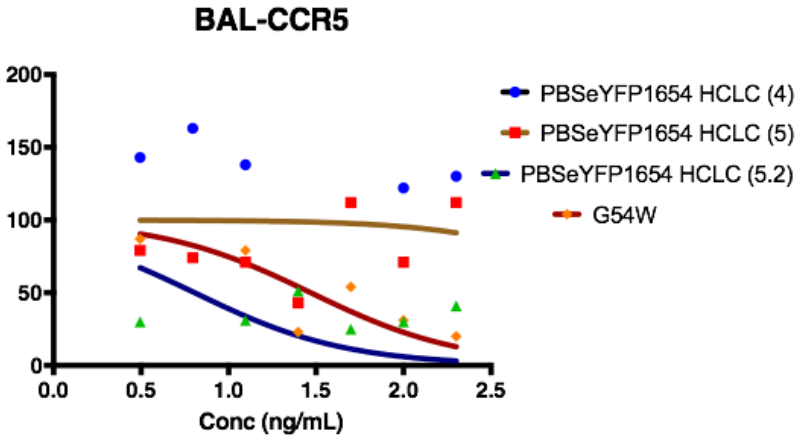


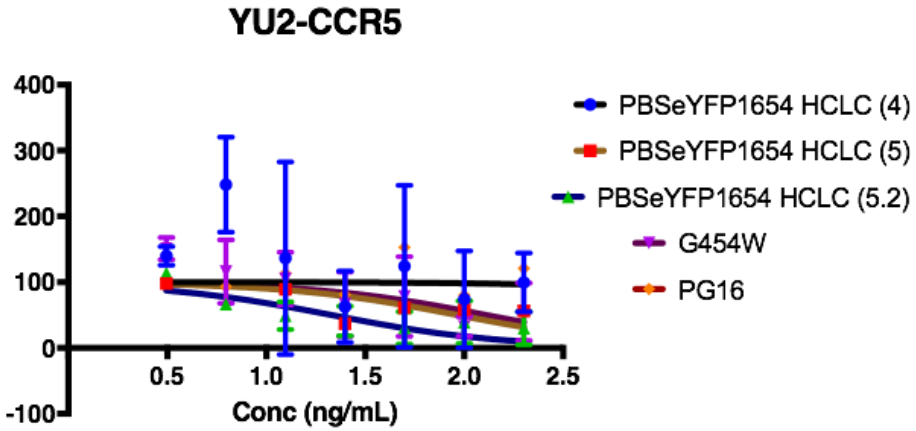
PNL4-3-CXCR4

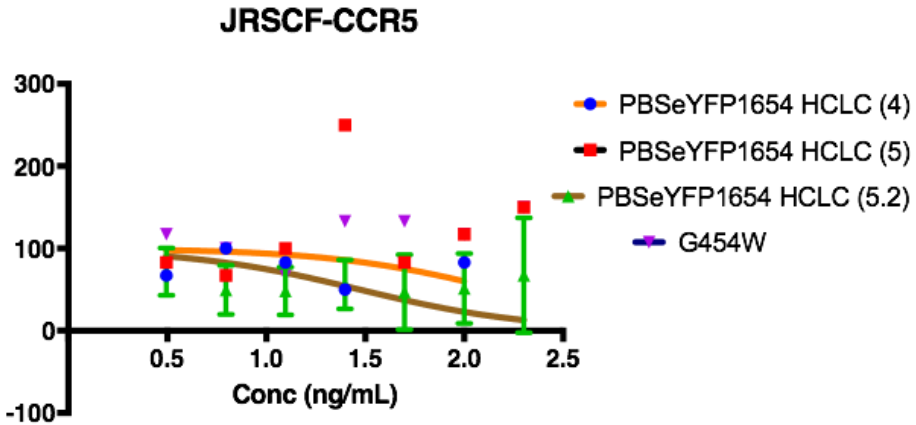


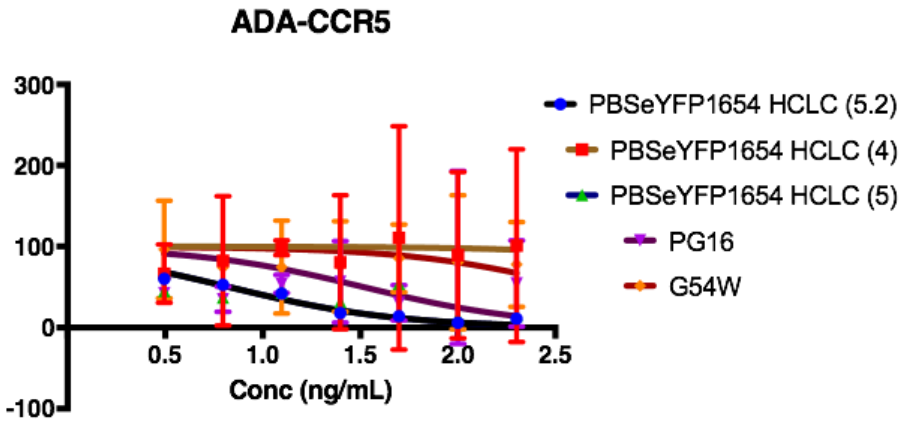
89.6-CXCR4

