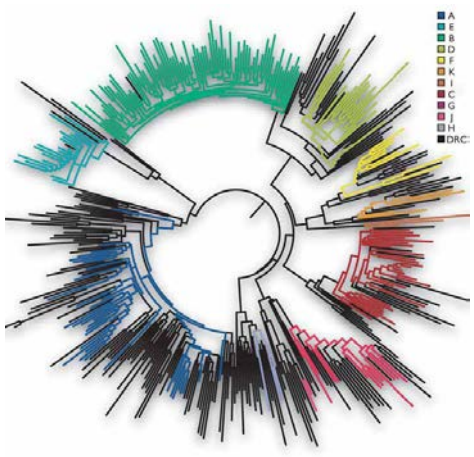
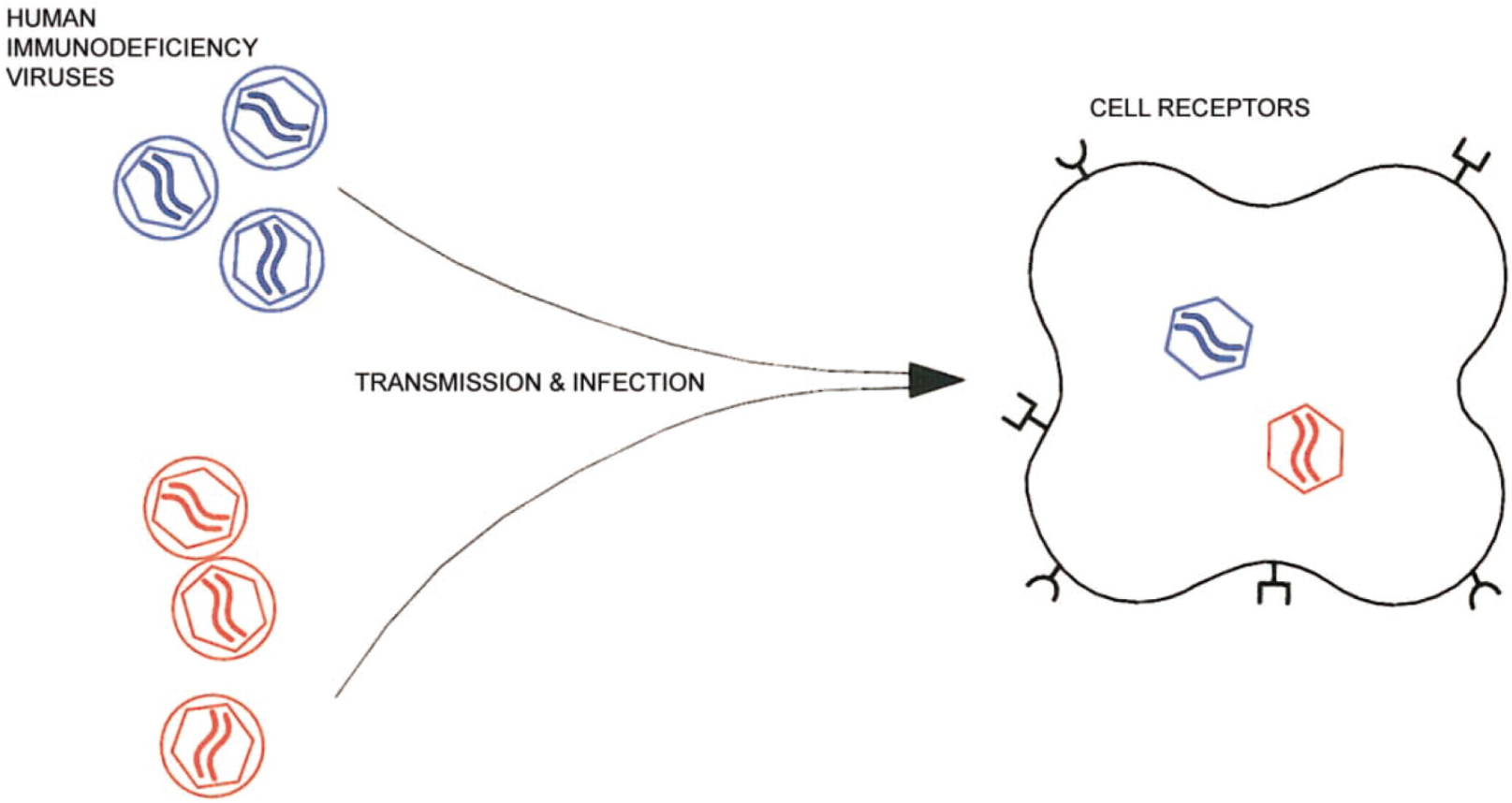


Detecting HIV-1super-infection in recently infected IDUs

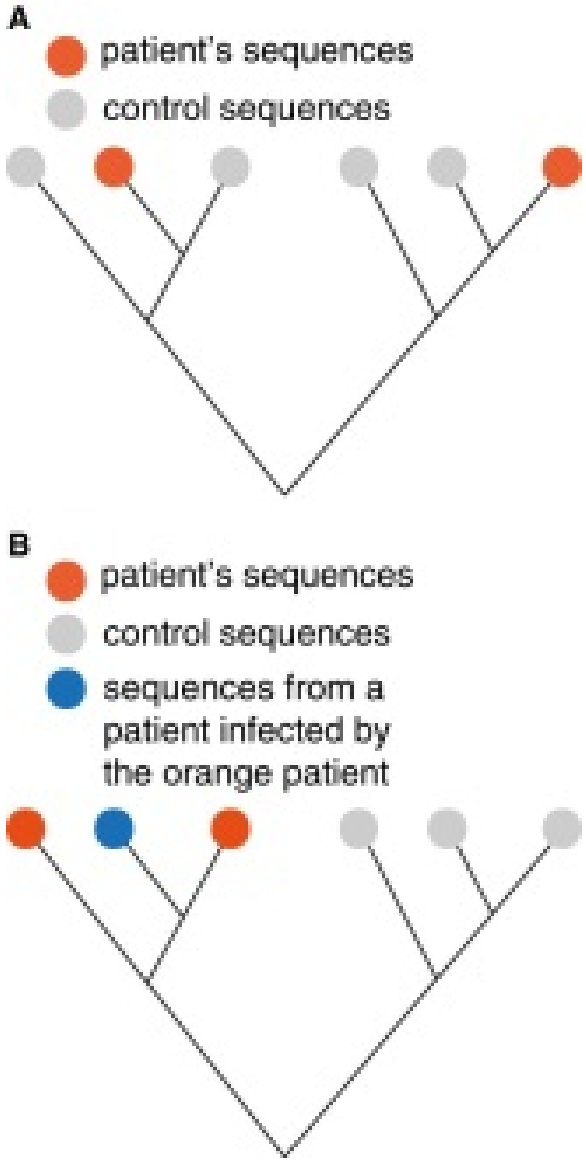
Simona Paraschiv, Bogdan Popescu, Leontina Banica, Eugen Radu and Dan Otelea

Bucharest, Romania

Co-infection vs super-infection



Jason T. Blackard et al. Clin Infect Dis. 2002;34:1108-1114



Dual-infection

Transmission cluster

Bartha I et al, BMC Infect Dis. 2013;13:537

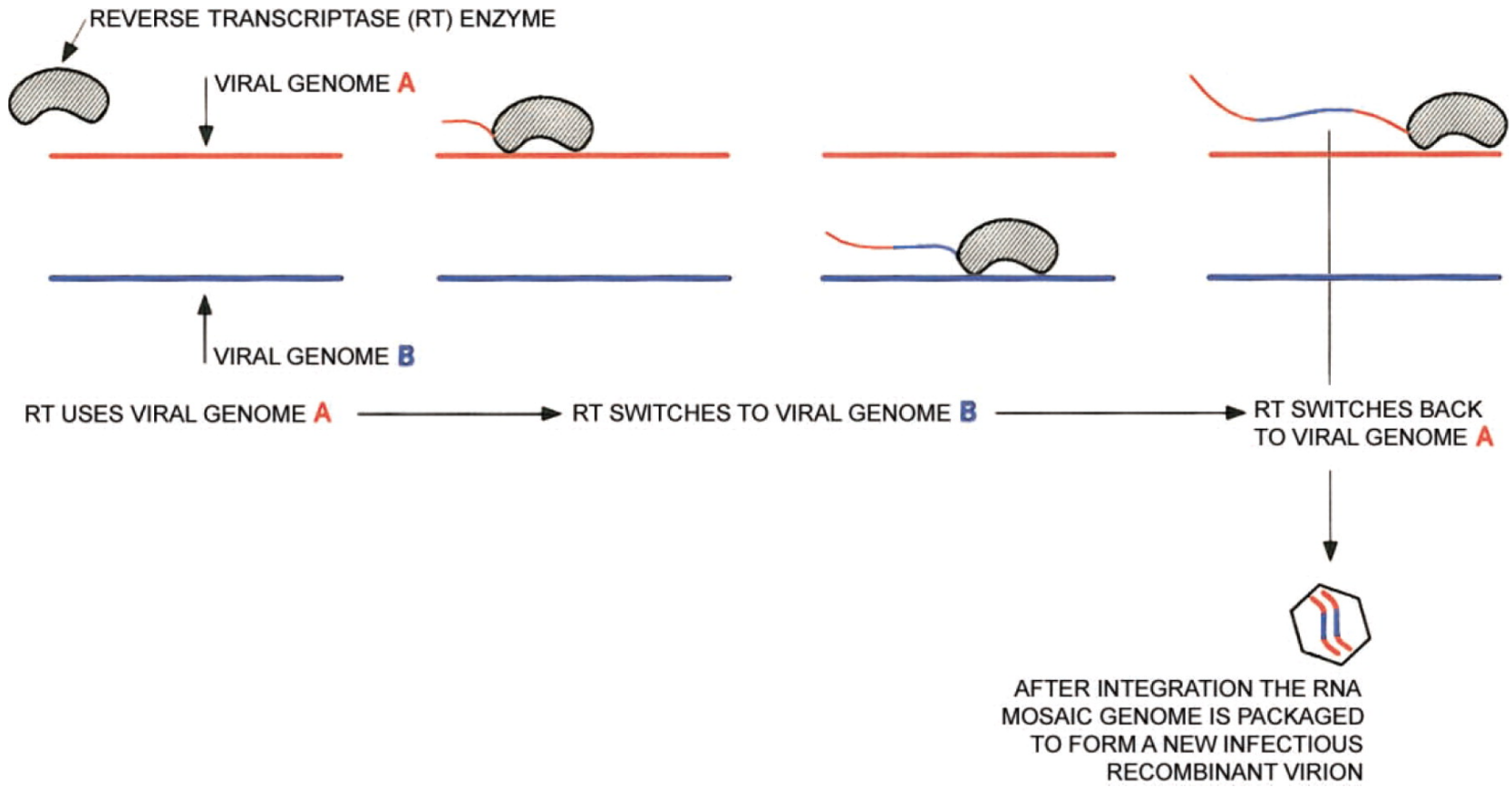
HIV super(co)-infections

- can involve different subtype viruses or same subtype viruses, from different sources
- Prevalence: 2%*- 39% ** (different sampling strategies, risk group populations)
- Transient super-infections are lost by infrequent sampling
- NGS and SGS are sensitive in detecting super-infection
- The appearance of the second virus is usually associated with CD4 count ↓and VL ↑
- Super-infection is the first step for recombination

