Distinct detection of intact and defective proviral DNA for HIV subtype B and C

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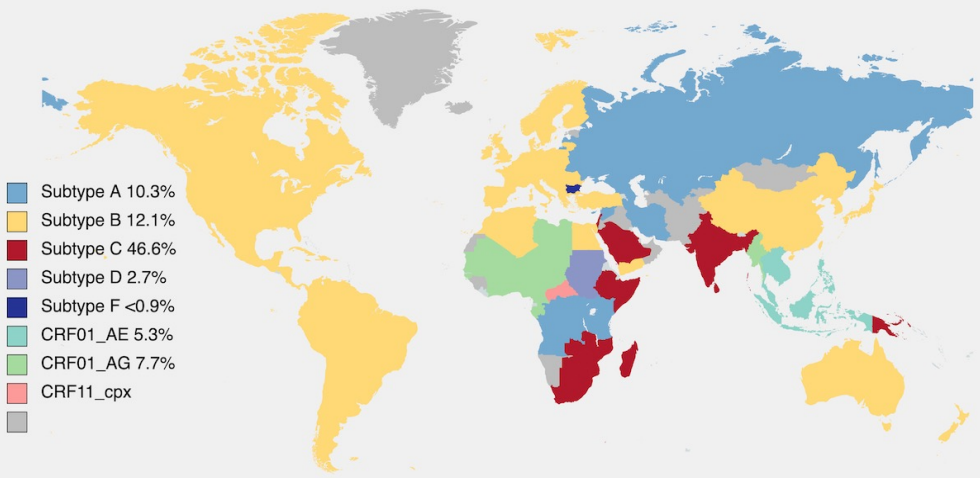
Introduction

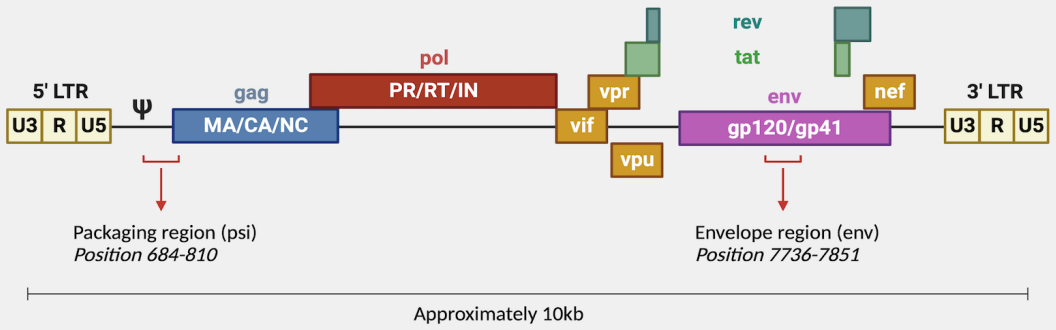
- The HIV reservoir is the main obstacle to a cure
- During treatment the reservoir can be replication competent (intact, 2.4%) or replication incompetent (defective, 97.6%)
- Reservoir quantification is essential to monitor cure interventions

To achieve a global cure it is important to quantify the reservoir of the most dominant HIV subtypes, B and C



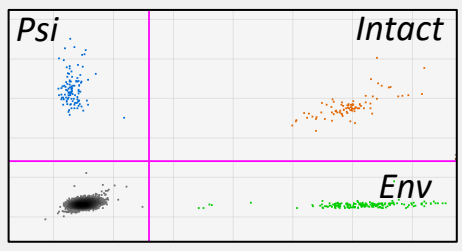
Methods

- Multiplex Digital Droplet PCR
- 2 primer/probe sets used to quantify the reservoir



Optimized protocol to distinguish intact and defective proviral HIV subtype B and C

- 1 fluorescent signal → defective proviral DNA (*Psi* or Env)
- 2 fluorescent signals → intact proviral DNA