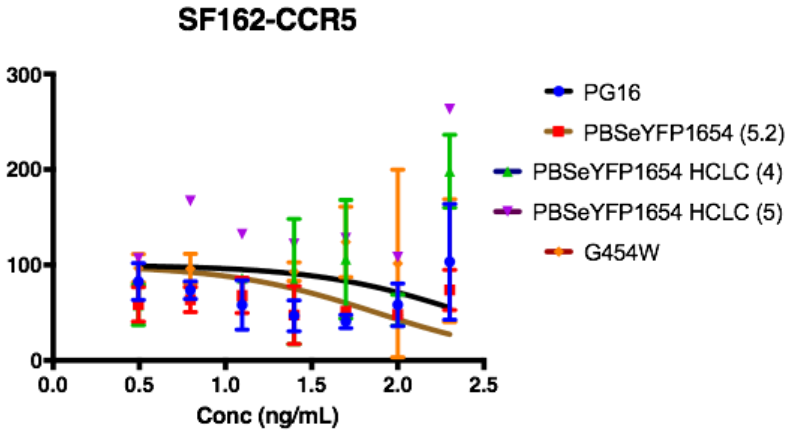


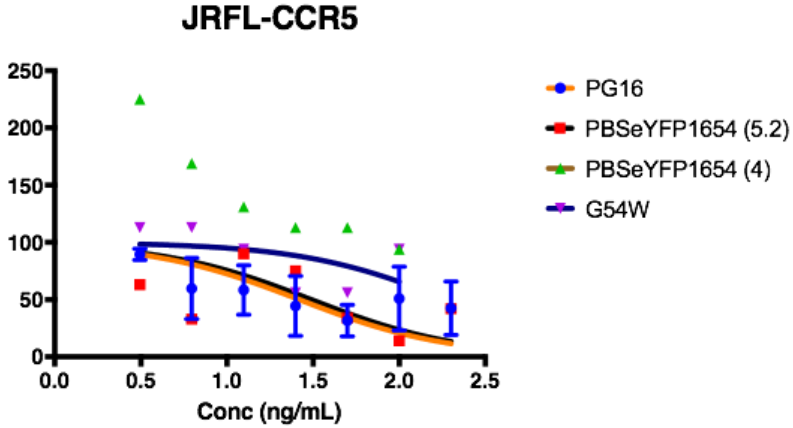

BAL-CCR5
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
Conc (ng/mL)
PBSeYFP1654 HCLC (4)
PBSeYFP1654 HCLC (5)
PBSeYFP1654 HCLC (5.2)
G54W