* Yerly S et al., AIDS 2004, 18:1413–1421

**Templenton AR et al, Retrovirology 2009; 6:54

HIV recombination



Jason T. Blackard et al. Clin Infect Dis. 2002;34:1108-1114

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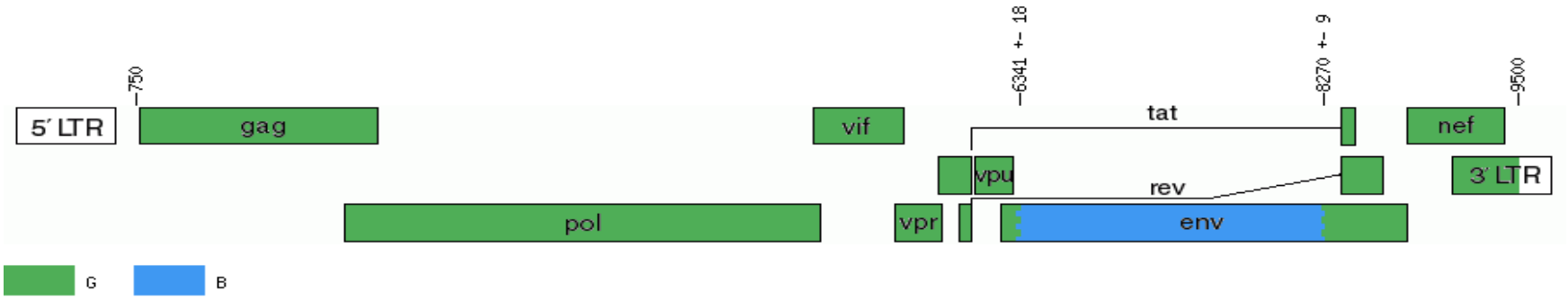
Recent HIV-1 Outbreak Among Intravenous Drug Users in Romania: Evidence for Cocirculation of CRF14_BG and Subtype F1 Strains

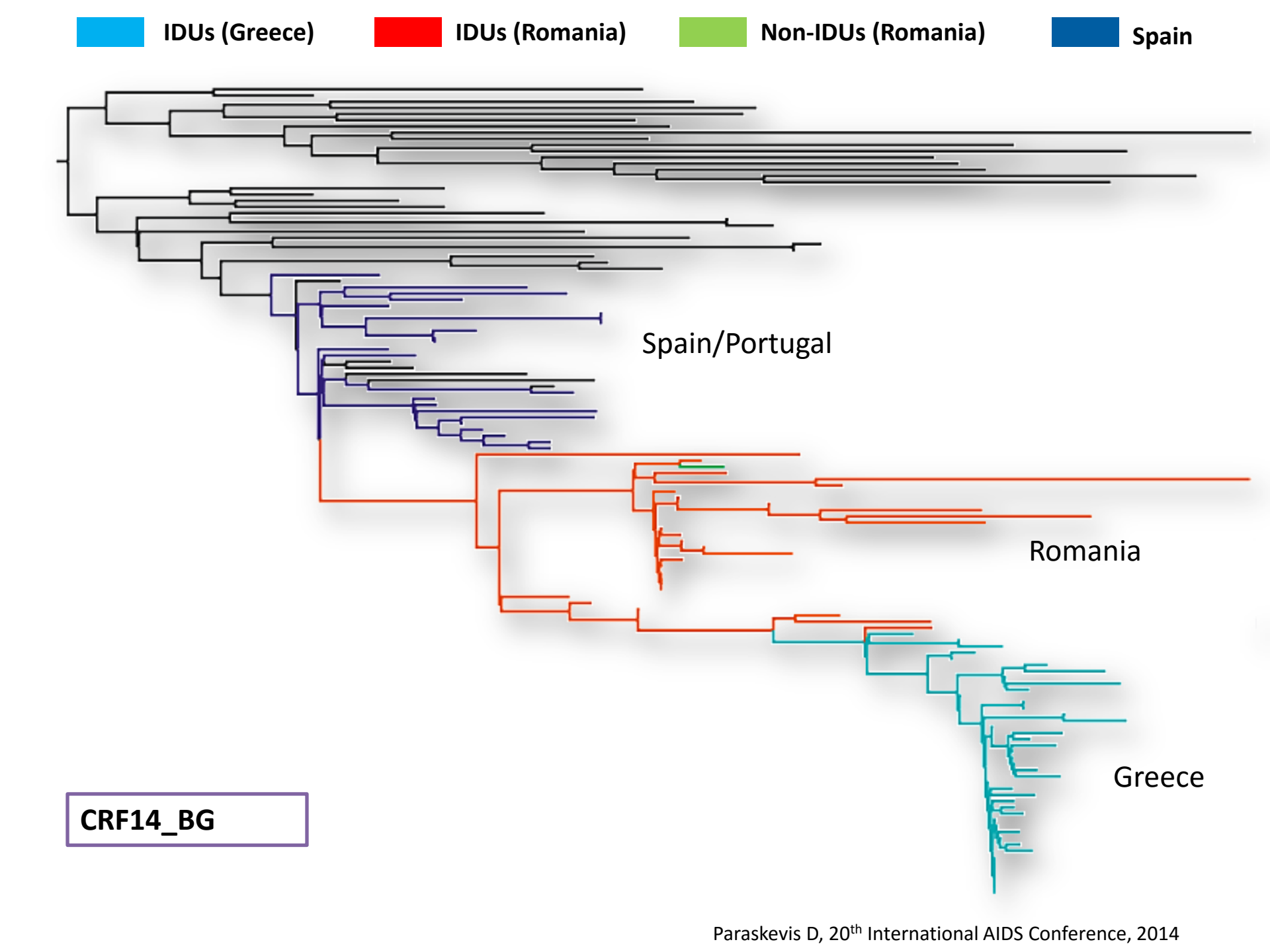
Iulia Niculescu,[1,*] Simona Paraschiv,[1,*] Dimitrios Paraskevis,[2] Adrian Abagiu,[1] Ionelia Batan,[1] Leontina Banica,[1] and Dan Otelea[1]

Characteristic	IDUs	Sexual contact	p-value	OR (95% CI)
No of patients	N= 138	N=58		
Age (years)				
Median [range]	29 [16-69]	34 [20-64]	0.014 (Mann-Whitney)	
IQR	7	14		
Gender- Male	113 (81.9%)	37 (63.8%)	0.009	2.5 (1.2-5.1)
Clinical stage				
A	102 (73.9%)	17 (29.3%)	<0.001	6.8 (3.4-13)
B	26 (18.8%)	28 (48.3%)	<0.001	0.2 (0.1-0.4)
C	10 (7.2%)	13 (22.4%)	0.006	0.2 (0.1-0.6)
CD4 count (cells/mm^3)				
<200	22 (15.9%)	18 (31%)	0.002	0.4 (0.2-0.8)
200-500	62 (44.9%)	29 (50%)	0.5	
>500	54 (39.1%)	11 (19%)	0.008	2.7 (1.3-5.7)
In prison				
Yes	47 (34.1%)	1 (1.7%)	<0.001	29 (3.9-219)
No	91 (65.9%)	57 (98.3%)		

