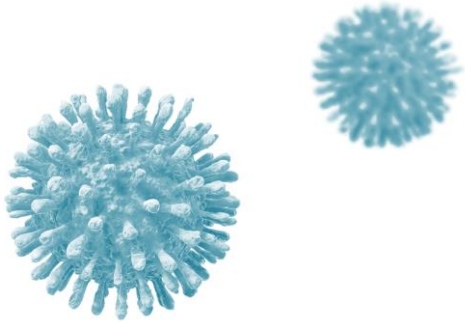
BREACH



# BREACH Symposium 2021

## Characterisation of the HIV-1 latent reservoirs

November 30th, 2021

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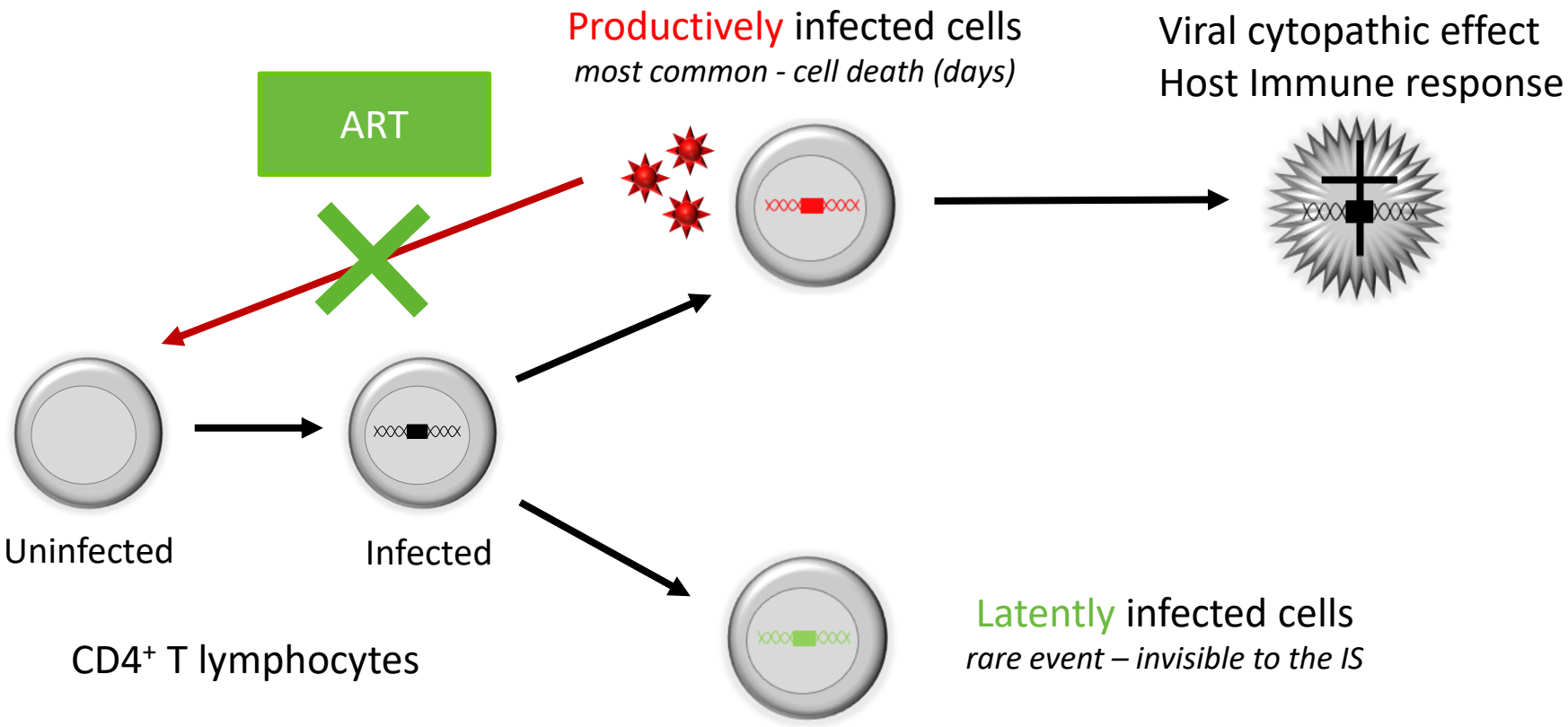
Department of Infectious Diseases and General Internal Medicine, University Hospital of Liège, Belgium