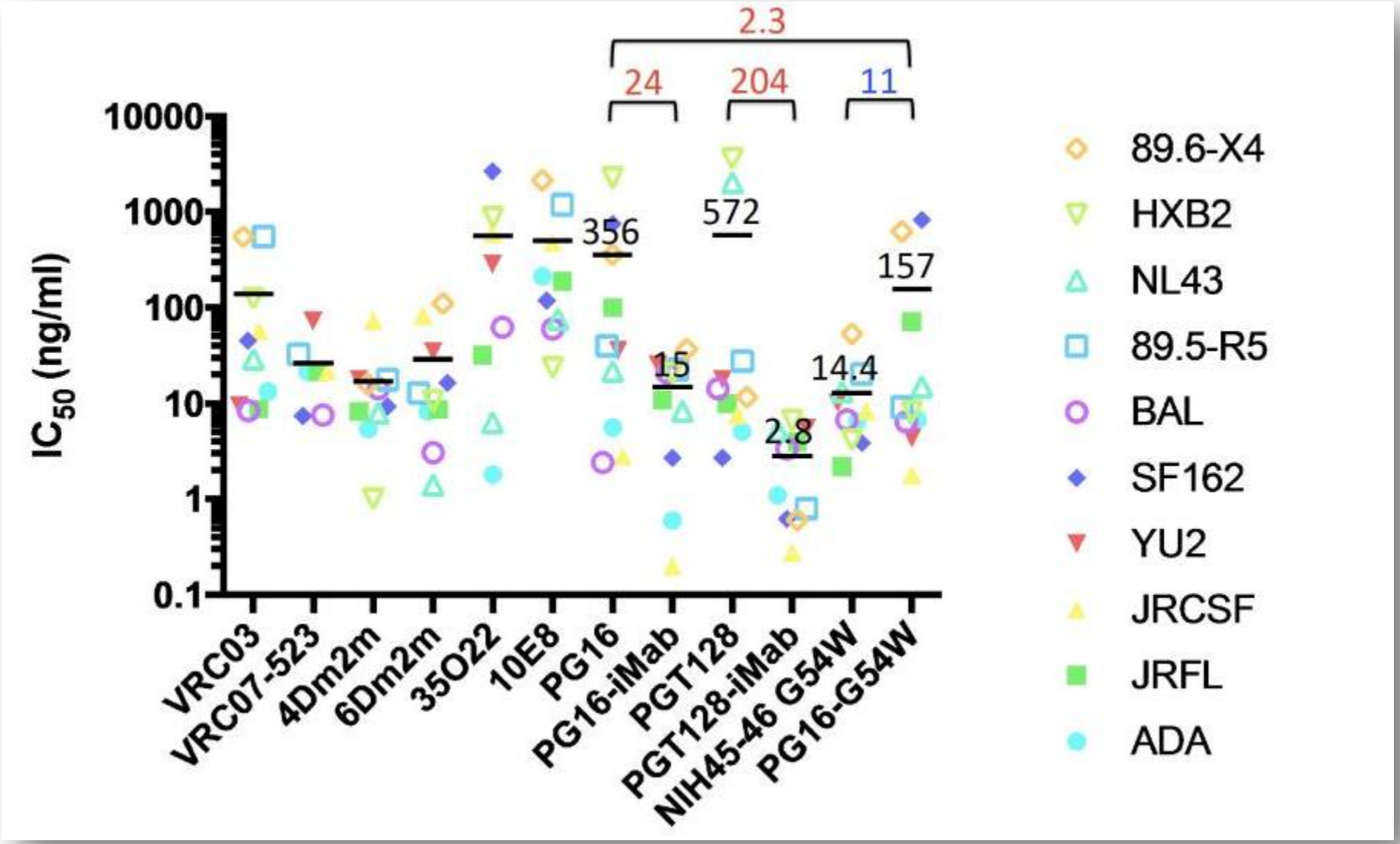

YU2-CCR5
400
300
200
100
0
-100
0.5
1.0
1.5
2.0
2.5
Conc (ng/mL)
PBSeYFP1654 HCLC (4)
PBSeYFP1654 HCLC (5)
PBSeYFP1654 HCLC (5.2)
G454W
PG16


JRSCF-CCR5
300
200
100
0
-100
0.5
1.0
1.5
2.0
2.5
Conc (ng/mL)
PBSeYFP1654 HCLC (4)
PBSeYFP1654 HCLC (5)
PBSeYFP1654 HCLC (5.2)
G454W


ADA-CCR5
300
200
100
0
-100
0.5
1.0
1.5
2.0
2.5
Conc (ng/mL)
PBSeYFP1654 HCLC (5.2)
PBSeYFP1654 HCLC (4)
PBSeYFP1654 HCLC (5)
PG16
G54W


SF162-CCR5
300
200
100
0
0.0
0.5
1.0
1.5
2.0
2.5
Conc (ng/mL)
PG16
PBSeYFP1654 (5.2)
PBSeYFP1654 HCLC (4)
PBSeYFP1654 HCLC (5)
G454W


JRFL-CCR5
250
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
Conc (ng/mL)
PG16
PBSeYFP1654 (5.2)
PBSeYFP1654 (4)
G54W


2.3
24
204
11
10000
1000
100
10
1
0.1
IC50 (ng/ml)
356
572
157
15
14.4
2.8
VRC03
VRC07-523
4Dm2m
6Dm2m
35O22
10E8
PG16
PG16-iMab
PGT128
PGT128-iMab
NIH45-46 G54W
PG16-G54W
89.6-X4
HXB2
NL43
89.5-R5
BAL
SF162
YU2
JRCSF
JRFL
ADA

