

HIV-1: Identification and Phenotypic Characterization of the Transmitted Virus

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AIDS Vaccine 2007

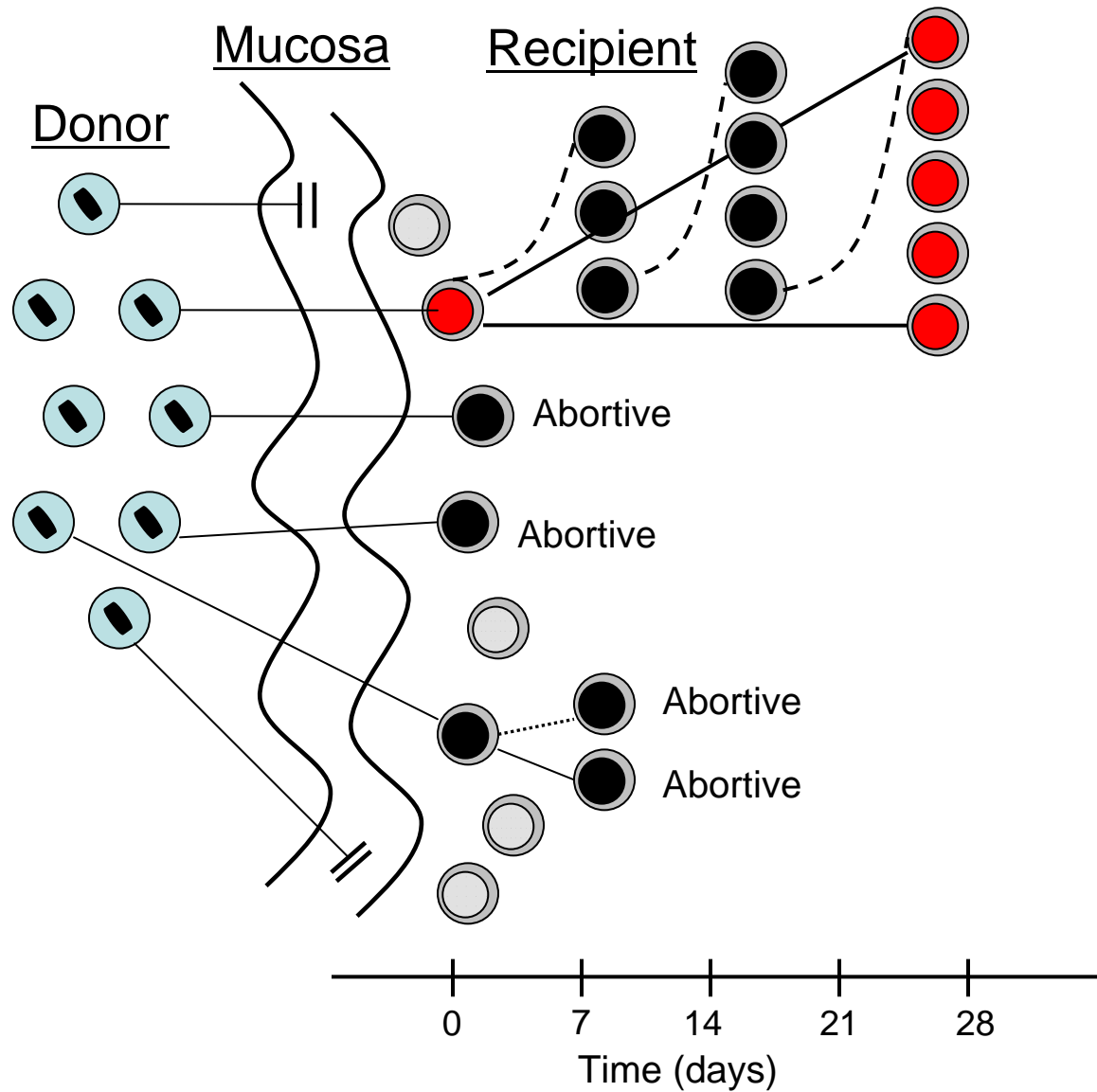
August 22, 2007

Salazar et al, Abstract P12-08

Keele et al, Abstract OA07-02

Korber et al, Symposium S07-04

HIV-1 Transmission Model



Can we identify the transmitted virus and its biological and antigenic properties?

Study Design

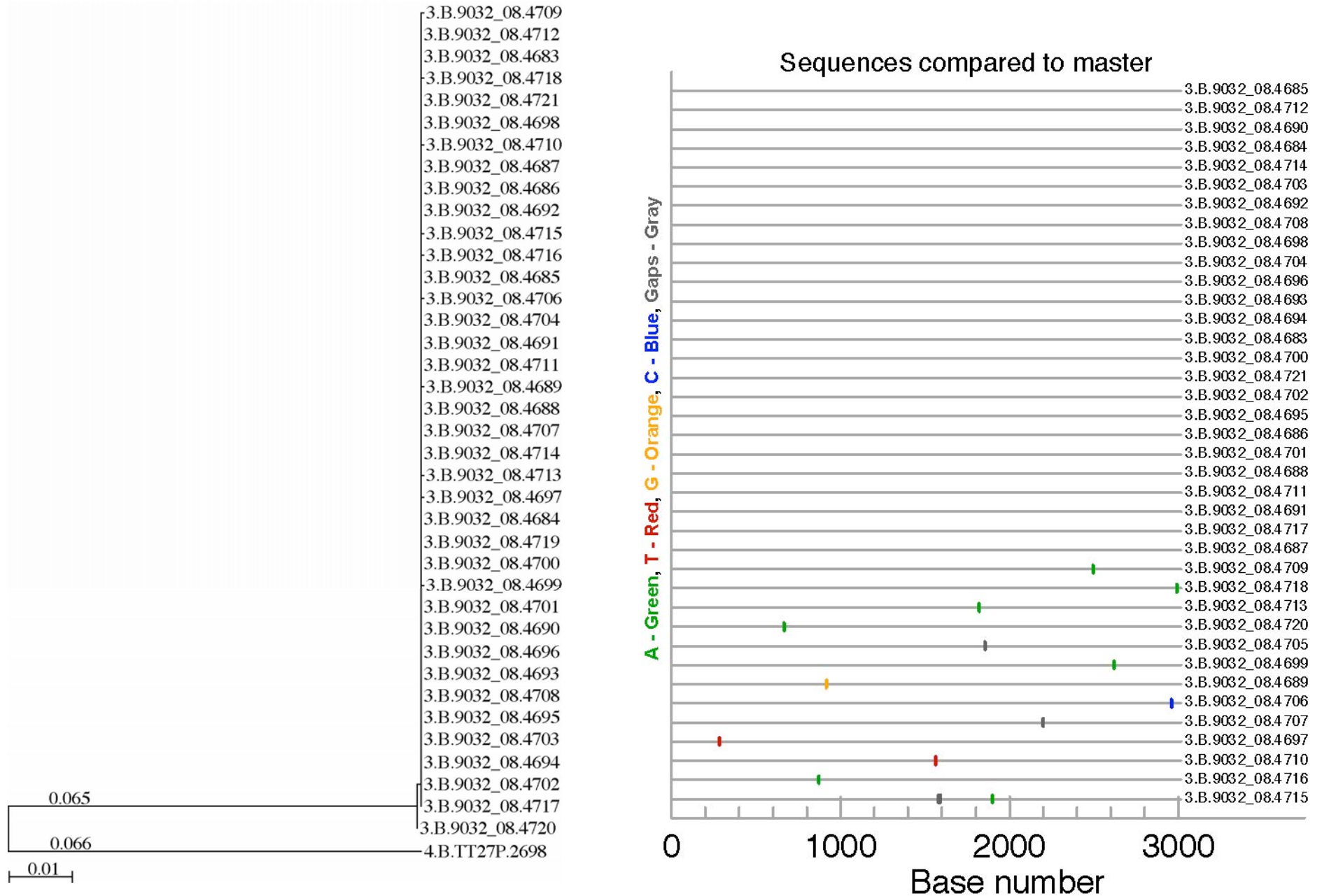
- Single genome amplification (SGA) of plasma viral RNA from 96 acutely infected subtype B patients
- Subjects classified by Fiebig Stage based on their vRNA and antibody status.
 - Fiebig I (vRNA positive): 1%
 - Fiebig II (vRNA and antigen positive/antibody negative): 51%
 - Fiebig III (ELISA pos/Wb neg): 17%
 - Fiebig IV (ELISA pos/Wb +/-): 8%
 - Fiebig V (Wb pos/p32 neg): 23%
 - Fiebig VI (Wb pos/p32 pos): 2%