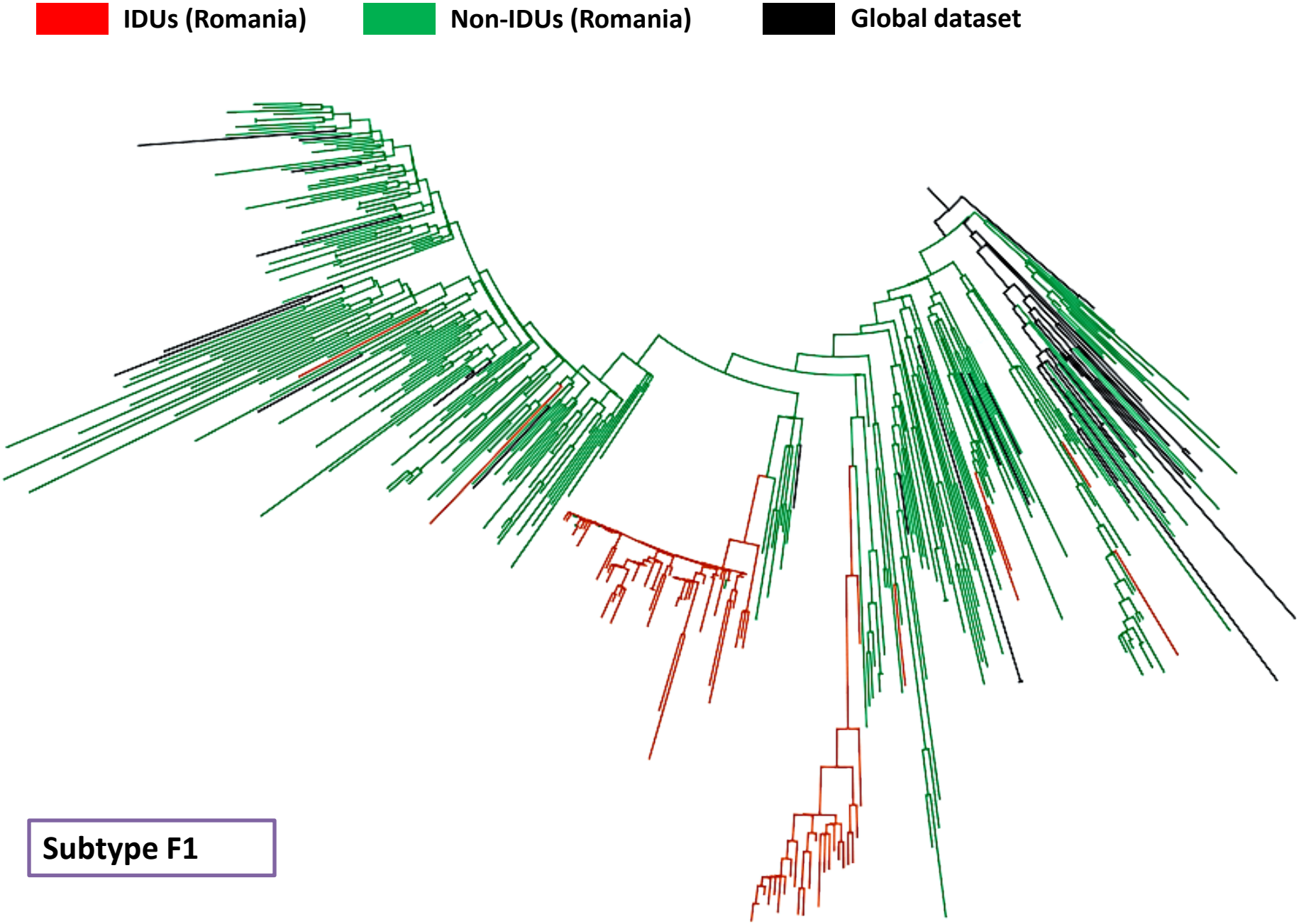
HCV co-infection	136 (98.6%)	6 (10.3%)	<0.001	589 (115-3013)
HBV co- infection	19 (13.8%)	11 (19%)	0.3	
VDRL	7 (5.1%)	3 (5.2%)	1	
TPHA	12 (8.7%)	14 (24.1%)	0.001	0.3 (0.1-0.7)
Opportunistic infections	10 (7.2%)	13 (22.4%)	0.006	0.2 (0.1-0.6)
Travels abroad	56 (40.6%)	24 (41.4%)	1	
Greece	15 (10.9%)	3 (5.2%)	0.1	
Spain	36 (26.1%)	3 (5.2%)	<0.001	7.7 (2.2-26.3)
HIV subtype				
F1	94 (68.1%)	51 (87.9%)	0.004	0.2 (0.1-0.6)
CRF14_BG	28 (20.3%)	1 (1.7%)	<0.001	14 (2-109)
B	8 (5.8%)	6 (10.3%)	0.3	
CRF02_AG	1 (0.7%)	0 (0.0%)		
CRF14BG_F1 recombinant	6 (4.3%)	0 (0.0%)		
B_F1 recombinant	1 (0.7%)	0 (0.0%)		



Delgado E et al, J Acquir Immune Defic Syndr. 2002



Paraskevis D, 20th International AIDS Conference, 2014



Paraskevis D, 20th International AIDS Conference, 2014

Objectives

- Intra host evolution of HIV, detect super-infection and possible recombinants

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- Target region: env (V2-V3), selective pressure – immune system

- Viral tropism prediction with NGS and specific algorithms; clinical implication

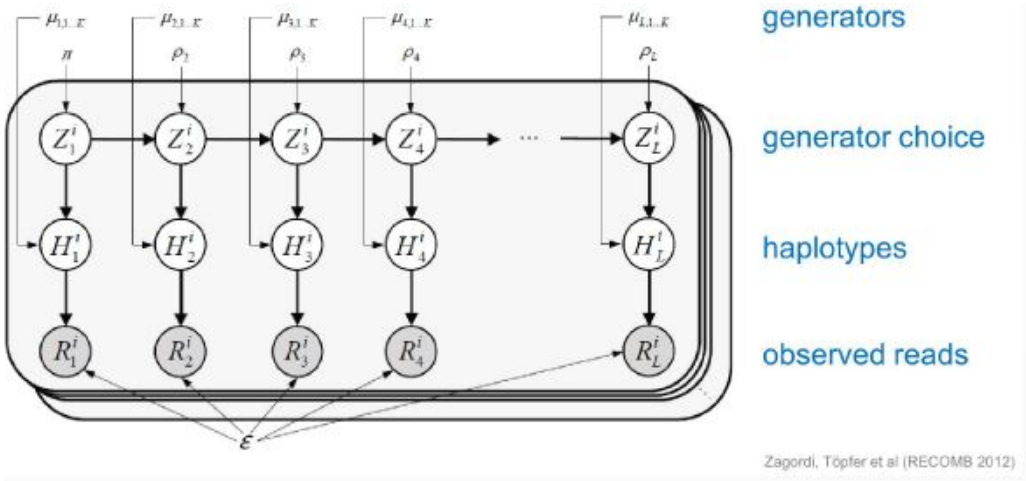
Methods

- 19 IDUs newly diagnosed with HIV-1 infection
- 20 heterosexually infected patients
- GSJunior 454 instrument
- The read mapping, error correction and viral variant reconstruction were performed in 2 different ways
- Phylogenetic analysis: ML trees (FastTree)
- Simplot, Hypermut
- Geno2pheno coreceptor algorithm

Pipeline #1: Probablistic method

- Error correction: InDelFixer
- Viral variant reconstruction: QuasiRecomb

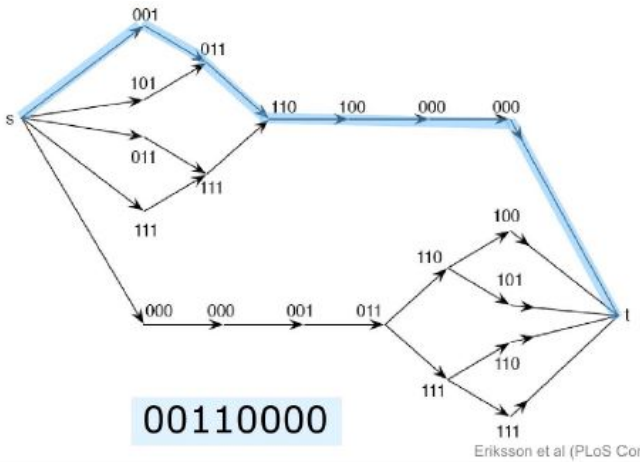
Jumping Hidden Markov Model

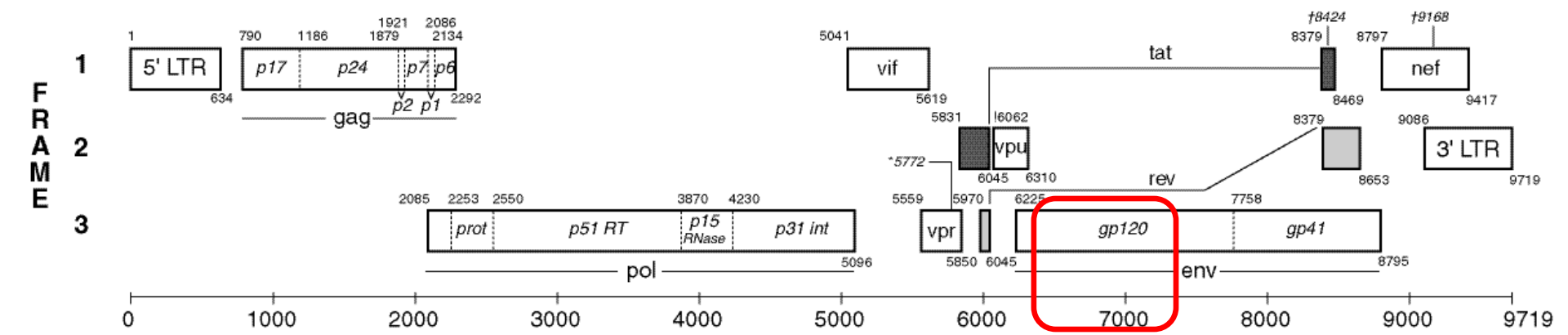


Pipeline #2: Combinatorial method

- Error correction:ReadClean454
- Haplotype reconstruction: QuRe v.0.99971

Metoda ReadGraph




FRAME
1
2
3
5' LTR
1
634
p17
790
1186
p24
1879
1921
p7
2086
p6
2134
p2
p1
2292
gag
vif
5041
5619
tat
†8424
8379
8469
nef
8797
†9168
9417
vpu
5831
6062
6045
6310
*5772
rev
8379
8653
9086
3' LTR
9719
prot
2085
2253
p51 RT
2550
p15
RNase
3870
p31 int
4230
5096
pol
vpr
5559
5970
5850
6045
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8795
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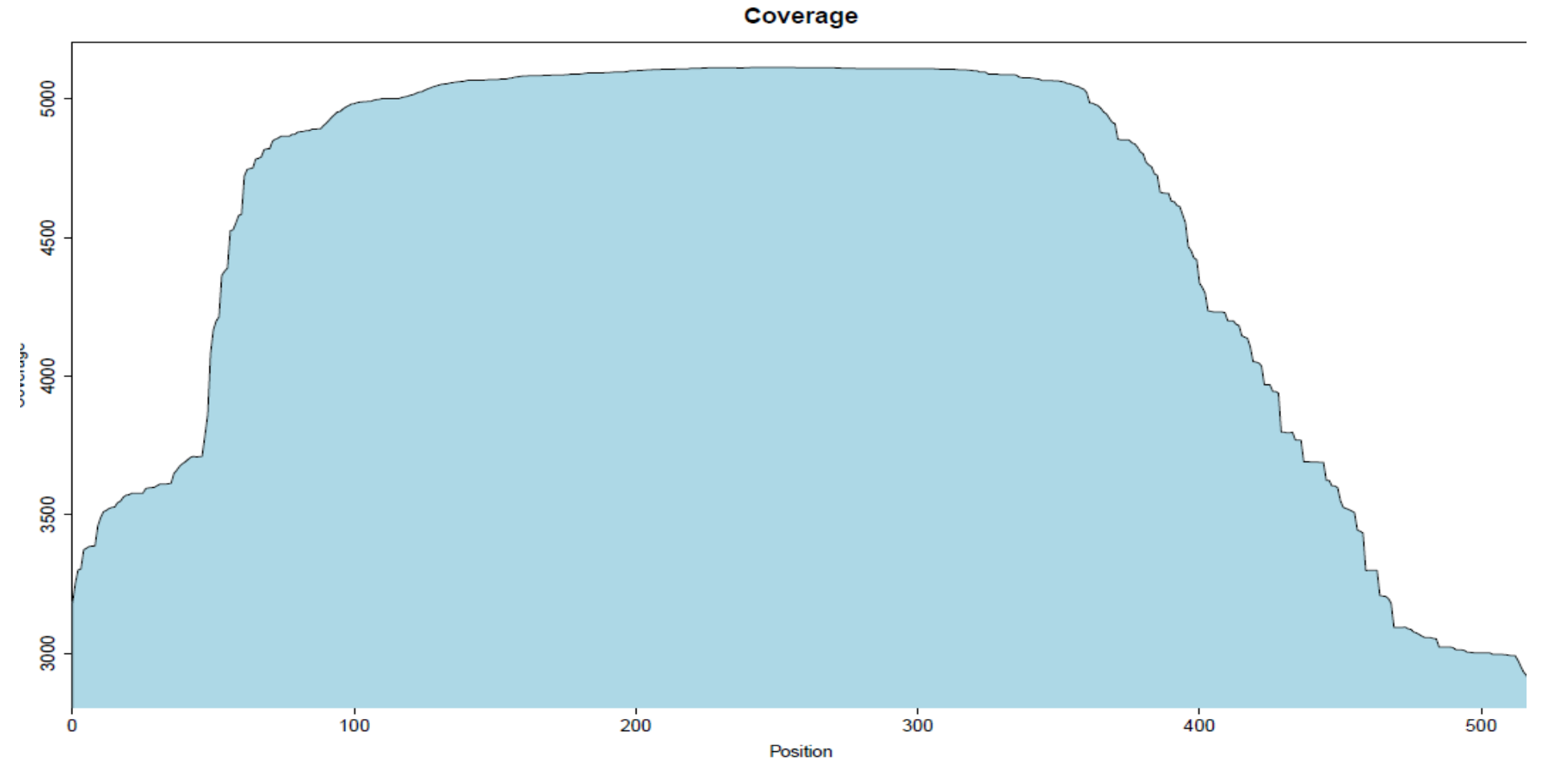
3000
4000
5000
6000
7000
8000
9000
9719