IC_{50} Karşılaştırma

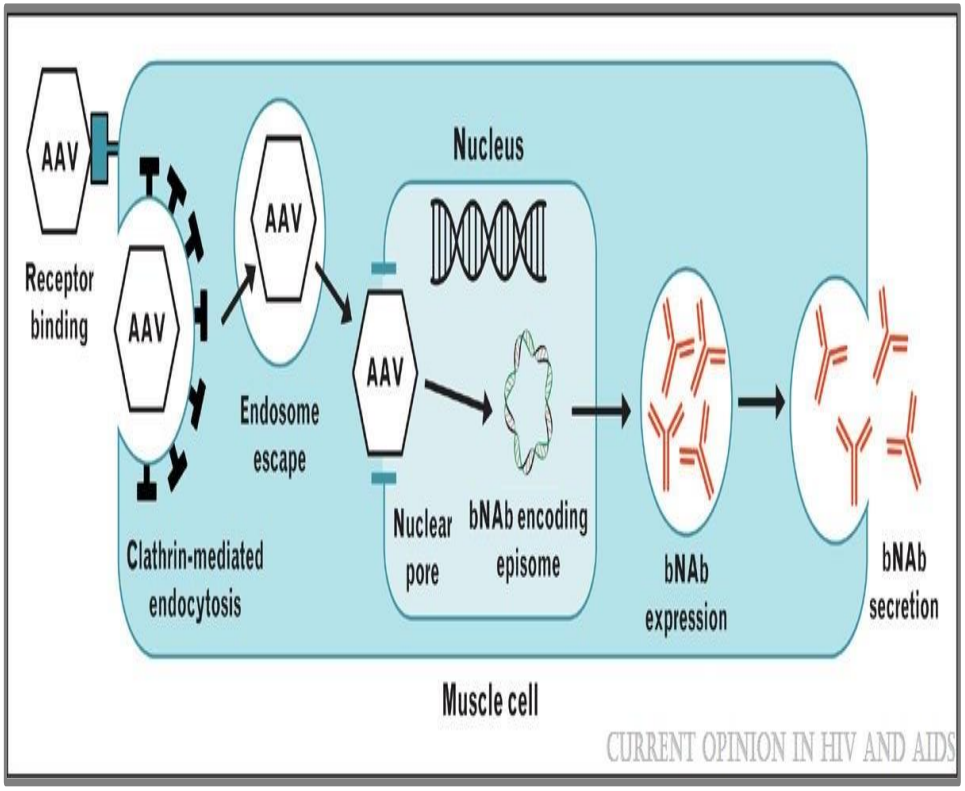
	CCR5 spesifik Pseudotype							CXCR4 spesifik Pseudotype			
	ADA	**JRFL**	**JRCSF**	**YU2**	**SF162**	**BAL**	**89.6-R5**	**PNL43**	**HXB2**	**89.6-X4**	**Ortalama**
PG16	5,6	99,3	2,8	36	749,2	2,4	40,1	21,6	2245	357,8	356
PG16-NIH45-46 G54W	6,7	71,1	1,8	4,3	822,4	6,4	9,2	14,9	8,2	624,9	157
Fold change	0	1.4	1.5	8.3	0	0	4,3	1,4	0,5	0,1	2.2
NIH45-46 G54W	6,5	2,2	8,5	10,1	3,9	6,7	20,2	28,1	4,1	53,6	14,4
PG16-NIH45-46 G54W	6,7	71,1	1,8	4,3	822,4	6,4	9,2	14,9	8,2	624,9	157
Fold change	1	0	4,7	2,3	0	1,1	2,2	1,9	0,5	0,1	0

Sorunlar

- Mutasyonlar
- Ağır Zincir (parental 50 kD, Bi-BNAb 85 kD)
- Glisin-Serin linker
- Her iki epitopa aynı zamanda bağlanmanın adaptasyon sınırlılığı yapması

Sonraki adım

- Biyofiziksel karakterizasyonu (Size-exclusion chromatogram)
- Vektör aracılıklı invivo Antiko ekspresyonu (Helper dependent Adenovirus)
- Yeni Bi-spesifik BNAb geliştirme



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Dinlediğiniz için teşekkürler