(Keele et al, Abstract OA07-02, Thursday 10:30 am Session 7)

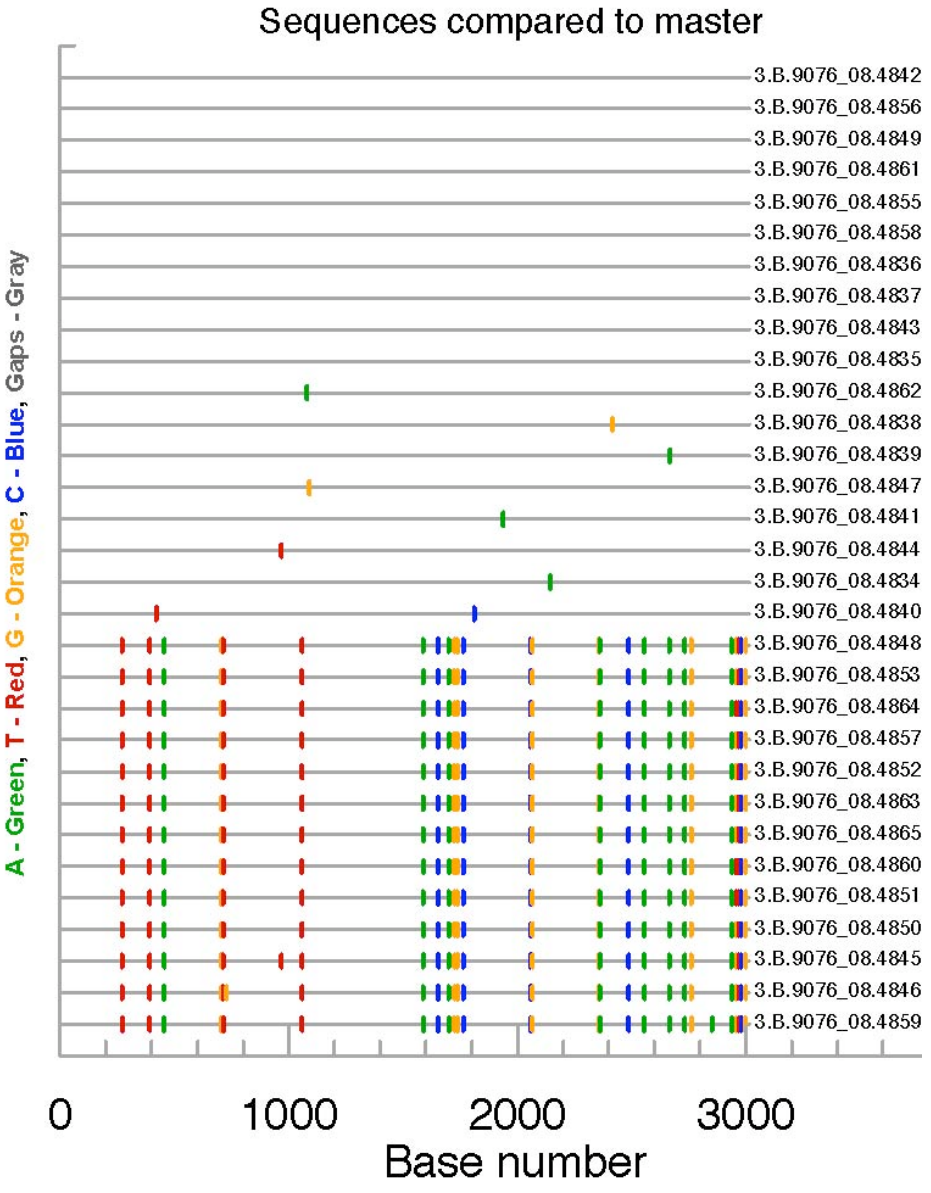
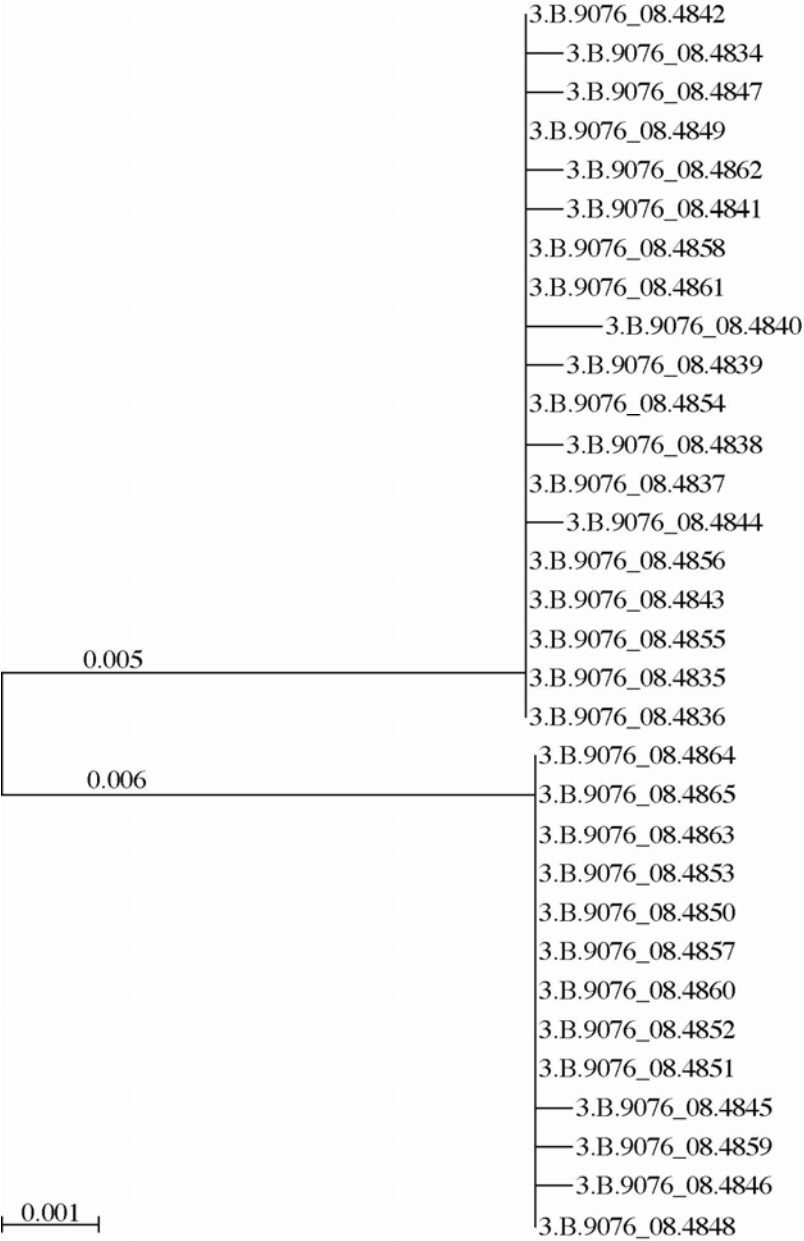
Study Design (cont'd)