Results

- Equal efficacy of the subtype B and C primers and probes was defined on Gblocks (fig.1)
- Limit of detection, which defines the sensitivity of the assay was determined at 7 copies with >95% certainty (fig.2)
- Limit of blank, which defines the specificity was determined at 0.90 copies at 60ºC and 1.23 copies at 55ºC (fig.2&3)
- Natural variation of 1 nucleotide change in *Env* can still be detected by lowering the annealing temperature to 55ºC (fig.3)

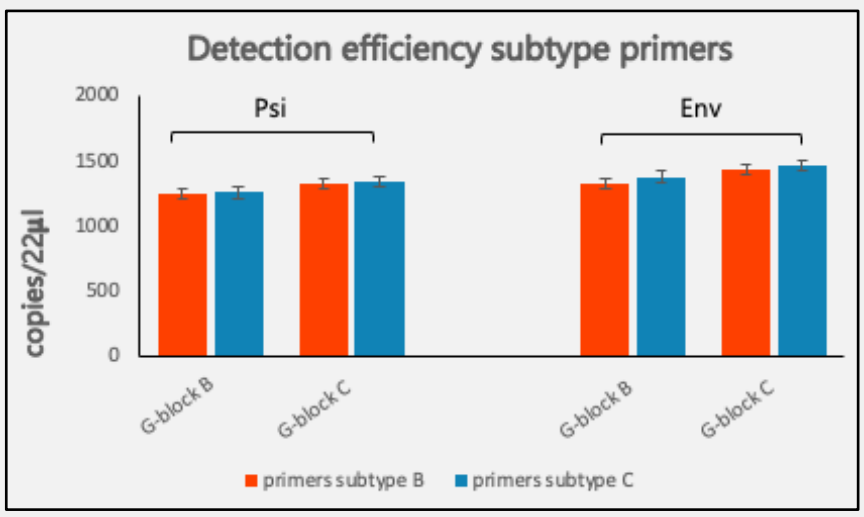


Fig 1. Detection efficacy of the psi and env region with both subtype B and C primers/probes on Gblocks.

Specificity and Sensitivity of HIV-1 IPDA with Gblocks

copy input	% of positive signals	mean copy output	standard deviation
6	97,5	6.38	3,68
5	92,5	4.22	2,97
4	90,0	3,50	2,36
3	95,1	3.21	2,22
2	83,3	1.91	1,63
1	61,4	1.11	1,19
0	14,0	0.12	0,48

LoB = mean $_{blank}$ + 1.645 * (SD blank LoD = LoB + 1.645 * (SD sample 95% positivity)

LoB = 0.12 + 1.645 * 0.48 = 0.90 LoD = 0.90 + (1.645 * 3.68) = 6.95

Fig 2 Specificity and sensitivity of the subtype B and C primers and probes at 60ºC . The Limit of Blank (LoB) was determined at 0.90 copies and the limit of detection (LoD) at 7 copies.

Specificity of the HIV-1 IPDA at 55°C		Annealing temperature			
LoB = 0.18 + 1.645 * 0.64 = 1.23		60°C	58 °C	55 °C	53 °C
Copies/22ul	Env intact	1293,6	1238,6	1001,0	1273,1
	Env probe G-A mutation 5th nucleotide	2,0	2,8	1021,4	1372,1
	Env probe G-A mutation 13th nucleotide	0,6	0,0	840,4	1177,7
	Env hypermutation probe	0,0	0,9	0,4	2,3

Fig 3. Detection of the subtype B and C primers/probes at the Env region with lower annealing temperatures, tested on Gblocks. The specificity of the assay at 55ºC was determined at 1.23 copies.

Discussion

- This IPDA is a specific and sensitive assay for the detection of subtype B and C defective and intact proviral DNA
- Decreasing the annealing temperature to 55ºC enables correct quantification of patient isolates with 1 nucleotide change in the *Env* gene
- This IPDA is most likely also able to correctly quantify subtype D and F, since these strains have no sequence differences in *psi* and *env*

The IPDA can form the basis for large-scale evaluation of global cure interventions in clinical studies

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