# A Versatile Peptide Library Spanning Immunogenic Fractions of the HIV Proteome with High Clade Coverage

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## Introduction

Recent findings of the RV144 vaccine trial imply that apart from vaccine regimen that do elicit neutralizing antibodies or cytotoxic T lymphocytes other mechanisms can confer protection against HIV-1 infection. As a consequence, attention has turned to activation of non-neutralizing antibodies and antibody-dependent cell-mediated cytotoxicity (ADCC) as possible mechanisms for vaccine-related protection. This also leads to an increased interest in defining new immunogenic regions presented by distinct HIV clades. Peptide microarrays are excellent tools for the identification of immunogenic protein regions and were also shown to enable monitoring of specific immune responses against HIV proteins (reported e.g. for the RV144 trial). The extreme variability of HIV protein sequences and the number of different clades make complex overlapping peptide libraries necessary to cover this diversity. The aim of this study was to design a peptide library presenting the immunodominant fractions of the HIV proteome while covering a high number of clades.

## Input Sequences

- Based on alignment of HIV transcripts from the LANL database version 2009[1] .
- Full length of GAG (p17and p24), TAT, ENV (gp120 and gp41), and NEF.
- Immunogenic regions of GAG p2p7p1p6, POL, VIF, and REV as published by LANL[2].

## Sequence Selection

- Consideration of most frequent clades A, B, C, D, G, CRF01_AE and CRF02_AG accounting for >90 % of global prevalence[3].
- From each of the frequent clades the best covering sequence was selected using Mosaic[4,5].
- A sequence cocktail containing these 7 best covering sequences was generated.
- 20 sequences with best coverage were identified without consideration of the clade and added to the sequence cocktail.
- Increase of coverage was calculated for each sequence and sequences gaining less than 0.75 % in coverage were removed from the cocktail (Fig. 1).

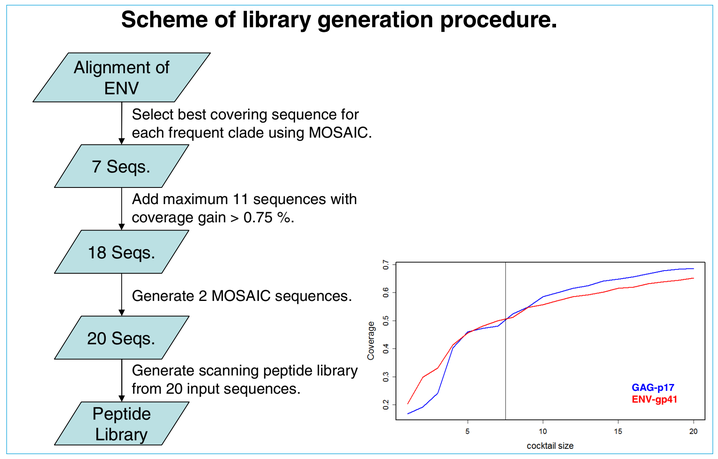


**Fig. 1**. Representation of coverage for cocktails of p17 (blue) and gp41 (red). The vertical line divides the cocktail into the 7 sequences for the frequent clades (left) and the added 13 sequences from the best covering sequences (right) as calculated against all HIV sequences irrespective of the clade.

## Generation of MOSAIC Sequences

- MOSAIC sequences are pseudo-protein sequences assembled from parts of proteins of a given library to reach optimal coverage[4].
- For each gene product 2 MOSAIC sequences were generated and added to the cocktail when the coverage gain was > 1 %.

## Final Library

- Unique overlapping peptides were generated.
- The final library consists of 5572 peptides with an average coverage of > 50 %.

| Protein | number of source sequences | coverage (%) |
|---|---|---|
| ENV gp160 | 2248 | 58 |
| GAG p17 | 3578 | 59 |
| GAG p24 | 3578 | 86 |
| NEF Nef | 2606 | 55 |

**Table 1**. Average coverage of presented full length proteins.

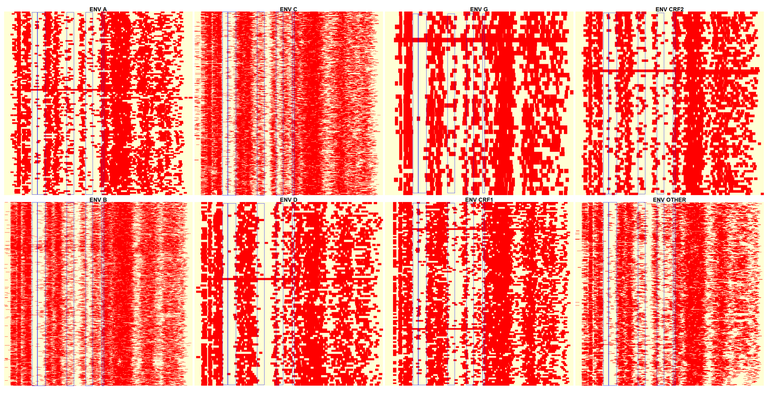


**Fig. 2**. Coverage of HIV1-peptide library for gp160 by clades (top row: A, C, G, CRF2; bottom row: B, D, CRF2, all other clades). x-axis: sequence of gp160, y-axis: all sequences for the respective clade from the alignment HIV1_ALL_2009_ENV_PRO.fasta are shown (total 2248). Blue boxes encircle V1-V5 loops.

## Application on Peptide Microarrays

- Peptide microarrays are excellent tools for monitoring humoral immune responses.
- Technology was successfully used in evaluation of patient samples in clinical trials[6,7].
- Library (5572 peptides) fits onto one peptide microarray.

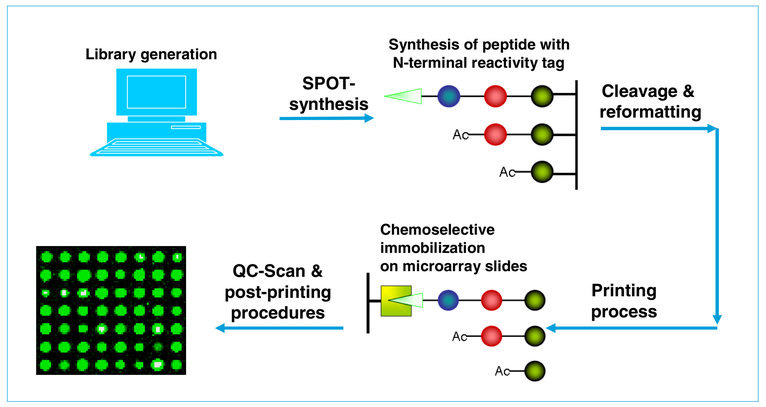


**Fig. 3.** Schematic representation of the array production process.

- **Development of a comprehensive HIV-1 peptide library with unprecedented coverage of diverse clades.**
- **Flexible microarray platform allows easy adaption to answer specific questions.**
- **Peptide microarrays are successfully used to study the humoral immune response on HIV infection.**

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