- Average number of *env* sequences per individual was 27 (range 10 – 62) derived from a single timepoints or serially.
- 2,590 complete *env* sequences analyzed.
- Analysis of *within patient* viral evolution done by visual inspection of nucleotide sequence alignments, phylogenetic tree construction, bioinformatics, and mathematical modeling.

Productive Infection with a Single Virus



Productive Infection with Two Viruses



Model Predictions of Envelope Quasispecies Diversity Following Infection

Assumptions:

Generation time: 2 days

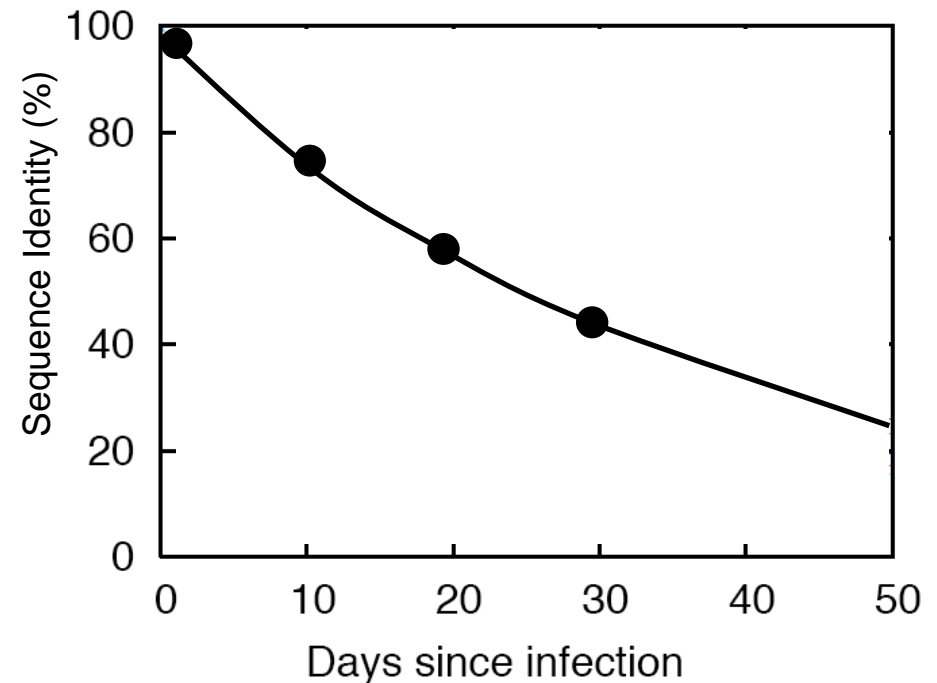
RT error rate: $\sim 2 \times 10^{-5}$
(1 in 20 envs with 1 bp change)

Predicted env identities:

~75% at 10 days

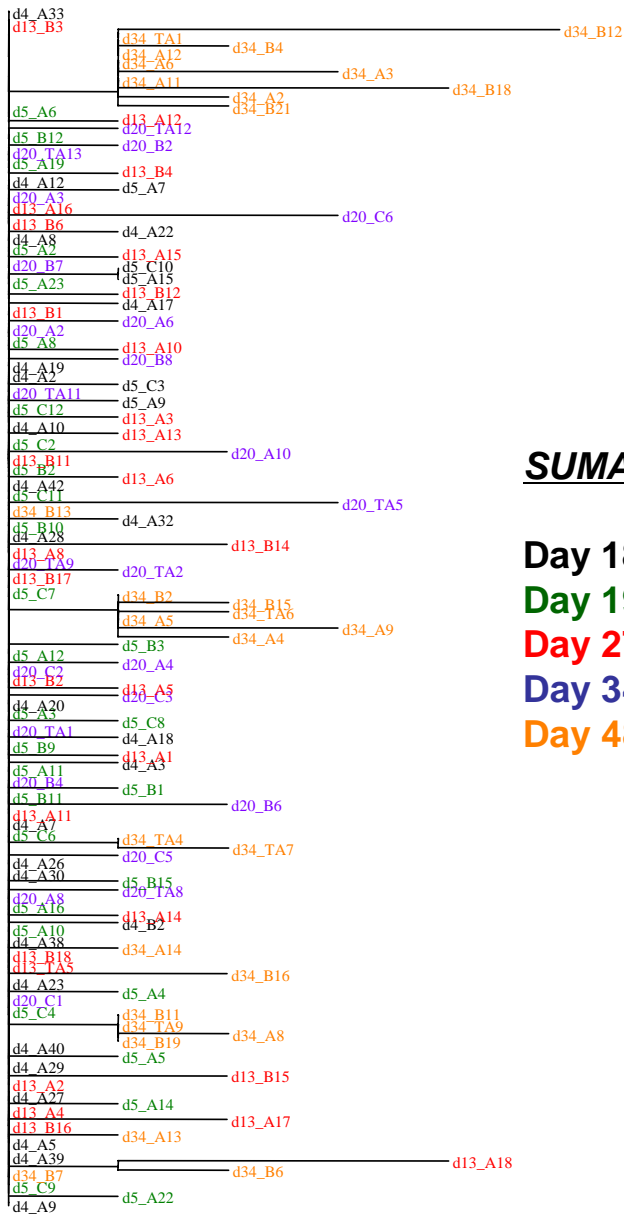
~60% at 20 days

~45% at 30 days



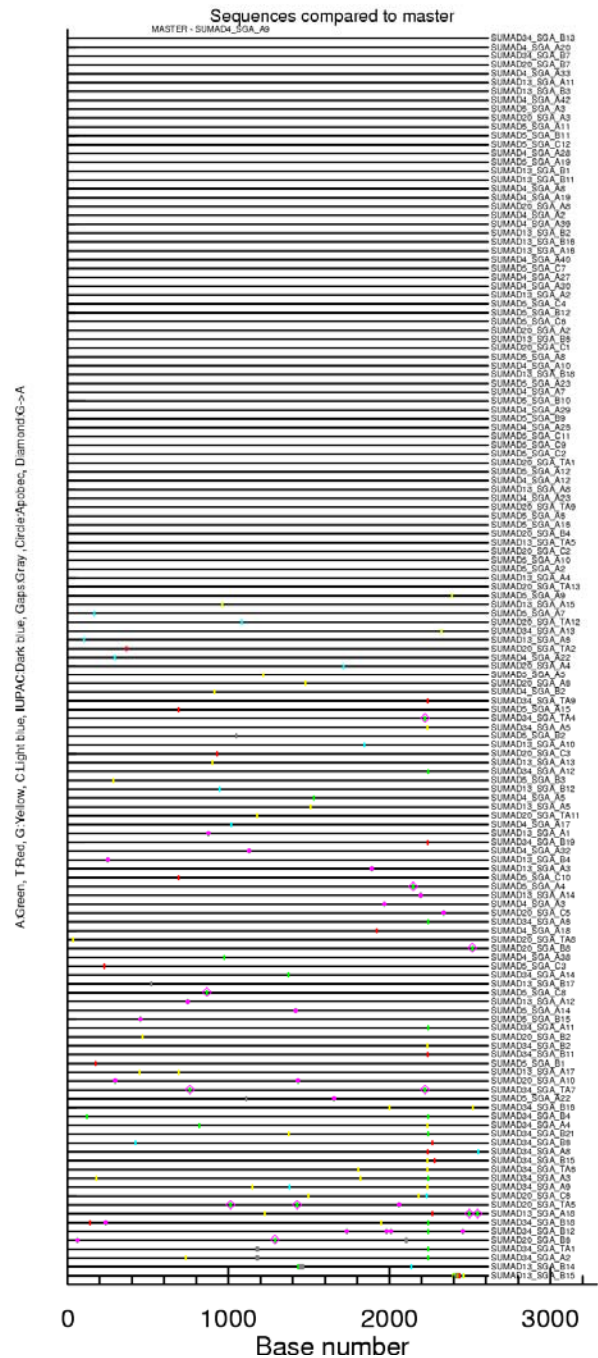
Hypothesis: The identical (consensus) plasma viral env sequences determined experimentally 14-42 days after infection represent the actual transmitted virus Env

Early Env Diversification in Subject SUMA Between Feibig Stage II and V

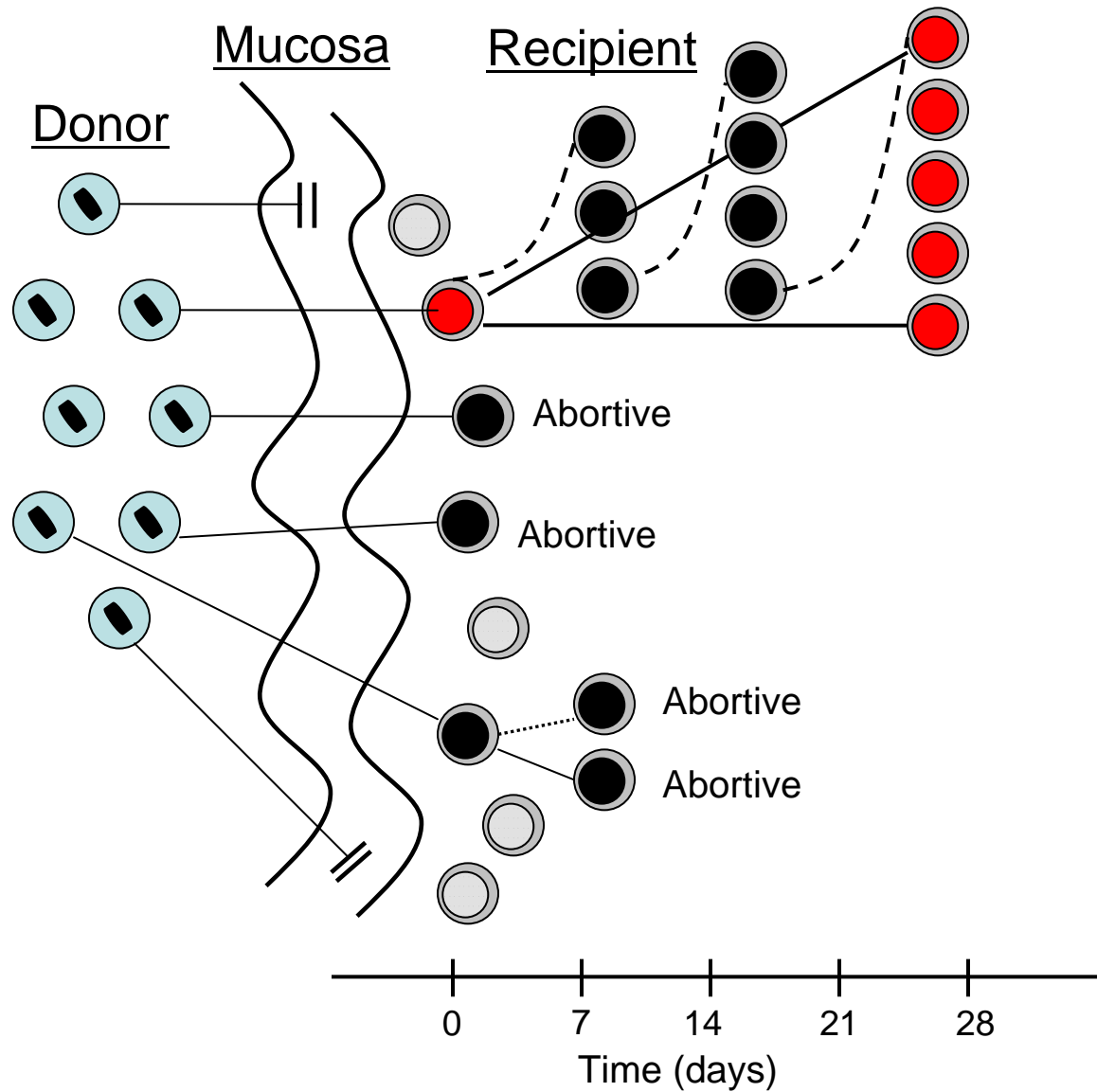


SUMA env evolution

- Day 18 - 77% identical
- Day 19 - 63% identical
- Day 27 - 48% identical
- Day 34 - 42% identical
- Day 48 - 7% identical