GIGA I3, University of Liège

# 1st source of residual viremia: HIV Latent Reservoirs




*Adapted from Gilles Darcis 's thesis, 2016*

# Characterisation of the HIV-1 latent reservoirs

## 1. PCIP-seq optimisation

Optimise “the Pooled CRISPR Inverse PCR sequencing” to HIV-1

- Comparison with Intact Proviral DNA Assay (IPDA)

## 2. Use PCIP-seq to characterise the HIV cellular reservoirs (CD32 study)

Characterisation of the HIV cellular reservoirs in specific T cell populations :

- CD32+ CD4+ T cells
- Various T cell subsets
- T cells expressing immune checkpoint molecules

## 3. Use PCIP-seq for a longitudinal study on cells

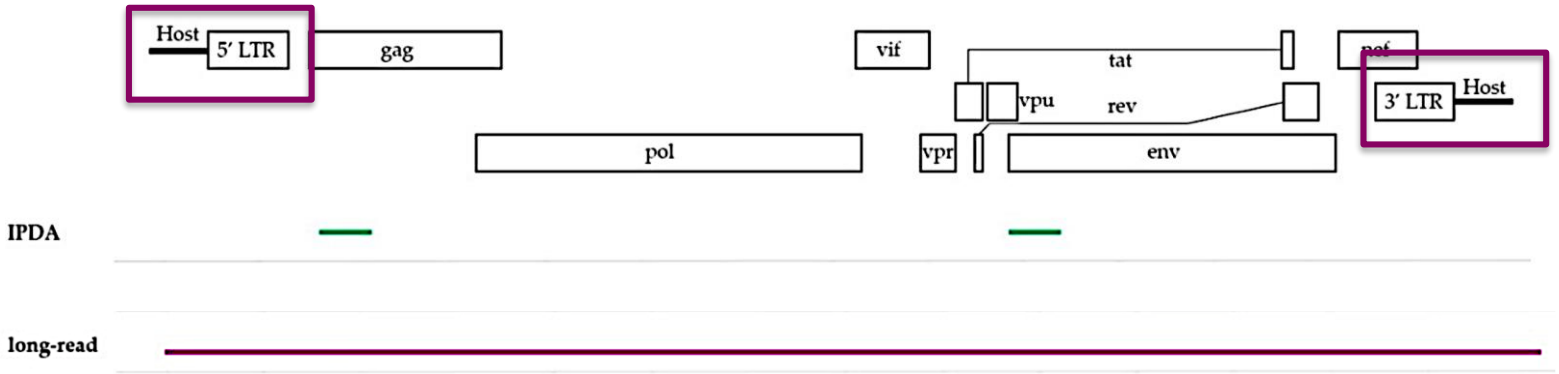
Analyse the evolution of the replication-competent HIV cellular reservoir overtime

# Characterisation of the HIV-1 latent reservoirs

## 1. PCIP-seq optimisation

Optimise the **Pooled CRISPR Inverse PCR sequencing (PCIP-seq)** to HIV-1

- Comparison with **Intact Proviral DNA Assay (IPDA)**




*Artesi et al., Genome Biology, 2021*
*Bruner et al., Nature, 2019*
*Lambrechts et al., Viruses, 2020*

