JACKHAMMER RT-PCR RECOVERS DIVERSE ARCHIVAL VIRAL GENOMES FROM KINSHASA, 1983

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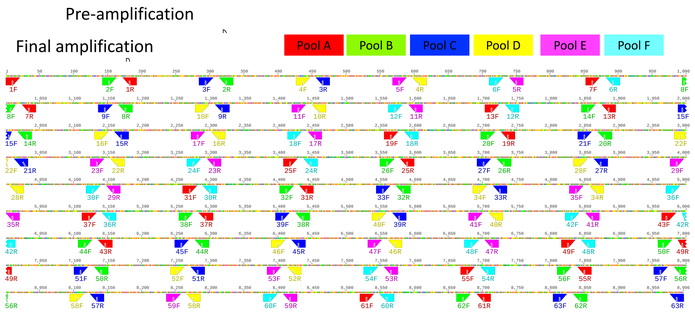
BACKGROUND

- We aimed to design a sensitive yet time-efficient RT-PCR procedure to characterize old, archival HIV genomes applicable to a wide diversity of HIV-1.

In 1983, AIDS was recognized for the first time in Africa (Kinshasa, Democratic Republic of Congo), and serum/plasma samples of these patients have been stored since[1]. Genomic data from such a relatively early phase in the epidemic are crucial for a better understanding of the long-term evolution of the HIV-1 pandemic. However, viral genomes from old, archival samples usually are degraded and fragmented, calling for very sensitive techniques suitable for HIV-1 group M's wide diversity.

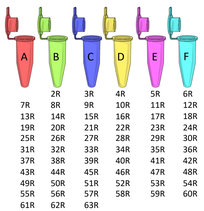
METHODS

- 48 serum/plasma samples from 1983 Kinshasa extracted, 45 deemed suitable after preliminary tests and were further pursued.
- Hemi-nested Jackhammer RT-PCR protocol[2]: see 3 steps below.
- 63 primer pairs targeting ~150-300 nt overlapping regions of HIV-1 group M, in multiplex pools for step 1 and 2.

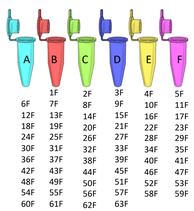


HIV-1 genome with scheme of Jackhammer primers colored by pool

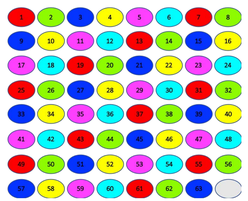
Step 1: Multiplex RT -> N/10 (here 6) pools of reverse primers at locus i



Step 2: Multiplex Pre-amplification PCR-> N/10 (here 6) pools of forward primers at locus i-1



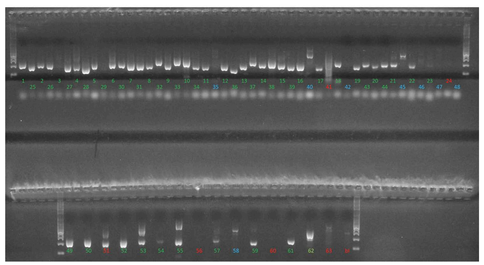
Step 3: N (here 63) individual PCR amplifications -> multiplexed pre-amplification products as template



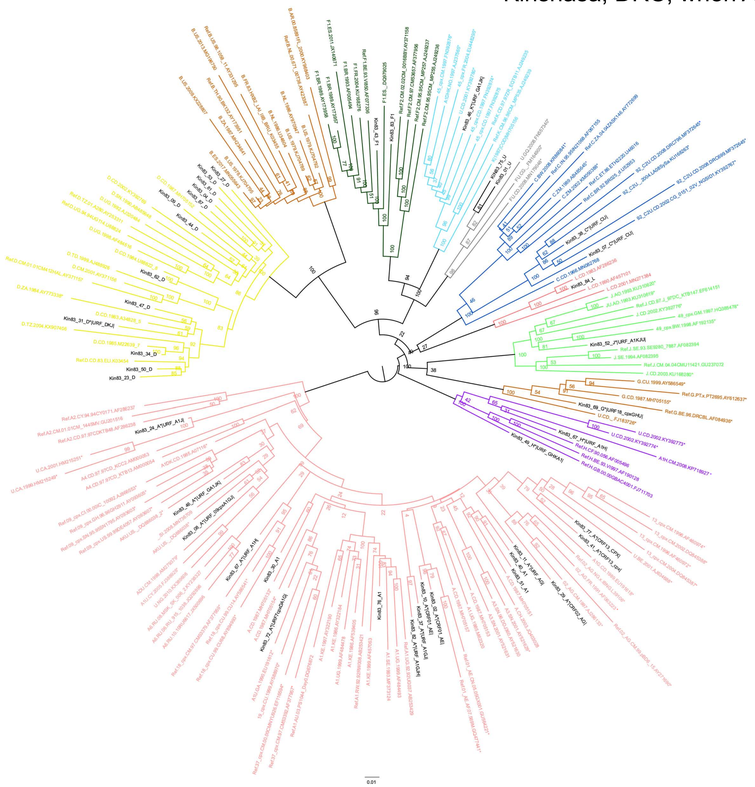
The ultra-sensitive **Jackhammer RT-PCR** procedure efficiently characterizes complete HIV-1 genomes from **archival specimens**. The 45 viral genomes recovered from the earliest known AIDS patient cohort in **1983 Kinshasa**, DRC, span almost the entire **HIV-1 group M diversity**.

RESULTS

- On average ~ 80% of PCRs produced Sanger sequences after a first hemi-nested jackhammer run with 63 broad-spectrum primer pairs.
- For the 45 near-complete genome samples, all gaps were further closed with additional primer pools, followed by sample-specific primer design – all organized in hemi-nested Jackhammer RT-PCR approach.
- The 45 genomes from Kinshasa contained:
  - 19 'pure' subtypes : A1, D, F1, L
  - 2 unclassified (U)
  - 6 known CRFs (01_AE, 02_AG, 13_CPX, 25_cpx)
  - 18 URFs



Example gel for 1 sample after Jackhammer RT-PCR procedure



Maximum likelihood tree of non-recombinant portions of recovered genomes from 1983 Kinshasa ("Kin83", in black) together with reference genomes of HIV-1 group M subtypes plus sequences with close blast hits to Kin83 sequences. Partial sequences indicated with stars.

CONCLUSIONS

- In an efficient <2 day procedure per sample, the hemi-nested jackhammer-RT-PCR protocol with a broad-spectrum 63 primer pair set can recover near-complete HIV-1 genomes of wide diversity.
- High quality full viral genomic data can still be retrieved from archival specimens previously deemed too degraded.

We characterized 45 complete HIV-1 group M genomes from specimens over three decades old. The recovered genomic diversity provides direct evidence that a broad range of HIV-1 group M diversity was already present in Kinshasa, DRC, when AIDS was first identified in Africa.

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