HIV-1 Transmission Model

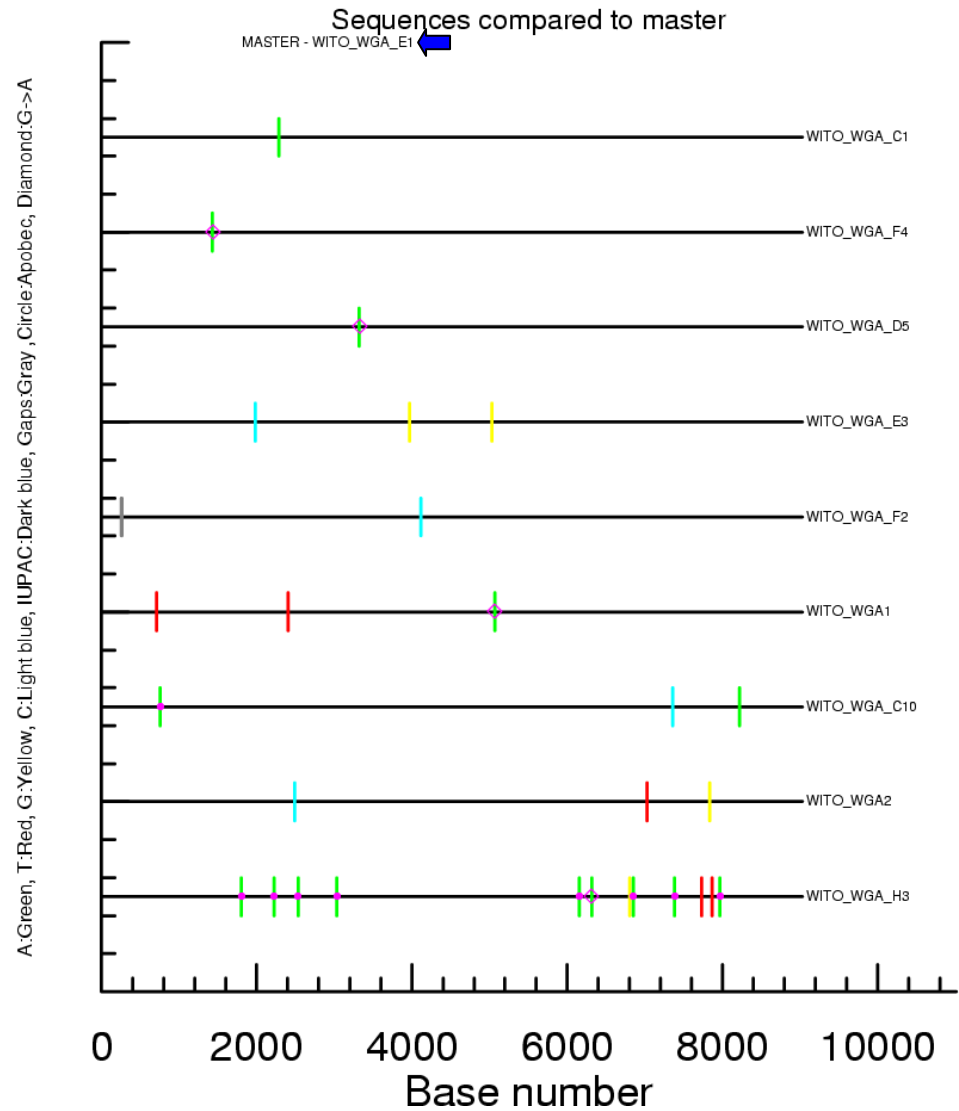
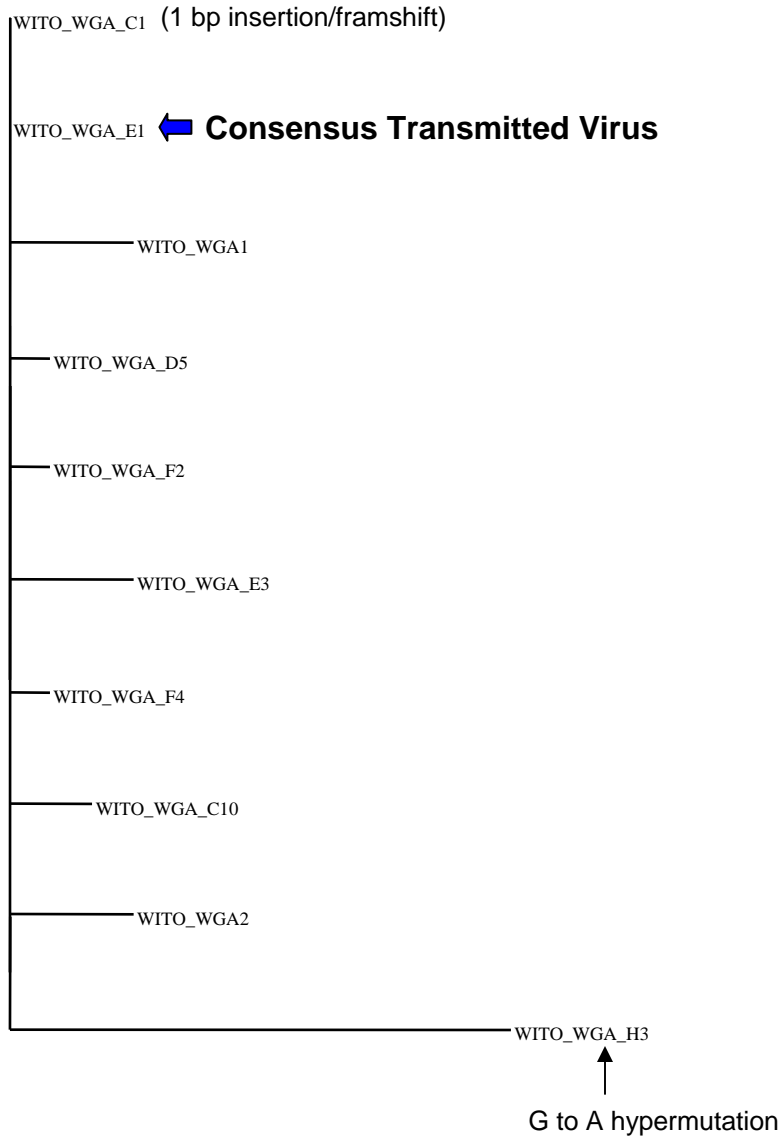


Conclusion: We can identify the transmitted virus Env and distinguish it from escape mutants

**Can we use SGA to identify the
transmitted full-length viral
genome . . . Yes**

Salazar et al, Abstract P12-08

WITO 4160 - Whole genome amplification of a transmitted virus



Transmitted Viral Proteome: WITO 4160

GAG

MGARASVLSGGELDRWEKIRLRPGGKKKYQLKHIVWASRELERFAVNPGLLETSEGRQILGQLQPSLQTGSEELKSLFNTVATLYCVHQKIDVKDTKEALDRIEEQNKSKIKAQQAADTGS
SQVSNQYPIVQNLQGMVHQAIAPRNLNAWVKVVEEKAFSPEVPMFSALEAGATPQDLNMTLNTVGGHQAAMQMLKETINEEAAEWDRHLPHIAGPIAPGQMREPRGSDIAGTTSTLQEQIGW
MTNPPPIVGEIYKRWILGLNKIVRMYSVPSILDIRQGPKEPFRDYVDRFYKTLRAEQASQDVKNWMTETLLVQANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEMSQTNST
AIMMQRGNFRTRQKPKVCFNCGKEGHIARNCRAPRKKGCWKCQKEGHQMKDCTERQANFLGKIWPSHKGRPGNFLQSRPEPTAPTAPPEESFRFGEEETTPPQKQETIDKEKYPLASLRSF
GNDPLSQ\$