Coverage
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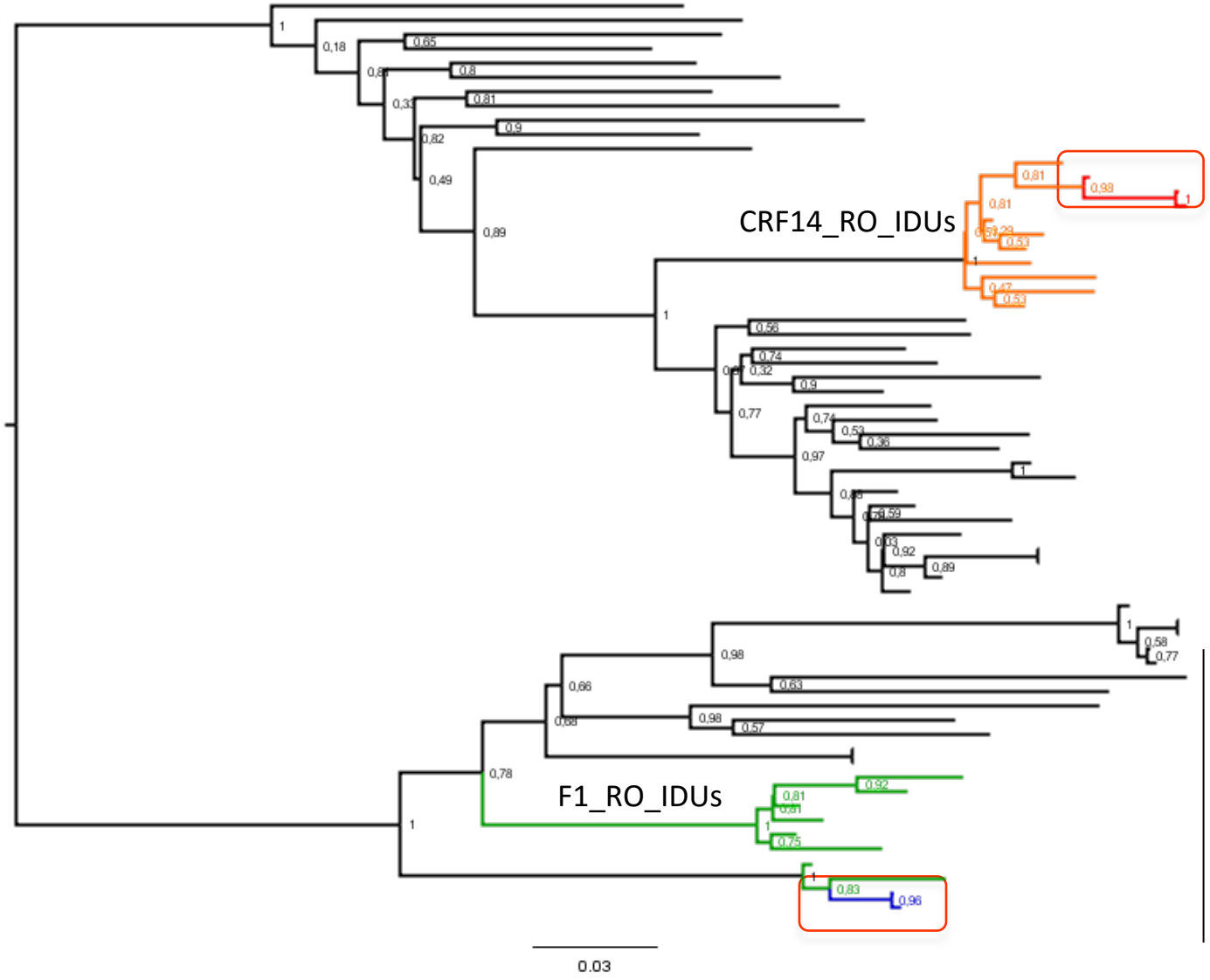
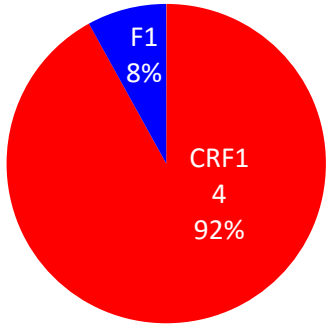
4500
4000
3500
3000
0
100
200
300
400
500
Position