# Characterisation of the HIV-1 latent reservoirs

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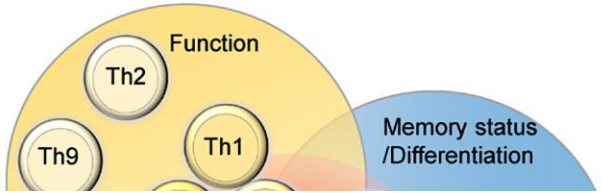
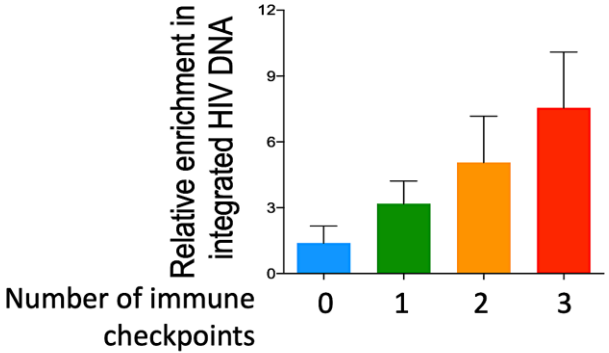
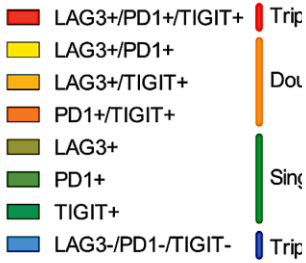
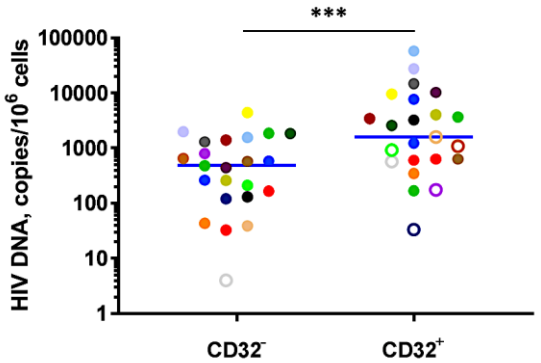
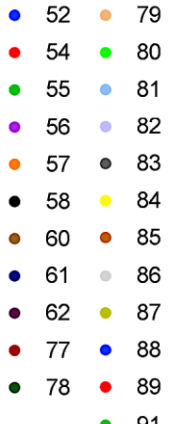