POL

FFRENLAFPQGGKAREFPSEQTRANSPNSPTGGELQVWGRDNNSPSEAGDNRQGEVVSFGFPQITLWQRPLVAIKIGQLKEALLDTGADDTVLEEMNLPGRWPKMIGGIGGFIKVRQYDQIPIE
CGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNFPISPIETVPVKLKPMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNTPVFAIKKDKSTKWRKLVDFRELNKRTQDFWEVQLGIPH
PAGLKKKSVTVLDVGDAYFSVPLDKDFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPIAFQCSMTKILEPFRKQNPDIYQYMDLLYVGSLEIGQHRTKIEELRQHLLRWGFTTPDKKHQK
EPPFLWMGYELHPDKWTVQPIVLPDKDSWTVNDIQKLVGKLNWASQIYAGIKVKQLCKLLRGAKSLTEVIPLTEEALELAENREILKEPVHGVYDPSKDLVAEIQKQGGQWQTYQIYQEPFKNL
KTGKYARMRGAHTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWEAWWMEYQATWIPEWVFNTPPLVKLWYQLEKEPITGAETFYVDGAANRETKLGKAGYVTDGRQKVVSLADTT
NQKTELQAIHLALQDSGSEVNIVTDSQYALGIIQAQPKSESEVNNQIIIEQLIKERVYLAWVPAHKGIGGNEQVDKLVSTGIRKVLFLDGIDKAQEEHEKYHNNWRAMASDFNLPPVVAKEIVASC
DKCQLKGEAIIHQVDCSPGIWQLDCTHLEGIILVAVHVASGYIEAEVIPAETGQETAYFILKLAGRWPKTIHTDNGSNFTSNTVKAACWWAGIKQEFIPYNPQSQGVVSMNNELKKIIGQVRD
QAEHLKTAVQMAVFIHNFKRKGGIGYSAGERIVDIIATDIQTKELQKQITKIQNFVYYRDSRDPLWKGPAKLLWKGEGAVVIQDNSEIKVPRRKAIIIRDYGGKQMGAGDDCVAGRQDED\$

VIF

MENRWQVMIVWQVDRMRIRAWNSLVKHHMYISKKAAGWFYRHHYESQHPKISSEVHIPIGEARLVITTYWGLNTGERDWHLGQGVSIWRKYYSTQVDPDLADQLIHLIYDFCFSESAINRIL
GHRVSPRCEYQAGHNKVGSLQYLALTAALIKPKKRKPLPSVAKLTEDRWNKPQRTKGRGSHMTMNGH\$

VPR

MEQAPEDQGSQREPYNEWTLELLEELKSEAVRHFRVWLHNLGQYIYETYGDTWAGVEAIRILQQLLFIHFRIGCQHSRIGIIRQRTRNGASRS\$

VPU

MQPLEILAVVALVVALILAVVWTIVYIEYRKIQKQKKIDRLIDRIRERAEDSGNESDGDQEELSALVEMGHHPWDVND\$

TAT

MEPVDPRLEPWKHPGSQPKTPCNCYCKKCCFHCQVCFTKKGLGISYGRKKRSQRRRTPNDSQDHQASLSKQPASQTRGDPTGQKKQKEKVERETEDPHD\$

REV

MAGRSGVSDELLTTVRIIKLLYQSNPPKPEGTRQARRNRRRRWRERQRQIRTISERILSNHLGRSAEPVPLQLPPLERLTLDCSEGCGDSGTQGVGNPQILVESPEVLESQKE\$

ENV

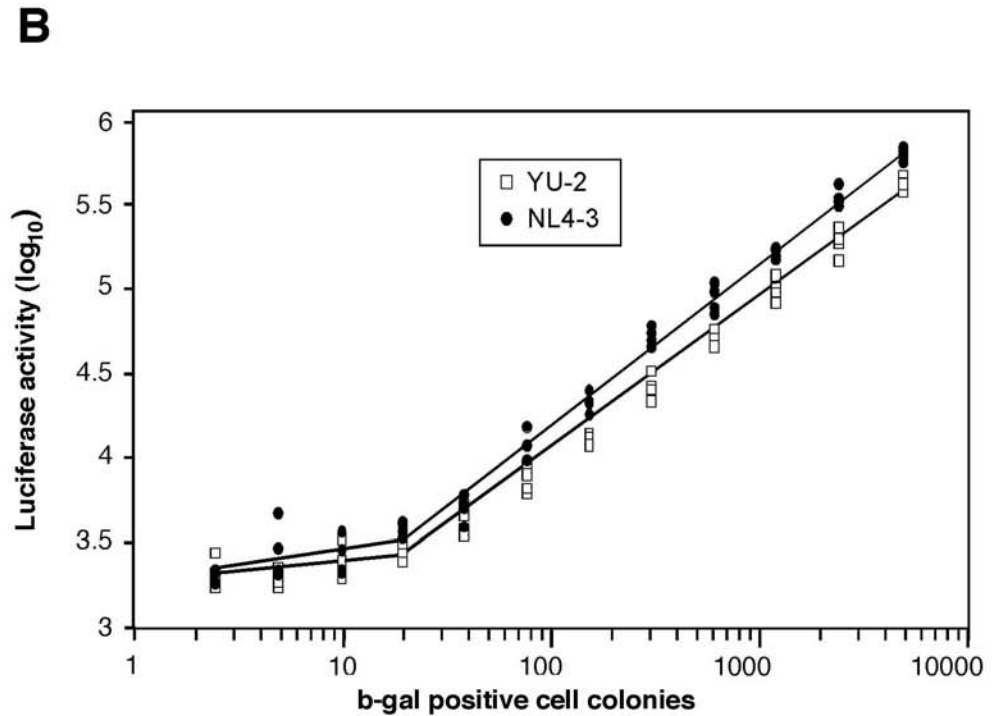
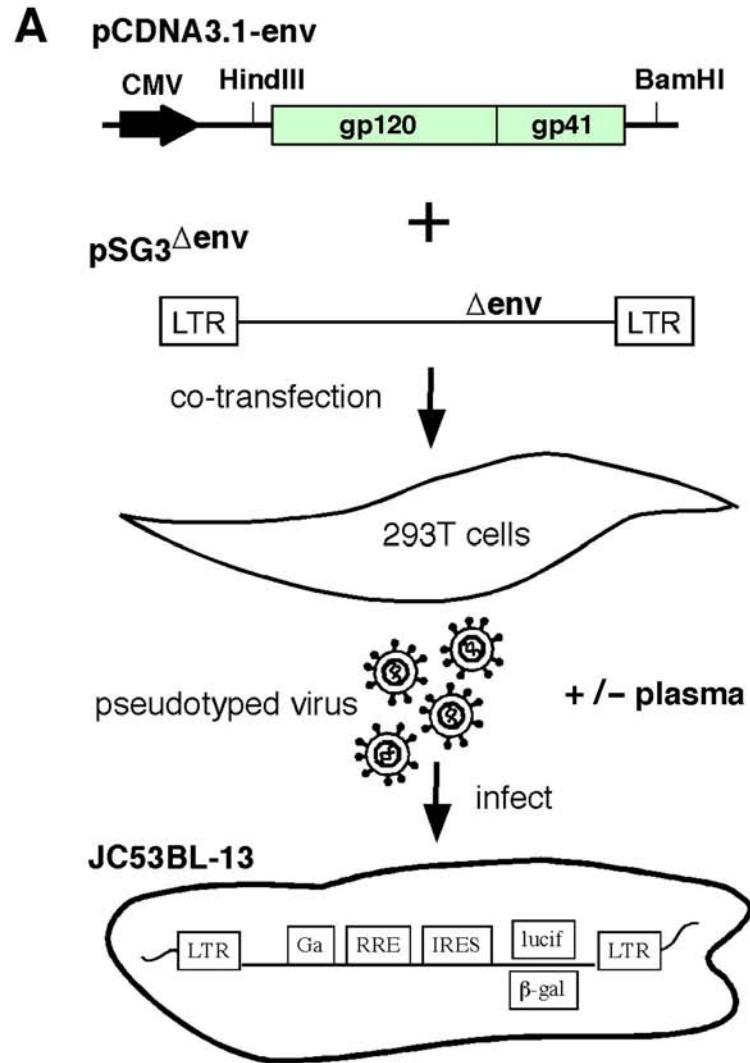
MKVMGTTKKNYQHLWRWIMLLGMLMSSAAEQWVTVYGYVVPVWREANTLFCASDAKAYDTEVHNWVATHACVPTDNPQEVVMGNVTEDFNMWKNMVEQMHEDIISLWDQSLKPCVK
LTPLCVTLHCTNVTISSTNGSTANVTMREEMKNCFNNTTVIDRKIQKEYALFYKLDIVPIEGKNTNTSYRLINCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNKTFNGKGPCRNVSTVQCTHGI
KPVVSTQLLLNGSLAEEIIIIRSENFTNNGKNIVQLKEPVKINCTRPGNNTRRSINIGPGRFYATGAIIGDIRKAHCNISTEQWNNLTQIVDKLREQFGNKTIIFNQSSGGDPEVVMHTFNCGGEFF
YCNSTQLFNSTWFNNGTSTWNSTADNITLPCRIKQVINMWQEVGKAMYAPPPIRGQIDCSSNITGLILTRDGGSNSSQNETFRPGGGNMKDNWRSELYKYKVVKIEPLGIAPTRAKRRVVQREKRA
VTLGAVFLGFLGAAGSTMGAASLTTLVQARLLSGIVQQSNLLRAIEAQQHMLQLTVWGIKQLQARVLAIERYKLDQQLLIWGCSSGKLICTTVPWNSTWSNKSYSYIWNMTWMQWEREID
NYTGFIYTLIEESQKNELELLELDKVASLWVFNITNWLWYIKLFIMIGGLVGLRIVCAVLSIVNRVQGYSPLSFQTRLPNPRGPDRETEEGGERDRDRSARLVNGLAIWDDLRLSL
CLFSYHRLRDLIVARVVEILGRRGWEILKYWWNLLKYWSQELKNSAVSLLNVTIAVAEGTDRVIEIVQRAVRAILHIPTRIRQGFERALL\$