Case #1 169bh2014

quasirecomb



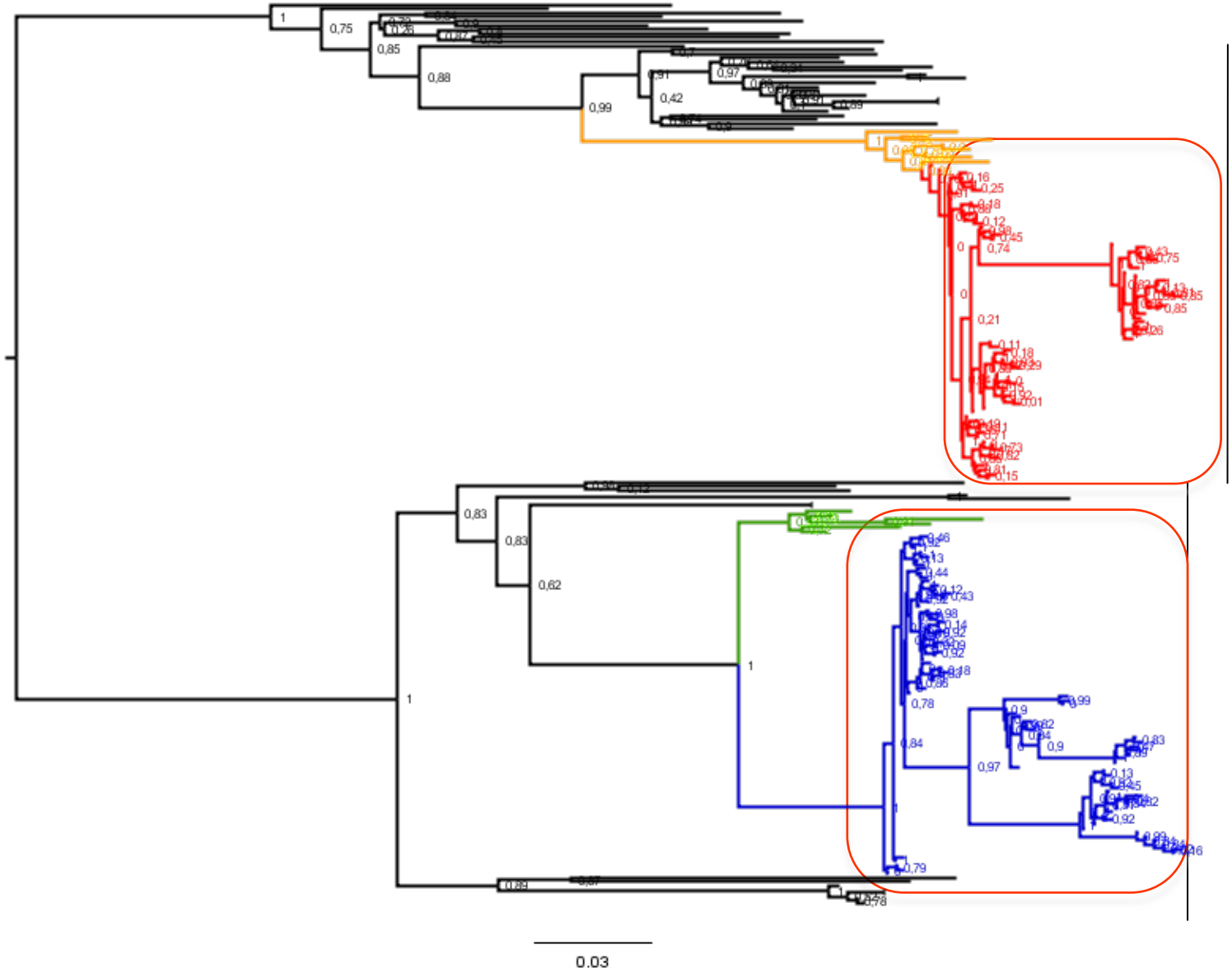
CRF14_BG cluster



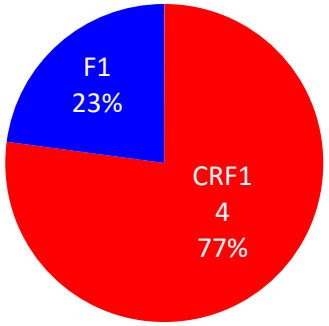
F1 cluster

Case #2 581bh2014

quasirecomb



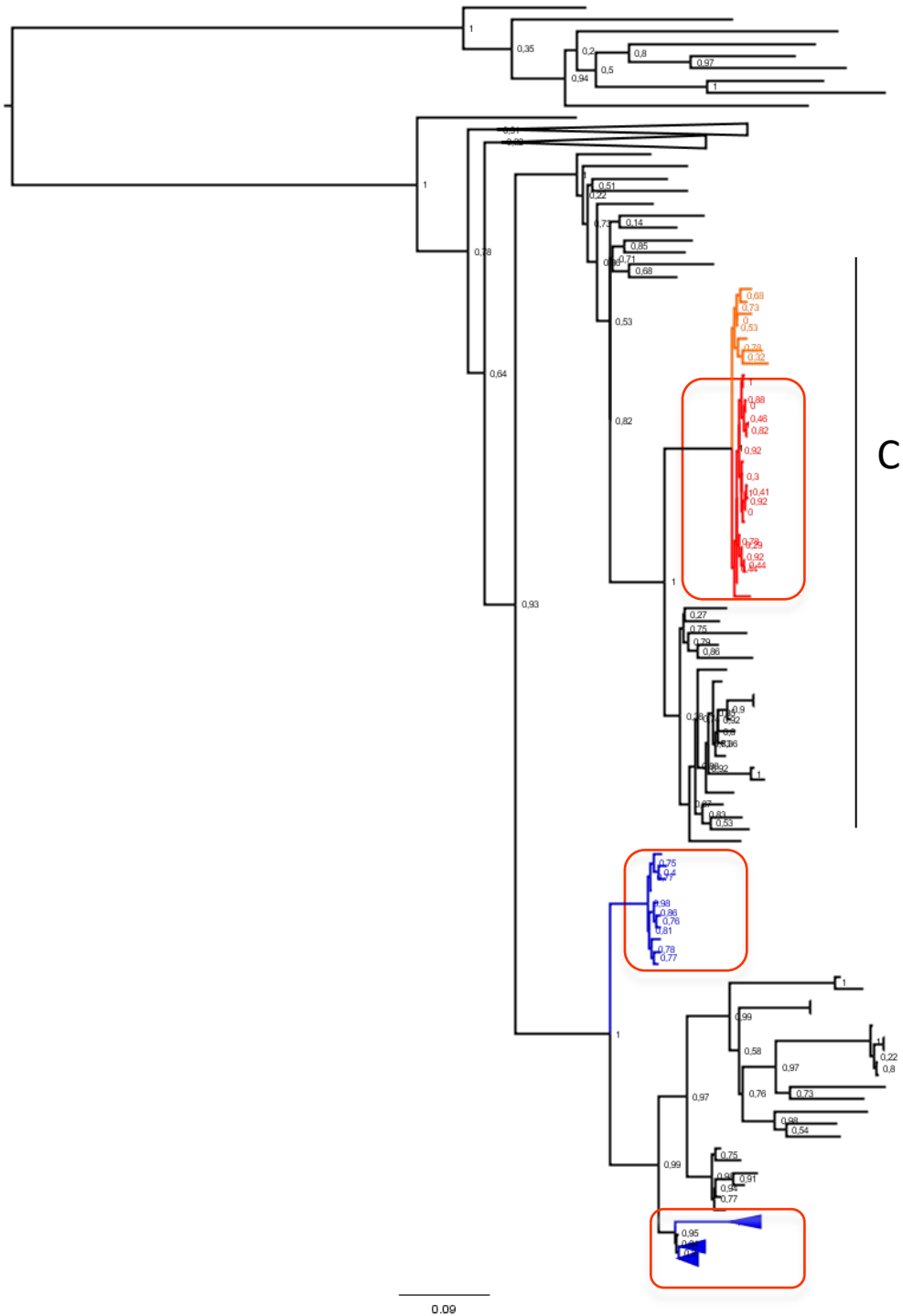
CRF14_BG cluster



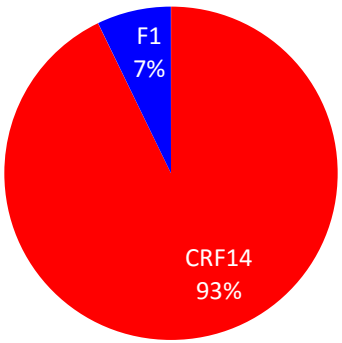
F1 subtype cluster

Case #3 3629bh2013

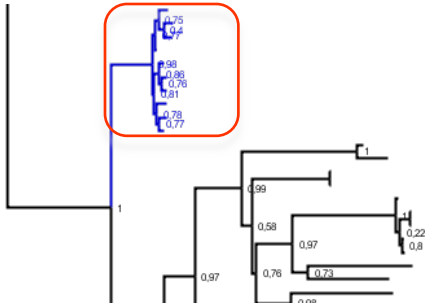
quasirecomb



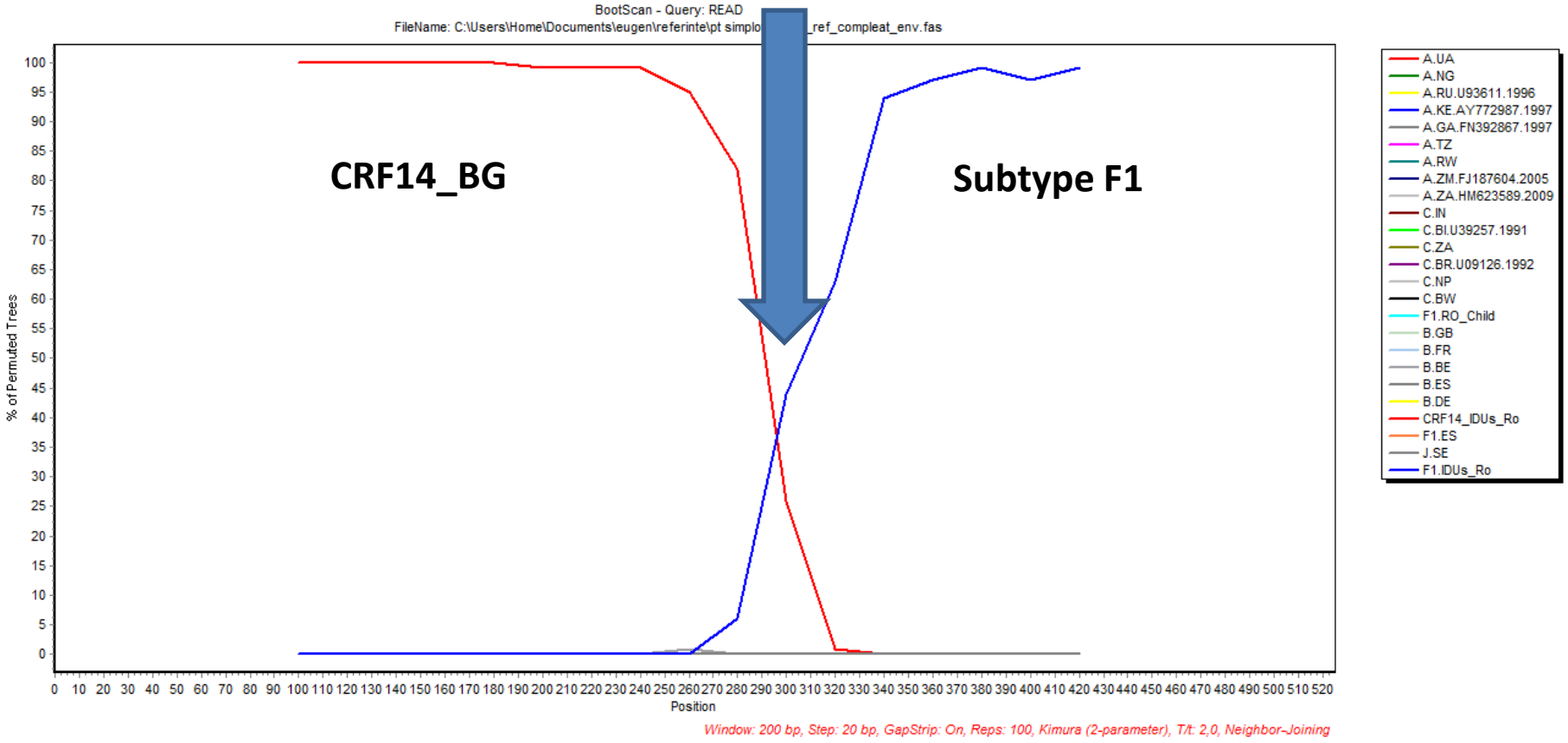
CRF14_BG cluster



F1 subtype cluster

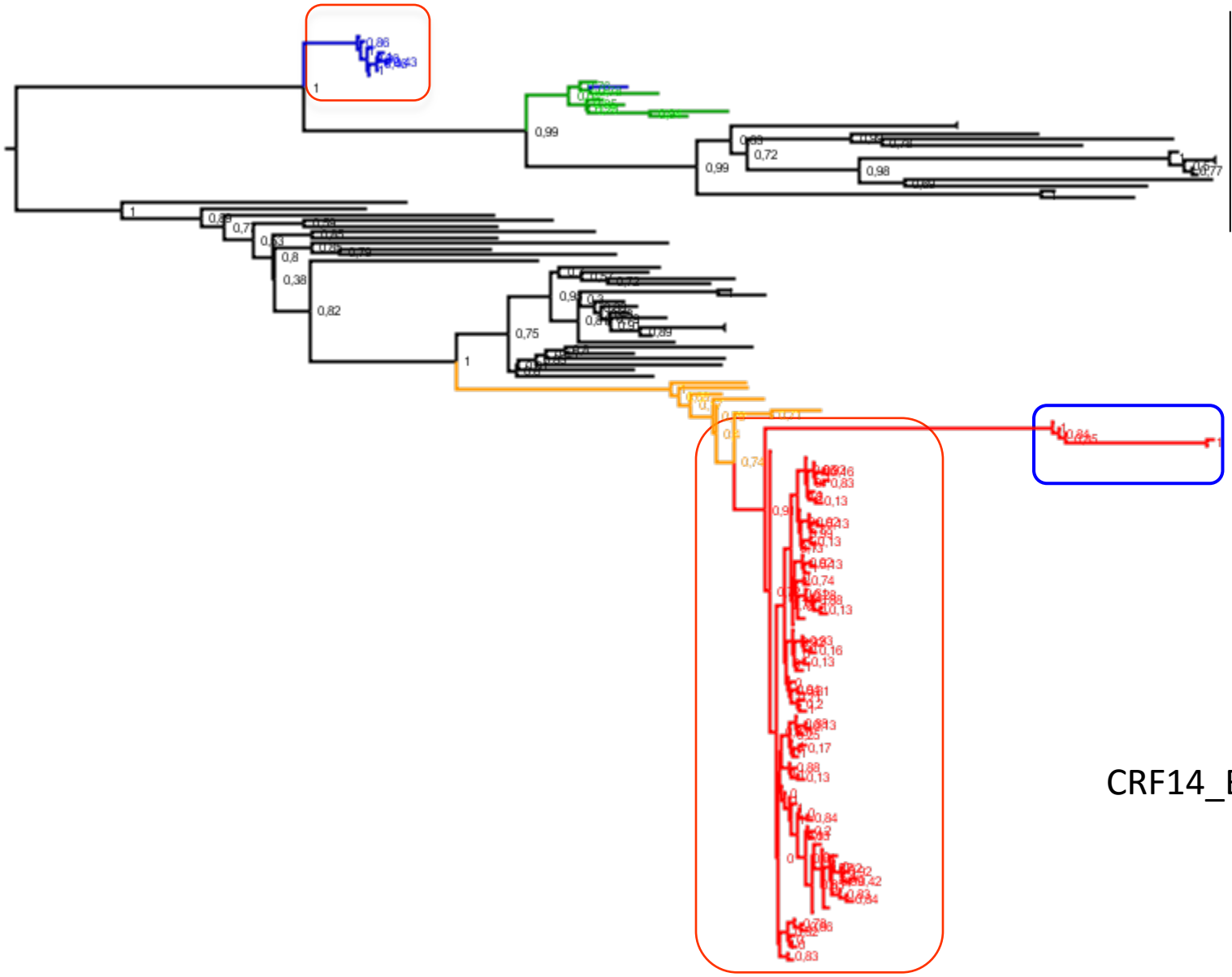


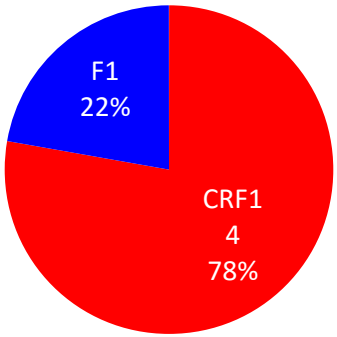
Recombination breakpoint



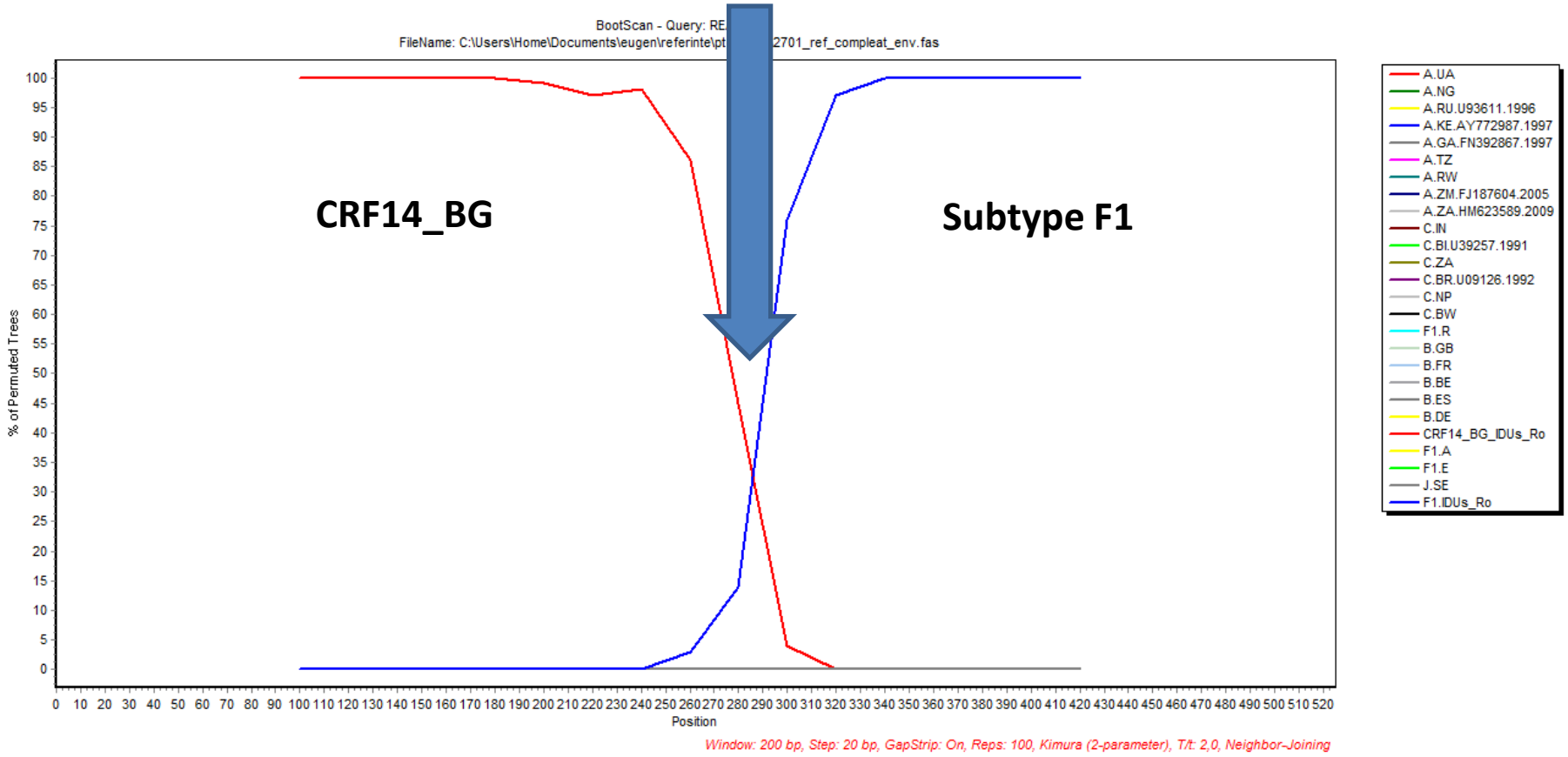
Case #4 2701bh2013

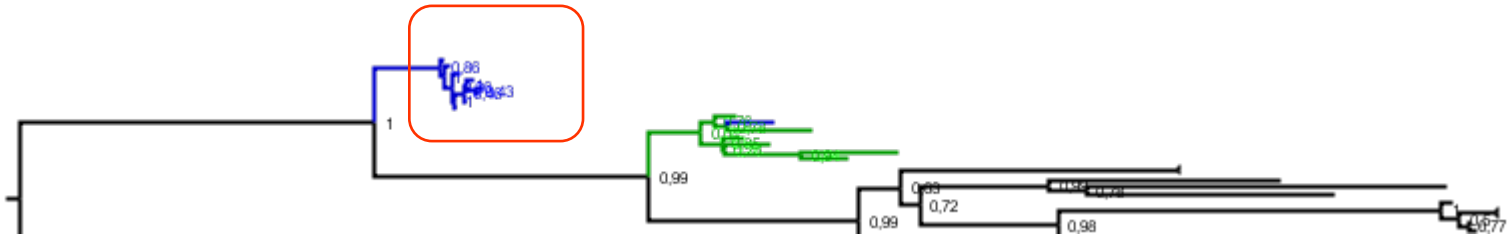
quasirecomb