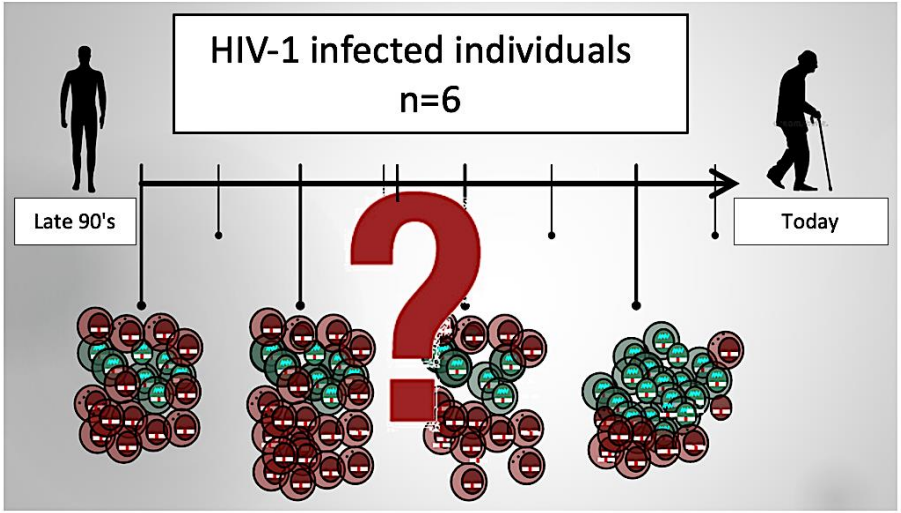

Darcis et al.,Cell Reports, 2020

# Characterisation of the HIV-1 latent reservoirs

## 3. Use PCIP-seq for a longitudinal study on cells

Analyse the evolution of the proviral landscape overtime

- Taking advantage of very old samples stored during late 90's



# Acknowledgments


**Infectious diseases department**

Gilles Darcis
Michel Moutschen
Patricia Dellot
Nicole Marechal

**AIDS Reference Laboratory**

Marie-Pierre Hayette
Dolores Vaira
Fabrice Susin

**GIGA**

**University of Amsterdam**

Alexander O. Pasternak
and all his team

LIÈGE université
GIGA

# Thank you for your attention!

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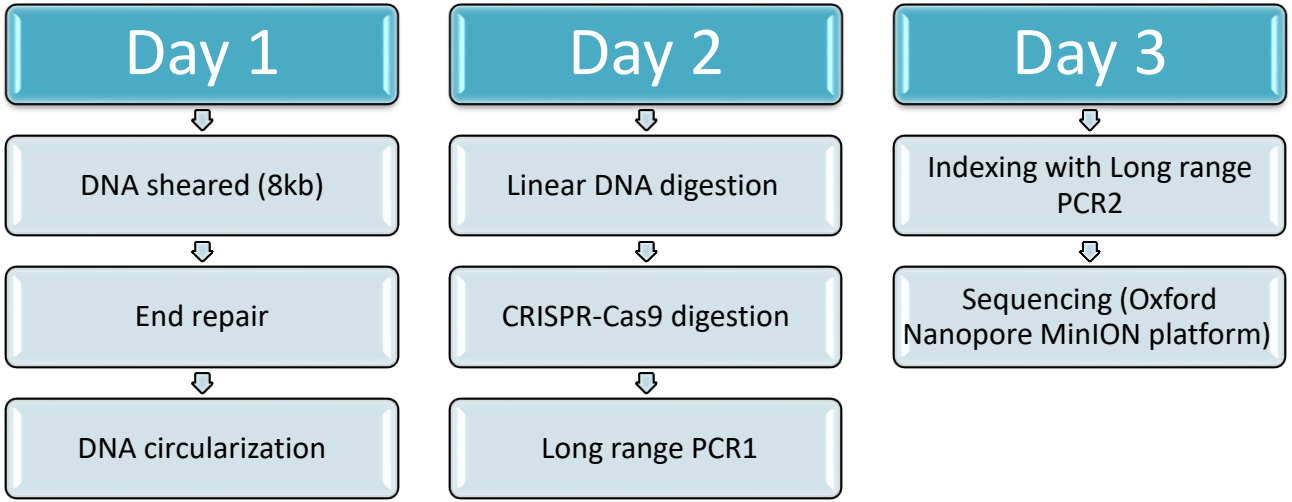
# PCIP-seq optimisation

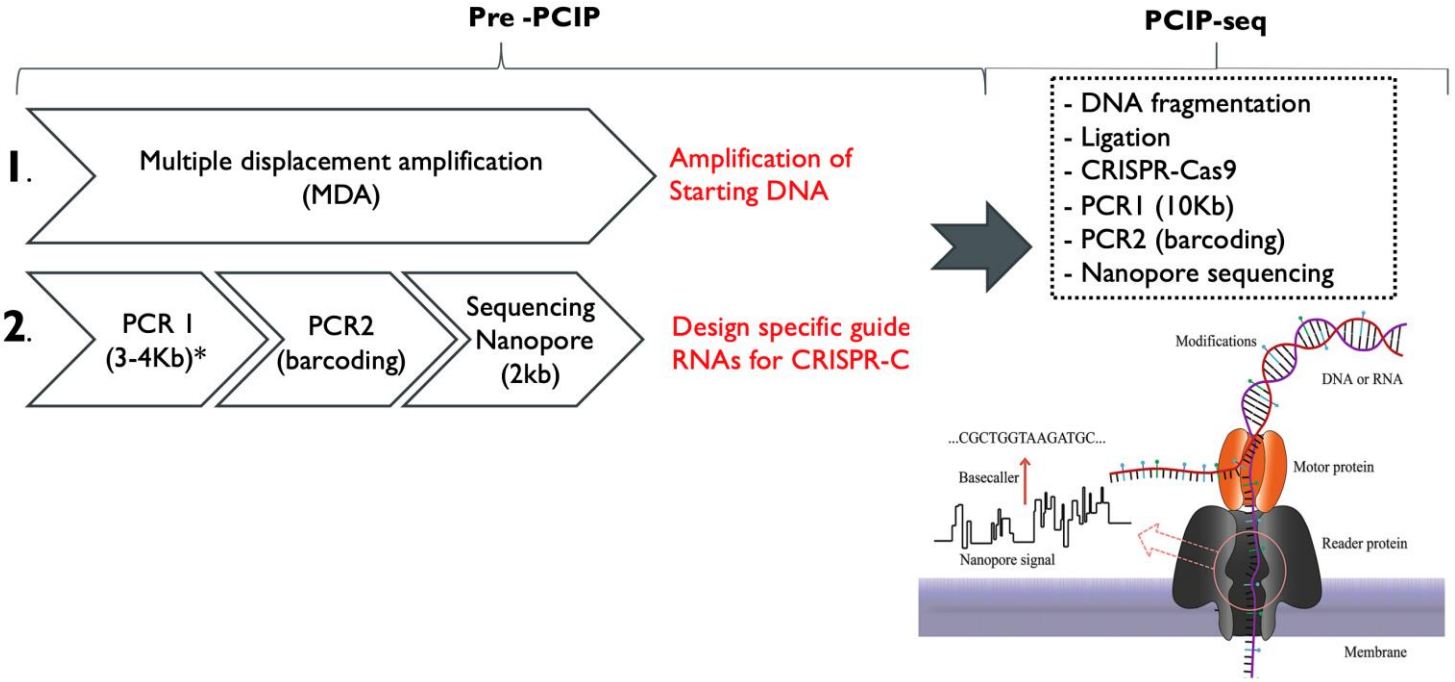
1. **Pooled CRISPR Inverse PCR sequencing (PCIP-seq) optimisation**: sequencing of both the HIV proviral genome and the associated site of integration