NEF

MGGKWSKSWKIGWPTVRERMRAEPEPAAVGVGAVSRDLERHGAVTSSNTATNNAWSAWLEAQAQEEEDNEVGFPVRPQVPRPMTYKAAVDLSHFLKEKGGLDGLIYSQQRQDILDVWVYN
TQGFPPDWQNYTPGPGTRYPLTFGWYKLVPEVEEVEKANEGENNSLLHPMGLHGMDDPEKEVLMWKFDSRLAFHHMAREKHPEFYKDC\$

What are the biological properties and antigenic characteristics of the transmitted virus?

Entry Assay for HIV-1



Wei, et al., *Nature* 422:307, 2003

Phenotype of Transmitted HIV-1 Envelopes

Transmitted Envs	sCD4 nM	HIVIG ug/ml	17b ug/ml	17b + sCD4 ug/ml	b12 ug/ml	2G12 ug/ml	2F5 ug/ml	4E10 ug/ml	Z13e1 ug/ml	447-52D ug/ml	447+sCD4 ug/ml	F425-B4e8 ug/ml	F425+sCD4 ug/ml	T-20 ug/ml	T-1249 ug/ml	antiCD4mab ug/ml	TAK-779 uM	coreceptor usage	
BORI-d9 4F8	306	461	>10	>10	0.8	>50	>50	9.8	>50	>25	0.2	>10	1.7	0.04	0.02	0.1	0.03	R5	
" " 4D7	343	240	>10	>10	2.1	>50	>50	8.1	>50	>25	16	>10	>10	0.03	0.01	0.04	0.12	R5	
" " 4F12	303	429	>10	>10	4.2	>50	>50	6.4	>50	>25	>25	>10	>10	0.11	0.03	0.1	0.02	R5	
" " 2F8	>1000	346	>10	>10	5.4	>50	>50	18.5	>50	>25	17.2	>10	>10	0.04	0.01	0.08	0.04	R5	
" " 2E12	589	413	>10	>10	0.7	>50	>50	6.5	>50	>25	5.1	>10	10	0.17	0.01	0.08	0.01	R5	
1051-12 C22	339	>1000	>10	>10	1	>50	9.8	31.7	>50	>25	5.9	>10	>10	0.09	0.05	0.06	0.13	R5	
" " TD12	85	890	>10	>10	0.4	>50	5	20	>50	>25	18.8	>10	>10	0.08	0.04	0.09	0.1	R5	
1006-11 C3	241	688	>10	>10	0.8	1.6	5.6	11.5	>50	>25	1.6	>10	7.9	0.16	0.05	0.05	0.06	R5	
1053-07 D6	428	322	>10	>10	>50	4.5	9.1	7.3	>50	>25	16.6	>10	0.8	0.02	0.02	0.11	0.03	R5	
1054-07 TC4	113	449	>10	>10	4.2	>50	>50	4.8	>50	>25	>25	>10	>10	0.07	0.03	0.11	0.05	R5	
1056-10 TA11	635	>1000	>10	>10	1.2	14.3	0.7	3.4	23.4	>25	>25	>10	>10	0.06	0.04	0.06	0.4	R5	
1012-11 TC21	331	>1000	>10	>10	>50	10.9	1.7	5.8	36.2	>25	>25	>10	>10	0.03	0.02	0.09	0.15	R5	
6240-08 TA5	478	>1000	>10	>10	>50	0.3	9.6	15.5	>50	>25	>25	>10	>10	0.09	0.03	0.06	0.14	R5	
6244-13 B5	254	455	>10	>10	>50	26	>50	2.3	15.4	>25	>25	>10	>10	0.35	0.05	0.05	0.09	R5	
62357-14 D3	83	655	>10	>10	47.8	19.3	5	8.7	33.5	>25	>25	>10	>10	0.04	0.02	0.2	0.03	R5	
9021-14 B2	378	618	>10	>10	2.7	45.9	6.4	9.7	31.4	>25	>25	>10	>10	0.75	0.03	0.05	0.04	R5	
9020-20 A13	217	44	>10	>10	30.9	11.6	0.6	1.4	>50	12.7	4.5	3.1	3.3	>5	0.03	0.05	0.13	R5	
SC51 4B2	122	32	>10	>10	0.1	2.7	0.2	0.9	5	>25	>25	>10	>10	0.15	0.01	0.1	0.13	R5	
" 4G3	282	107	>10	>10	1.1	3.5	0.1	0.3	6	>25	>25	>10	>10	0.25	0.01	0.11	0.21	R5	
TT29P 3A1	207	373	>10	>10	0.7	38.8	3.4	4.9	23.6	>25	>25	>10	>10	0.24	0.02	0.06	0.12	R5	
TT31P 2G1	288	110	>10	>10	0.1	3.7	0.8	4.1	13.3	>25	>25	>10	>10	1.71	0.03	0.04	0.14	R5	
" 2F10	246	311	>10	>10	0.1	4.7	2.5	6	29	>25	>25	>10	10	0.55	0.02	0.08	0.13	R5	
WEAU-d15 4-10	72	507	>10	>10	1.2	0.3	0.8	1.1	>50	>25	>25	>10	>10	0.01	0.01	0.09	4.14	R5/X4	
9014-01 TB1	>1000	>1000	>10	>10	7.6	>50	2.2	11	>50	>25	17.3	>10	10	0.08	0.02	0.06	0.54	R5	
9015-07 A1	>1000	413	>10	>10	3.5	4	2.6	4.9	>50	>25	>25	>10	>10	0.03	0.03	0.08	0.19	R5	
62615-03 P4	354	>1000	>10	>10	10.5	>50	>50	25	>50	>25	>25	>10	>10	0.02	0.03	0.14	0.11	R5	
63068-05 A1	>1000	565	>10	>10	18.2	5	7.9	15	>50	>25	2.6	>10	4.3	0.05	0.06	0.07	0.14	R5	
700010040 C9	97	441	>10	>10	0.7	>50	5.1	9	>50	>25	>25	>10	>10	0.04	0.03	0.17	0.03	R5	
PRB931-06 TC3	1000	440	>10	>10	22.7	1.4	7.2	20.9	>50	>25	14.5	>10	7.6	0.2	0.04	0.12	0.16	R5	