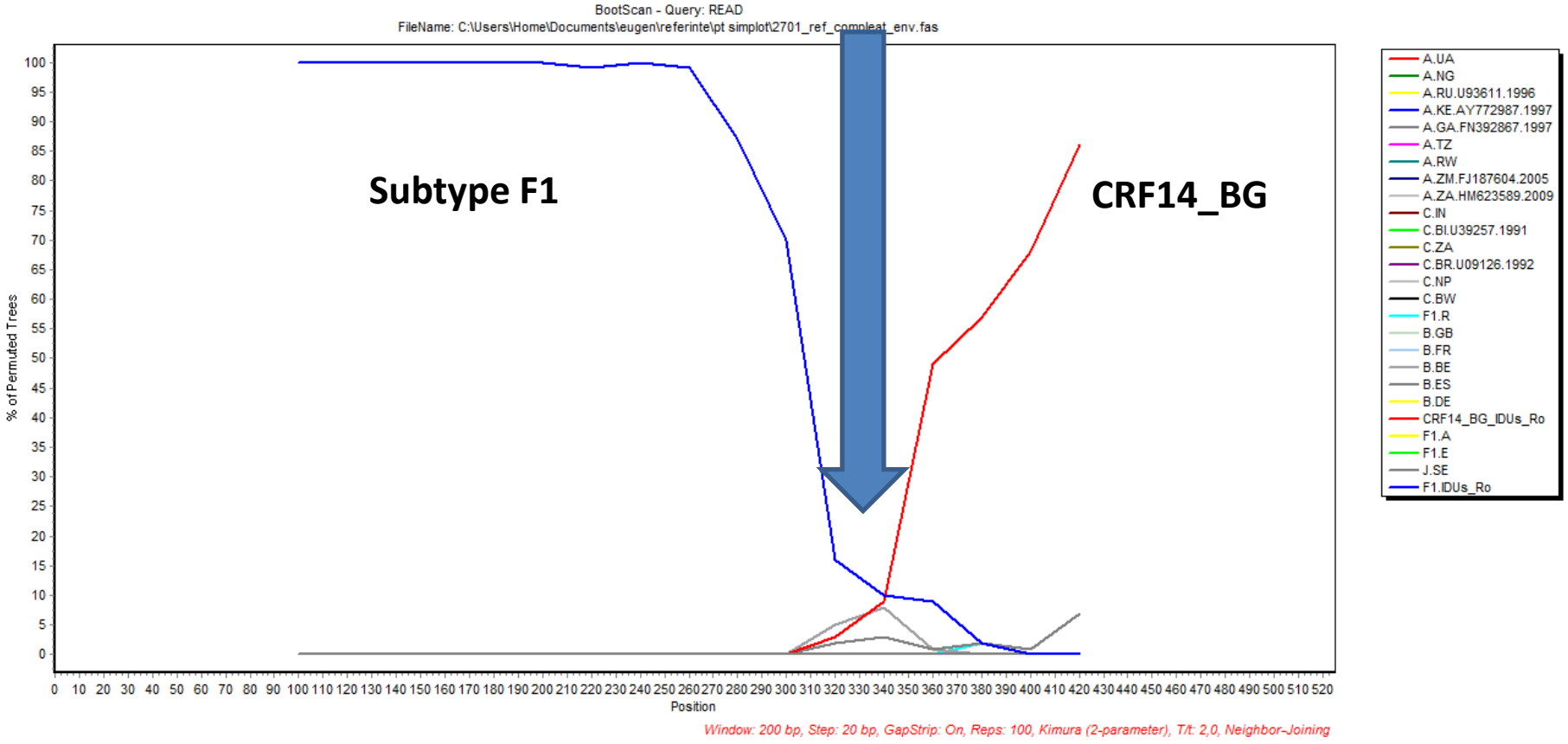


Recombination breakpoint





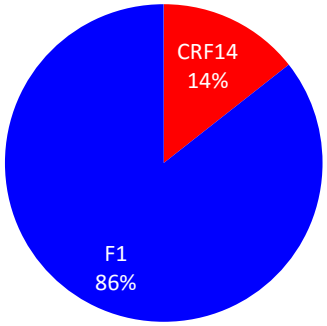
Recombination breakpoint



Case #5 3470bh2013

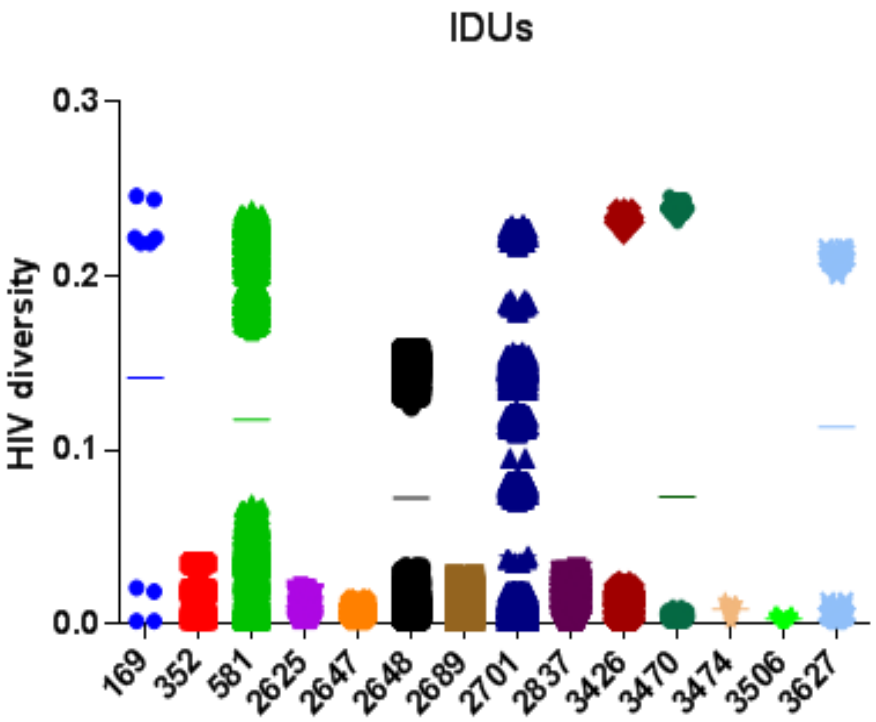
quasirecomb

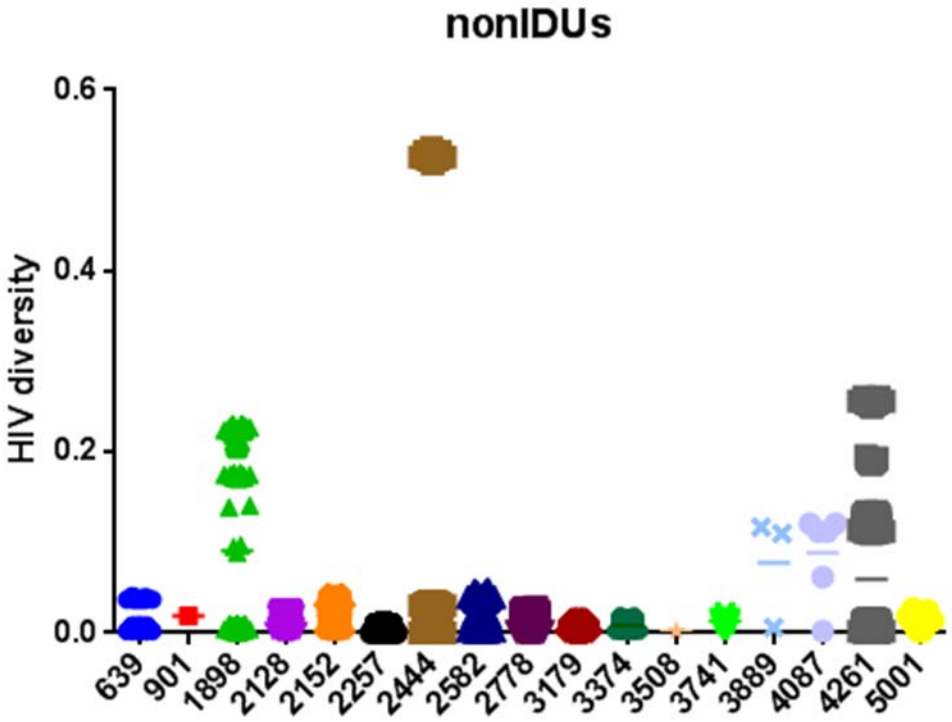
F1 cluster



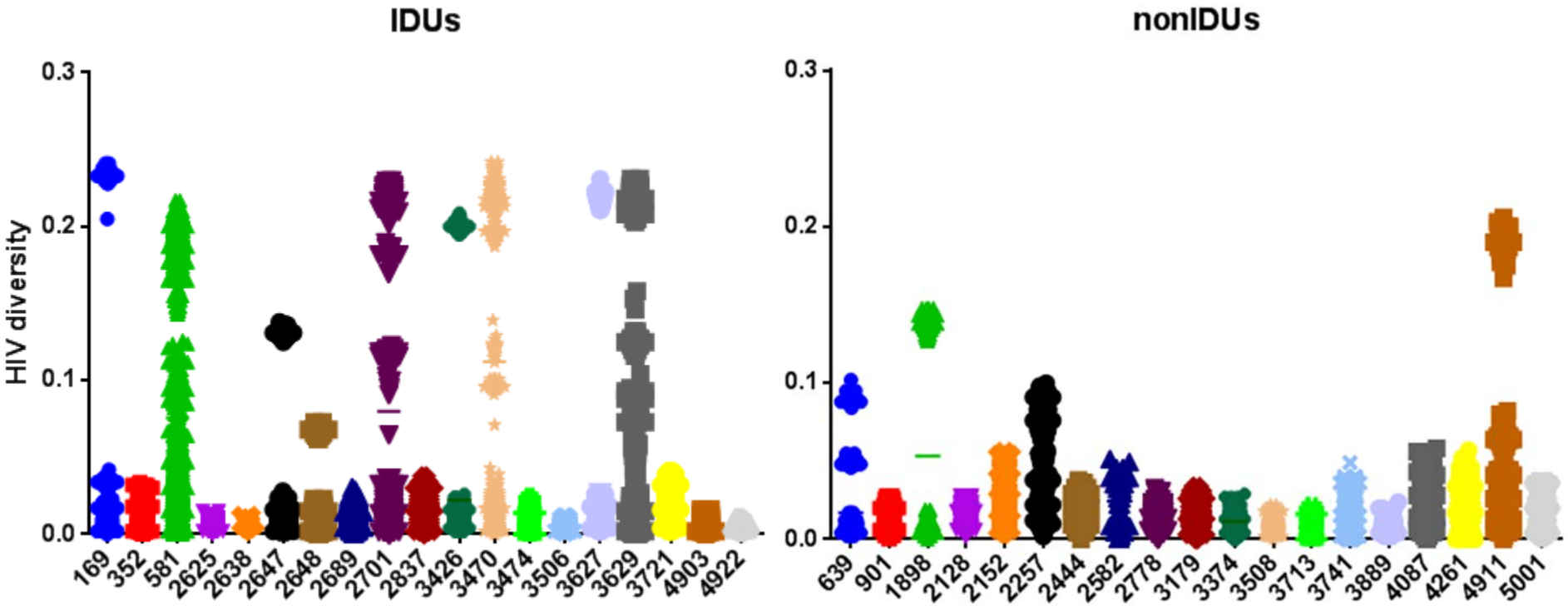
CRF14_BG cluster

Intrahost diversity
Pipeline #1: InDelFixer + Quasirecomb

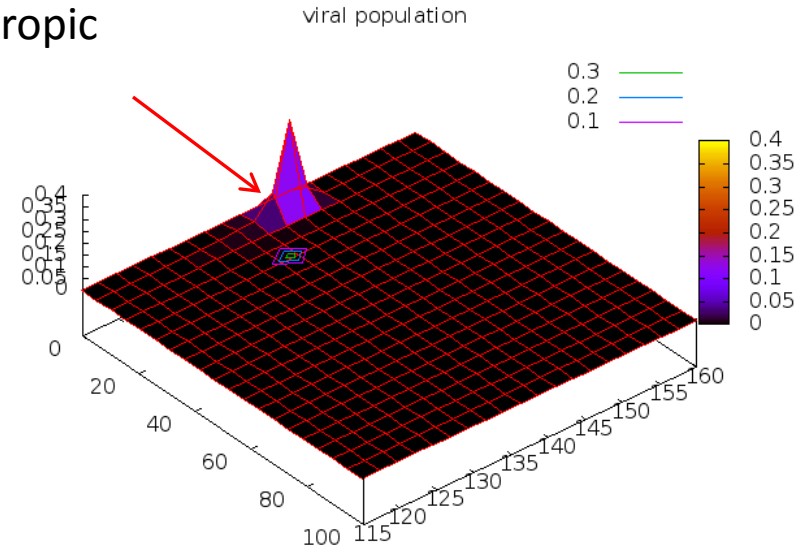




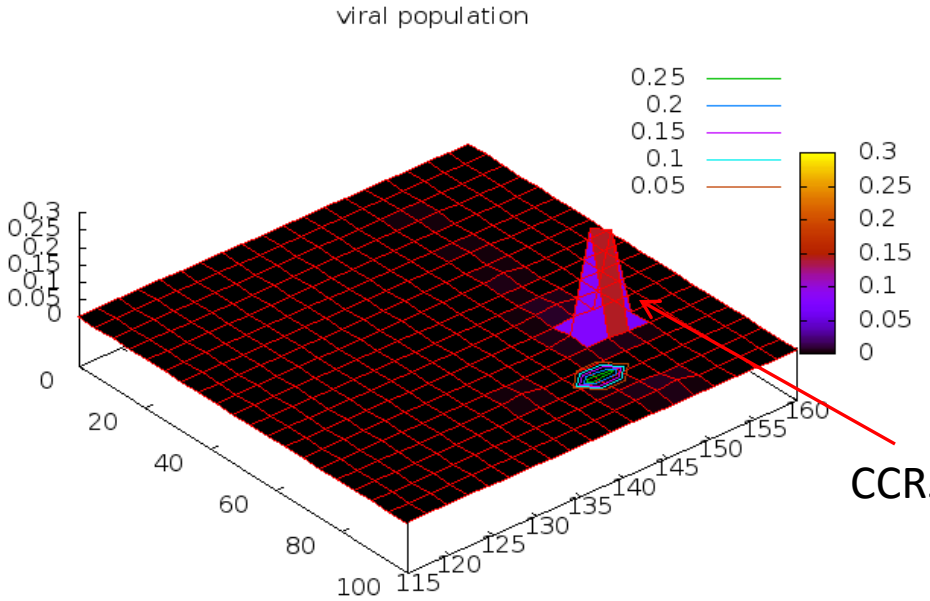
Intrahost diversity
Pipeline #2: RC454 + Qure



CXCR4 tropic

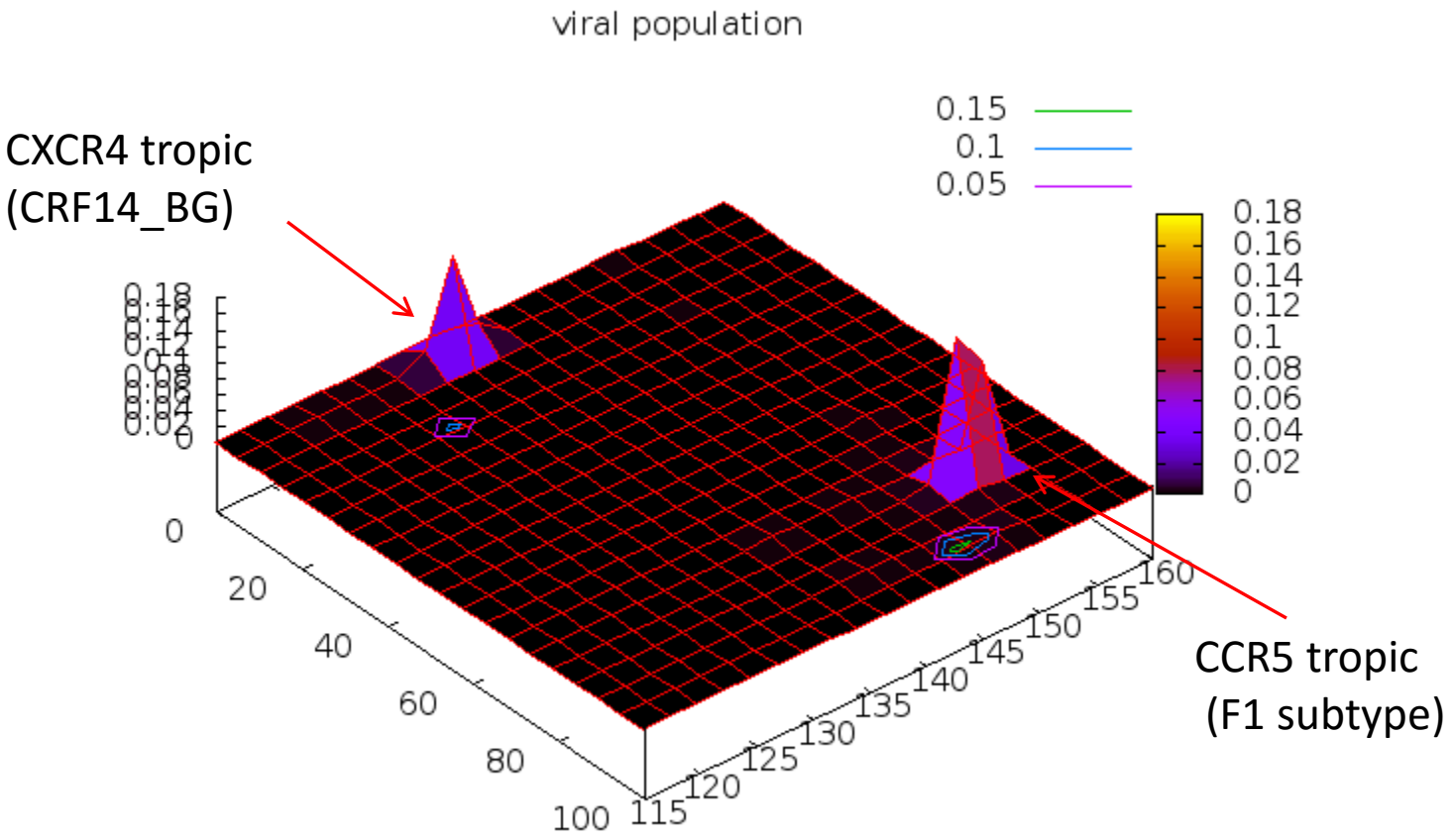


Tropism prediction by NGS experiments



CCR5 tropic

Tropism prediction in super-infected IDU



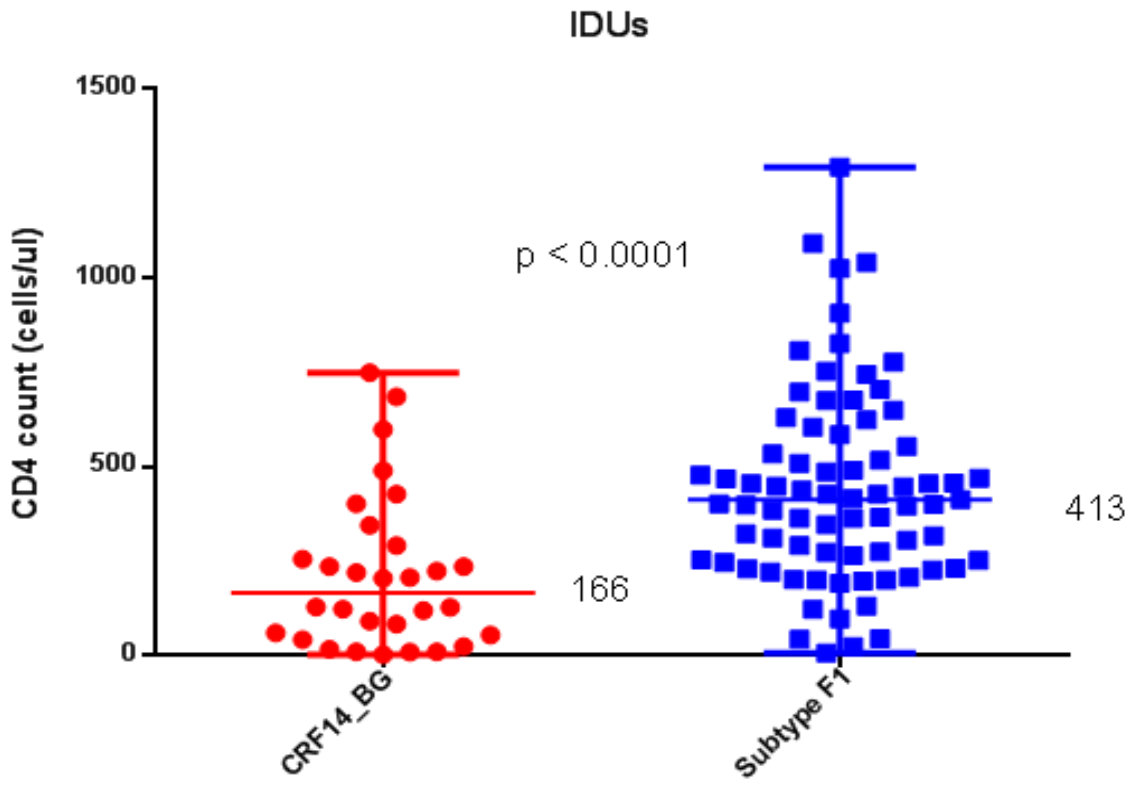