2. **Intact Proviral DNA assay (IPDA)**: separately quantifies intact and defective proviruses

# PCIP-seq optimisation

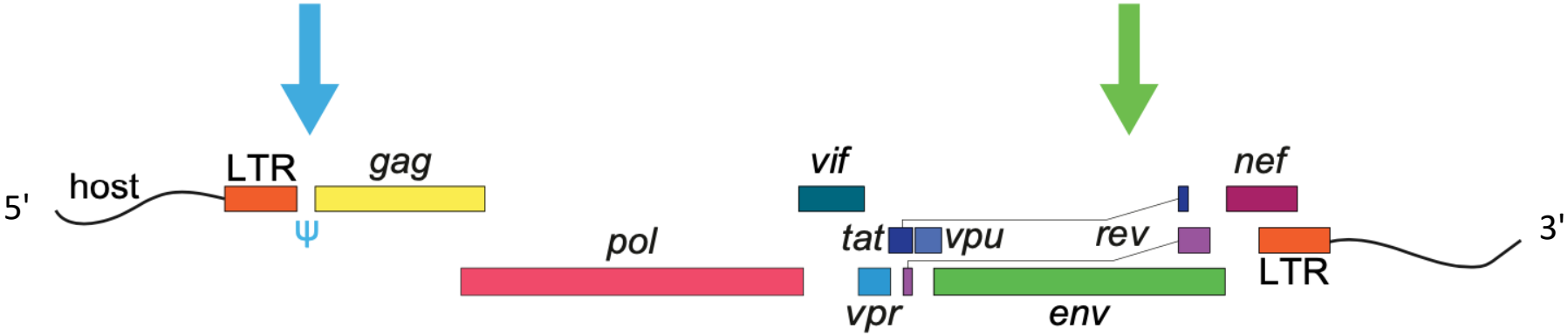
1. Extremely low proviral load → multiple displacement amplification (MDA)
2. High mutation rates → design specific guide RNAs for CRISPR-Cas9




*Artesi M. et al., Genome Biology,2021*

# Intact Proviral DNA Assay (IPDA)

- Duplexed droplet digital PCR (ddPCR) to distinguish and separately quantify intact proviruses from defective ones
- Evaluate IPDA performance by comparing it with PCIP-seq in our cohort




- Natural HIV-1 polymorphism in prime/probe binding regions → IPDA detection failure

*Bruner et al., Nature, 2019*
*Simonetti et al., Microbiology, 2020*