PRB956-04 B20	152	426	>10	>10	1.7	2.5	1.8	5.1	>50	>25	4.9	>10	4	0.11	0.04	0.05	0.6	R5	
SC22 3C2	514	453	>10	>10	5.3	2.1	>50	5.8	>50	>25	>25	>10	>10	0.32	0.02	0.06	0.18	R5	
SC33 4H1	61	339	>10	>10	0.6	2.9	1.8	3.2	50	25	8.2	4.3	9.3	1.03	0.11	0.11	0.53	R5	
SC33 4A4	928	461	>10	>10	0.651	>50	2.6	2.9	30.8	>25	>25	>10	>10	0.15	0.03	0.05	0.12	R5	
SC05 8C11	267	323	>10	>10	1.9	19.1	1.4	1.7	28	>25	>25	>10	>10	0.64	0.02	0.11	0.09	R5	
SC45 4B5	268	209	>10	>10	0.7	7.4	0.8	3.2	50	>25	5.1	9.6	6.5	0.03	0.01	0.09	0.28	R5	
1058-11 B11	298	642	>10	>10	3	>50	2.4	2.4	34	>25	>25	>10	>10	1.1	0.01	0.13	0.02	R5	
1059-09 A4	201	55	>10	>10	1	11.4	0.3	.6	22	>25	15.9	>10	6.5	0.02	0.01	0.13	0.09	R5	
TT27P 8H1	1000	393	>10	>10	1.6	>50	0.7	1.5	>50	>25	>25	>10	>10	0.42	0.01	0.08	0.22	R5	
" 8C2	188	496	>10	>10	0.643	>50	1.3	1.1	>50	>25	>25	>10	>10	0.04	0.01	0.08	0.24	R5	
TT34P 8C11	129	227	>10	>10	>50	8.5	2.1	2.5	20	>25	>25	>10	>10	0.45	0.02	0.15	0.05	R5	
TT35P 11H8	643	228	>10	>10	>50	>50	1.8	3.5	30	>25	3	>10	>10	0.33	0.01	0.11	0.34	R5	
63358-04 P3	538	1000	>10	>10	>50	>50	3.1	4.0	>50	>25	>25	>10	>10	0.04	0.04	0.05	0.03	R5	
Control Envs																			
YU-2	10	508	>10	>10	3.5	>50	24	25	>50	3.9	0.2	>10	0.7	0.27	0.09	0.28	0.029	R5	
NL4.3	2.4	99	0.6	1.2	0.02	1.1	0.4	0.7	35	0.02	0.02	6.4	5.4	0.91	0.01	1.9	>10	X4	
SC03C 8A9	818	393	>10	>10	3.3	1.7	1.2	1.9	17.6	>25	>25	>10	>10	0.05	0.01	0.07	1.92	R5	
SC02C 3D9	442	654	>10	>10	3.5	1.7	1.3	2.5	17.9	>25	>25	>10	>10	0.13	0.04	0.06	0.03	R5	
MLV	>1000	>1000	>10	>10	>50	>50	>50	>50	>50	>25	>25	>10	>10	>5	>5	>10	>10	-/-	

Total subjects = 34
Total Envs = 42

Results: Consensus *env* genes from 34 subjects were cloned, expressed, and tested for entry into JC53 bl-13 cells. All Env proteins were functional and utilized CCR5 for entry. Transmitted Envs exhibited neutralization profiles typical of primary virus isolates. Notably, transmitted Envs were generally insensitive to CD4i and V3 mAbs but sensitive to CD4bs and MPER mAbs.

Conclusions

- We have developed a strategy for identifying HIV-1 *envs* and full-length genomes corresponding to virus(es) at or near the moment of transmission.
- This strategy allows for a detailed analysis of viral properties necessary and sufficient for productive clinical infection and a molecular analysis of early virus diversification and evolution.
- Phenotypic analysis of 42 transmitted/early *envs* from 34 patients indicated that:
 - All *Envs* were functional.
 - All *Envs* utilized CCR5; 1 utilized CCR5/CXCR4.
 - All *Envs* exhibited neut profiles typical of primary viruses, including resistance to CD4i and V3 mAbs and susceptibility to CD4bs, 2G12, and/or MPER mAbs.

Acknowledgements

UAB

Beatrice Hahn

George Shaw

Jesus Salazar

Kimmy Pham

Richard Sun

Maria Salazar

Xiping Wei

Shuyi Wang

Yalu Chen

Truman Grayson

Julie Decker

David McPherson

Duke

Feng Gao

Chunlai Jiang

Jennifer Kirchherr

Jennifer Hamilton

Amit Kumar

Sheri Chen

Barton Haynes

UNC

Ron Swanstrom

Li-Hua Ping

Jeffrey Anderson

Nobutoki Takamuni

Yuyang Tang

LANL

Bette Korber

Tanmoy Bhattacharya

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Marcus Daniels

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