CRF14BG characteristics:

-Baseline CXCR4 tropic viruses

-Correlates with a more rapid progression of disease*

* Bártolo I et al, 2011, PLoS One; Origin and epidemiological history of HIV-1 CRF14_BG.

Lower CD4 counts in CRF14_BG infected IDUs at baseline



Banica L, abstract #12, 11th International Workshop on co-infection HIV & Hepatitis, London, 2015

Conclusions

- NGS can provide, with the help of phylogenetic analysis, important insights about the intra-host sub-population structure

- Within IDUs, the viral populations were more diverse then in heterosexuals, super-infections being identified; recombination between F1 and CRF14 was observed

- CRF14_BG was the predominant strain in dual infected patients

- In dual infected patients, the CRF14_BG variants were predicted as CXCR4 tropic, in contrast with the F1 variants that were CXCR5 tropic.

Future plans

- Include more IDUs: prevalence of dual infection
- To collect and analyse longitudinal samples from IDUs - molecular clock analysis: which strain was first
- Complete genome sequencing of the new recombinant form identified circulating among IDUs
- Extend the perspective: immunological and clinical

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Leontina Banica
Ionelia Nicolae
Emil Neaga
Dan Otelea

The 8th National HIV/AIDS CONGRESS

3rd Central European HIV FORUM

Sibiu, 5-7th May 2016