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HIV-1 Clones from HLA-typed Individuals

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Virus Cloning Protocol:

Virus isolate from HIV-infected subject is serially diluted appropriately in a 96-well plate in 100 μL Cloning Medium + 1×10^5 per well of PBMCs stimulated with PHA or with anti-CD3/CD8 antibody 2 to 6 days before and incubated at 37°C and 5% CO_2 in a humidified incubator for 1 week. 100 μL supernatant/well is removed and replaced with 100 μL fresh Cloning Medium/well and the plate incubated a second week. 100 μL supernatant/well is removed and tested for p24. If no wells are considered clonal, one positive well is selected, diluted appropriately, and used to repeat the cloning process in a fresh 96-well plate. Wells considered clonal are expanded in 5 mL either Cloning Medium or R20-50 in a flask with 5×10^6 PBMCs stimulated with PHA or with anti-CD3/CD8 antibody 2 to 6 days before. At 0.5 weeks after transfer to a flask and weekly thereafter, culture is fed by removing half the volume without resuspending cells and replacing with an equal volume of medium—or a greater volume if a larger culture volume is desired. At one week after transfer to a flask and once a week subsequently, the culture is split by resuspending the cells, removing half the volume, and replacing with an equal volume of fresh medium containing 5×10^6 PBMCs stimulated with PHA or with anti-CD3/CD8 antibody 2 to 6 days before. When the p24 indicates a sufficient concentration of virus, the culture is harvested by pelleting the cells at 1500rpm for 10 minutes and removing the supernatant with a pipet.

Cloning Medium:

IMDM (Iscove's mod. Dulbecco's Med.)	500 mL
FBS (heat-inactivated 1 hr. at 56°C)	55 mL (<i>approximately</i>)
Polybrene (0.5 mg/mL)	5 mL (<i>medium thus $5\mu\text{g}/\text{mL}$</i>)
L-Glutamine (200 mM)	5 mL
Penicillin/Streptomycin	5 mL
<i>(Penicillin 10,000 I.U./mL, Streptomycin 10,000 $\mu\text{g}/\text{mL}$ stock)</i>	
IL-2	280 μL of 1×10^5 units/mL, 0.22 μ filter-sterilized
<i>(Medium is therefore approximately 50 units/mL)</i>	

HIV Clones (Thailand)			
<i>Cat No.</i>	<i>Reagent Name (Code)</i>	<i>p24/mL (pg)</i>	<i>TCID 50/mL</i>
11655	307: RPW-0510, Clone B11 E3	93,603	1.7E+05
11656	309: NKR-0512, Clone H6 G8	139,827	3.0E+05
11657	310: PWJ-0513, Clone G8	122,643	2.4E+05
11658	311: KSS-0514, Clone A6 B4 E2 F7	75,083	1.3E+05
11611	312: PKT-0515, Clone D8	148,921	3.3E+05
11612	317: JJS-0520, Clone G7	138,051	2.9E+05
11613	322: SPK-0525, Clone C2 E6 F8	126,483	2.5E+05
11614	322: SPK-0525, Clone C2 H8	117,532	2.3E+05
11615	330: EUN-0533, Clone G6	30,281	7.8E+04
11616	335: DPS-0538, Clone F9	18,016	6.7E+04
11617	361: STP-0553, Clone A9	141,182	3.0E+05
11618	364: TLK-0557, Clone C11	119,632	2.3E+05
11619	368: TPT-0562, Clone A4 B3 F8 F7	103,717	1.9E+05
11620	368: TPT-0562, Clone A4 B3 F8 H8	30,262	7.8E+04

HIV Clones (China)			
<i>Cat No.</i>	<i>Reagent Name (Code)</i>	<i>p24/mL (pg)</i>	<i>TCID 50/mL</i>
11621	375: CXA-056, Clone B9 H10 A11	231,574	9.1E+05
11622	375: CXA-056, Clone B9 H10 G12	258,793	1.3E+06
11623	377: CBJ-008, Clone D11 H6 A9	72,179	1.3E+05
11624	379: CBJ-011, E11 H1 D6	49,636	9.8E+04
11625	381: CBJ-010, Clone F8 A6 E5	109,702	2.0E+05
11626	381: CBJ-010, Clone F8 A6 G6	91,909	1.6E+05
11627	382: CXA-037, Clone D11 B8	87,747	1.6E+05
11628	383: CXA-022, Clone D10 D7	239,012	9.9E+05
11629	386: CBJB-033, Clone F10 A11	138,638	2.9E+05
11630	386: CBJB-033, Clone F10 D9	108,417	2.0E+05
11632	390: CBJA-028, Clone B11 H2 E7	74,927	1.3E+05
11631	392: CXA-026, Clone A12	134,750	2.8E+05
11633	399: CBJA-020, Clone D12 B6 B2 F11 A10	137,532	2.9E+05
11634	399: CBJA-020, Clone D12 B6 B2 F11 F9	29,171	7.7E+04