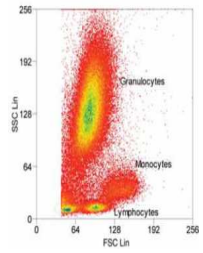

SELECTION OF MOLECULAR APTAMERS FOR IDENTIFICATION OF LIVE CELLS OF *RALSTONIA SOLANACEARUM*: A NEW POTENTIAL METHOD IN PLANT PATHOLOGY

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UF UNIVERSITY of FLORIDA
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MADISON

Outlines

- Introduction
- Objective
- Method & protocols
- Results
- Conclusion & perspectives
- Acknowledgements

Introduction

- *R. solanacearum* causes bacterial wilts on a wide range of planted crops, ornamentals and weeds worldwide.

- "Species complex".

  5 races

  5 biovars

  4 phylotypes

- Race 3 biovar 2 (R3bv2).

  World wide distribution except US and Canada

  Major disease of potato

  Introduction in the US in latently infected geranium cuttings

  Strictest biosecurity regulations

➡ **Critical need for unambiguous identification of R3bv2.**
Development of fast, sensitive, and specific detection methods.

Objective

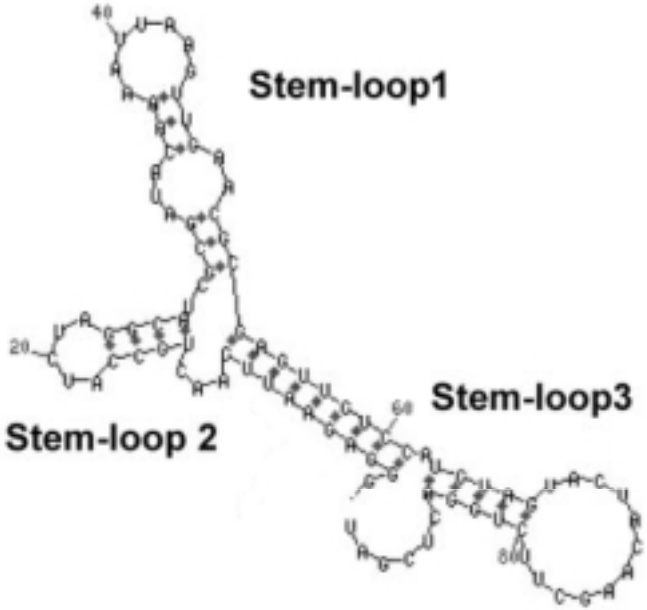
Evaluate the effectiveness of cell-SELEX* to produce molecular aptamers for differentiation of strains of *R. solanacearum* (detection of live cells).

*System Evolution of Ligands by EXponential enrichment

Method & protocols

- Molecular aptamers.

  - Single-stranded oligonucleotides.
  - Bind with high affinity to their target.
  - Detection of whole cells.
  - Small molecules, chemically stable.
  - Molecular probes for cancer studies.
  - Blocking molecules: reduced virulence in *M. tuberculosis*.



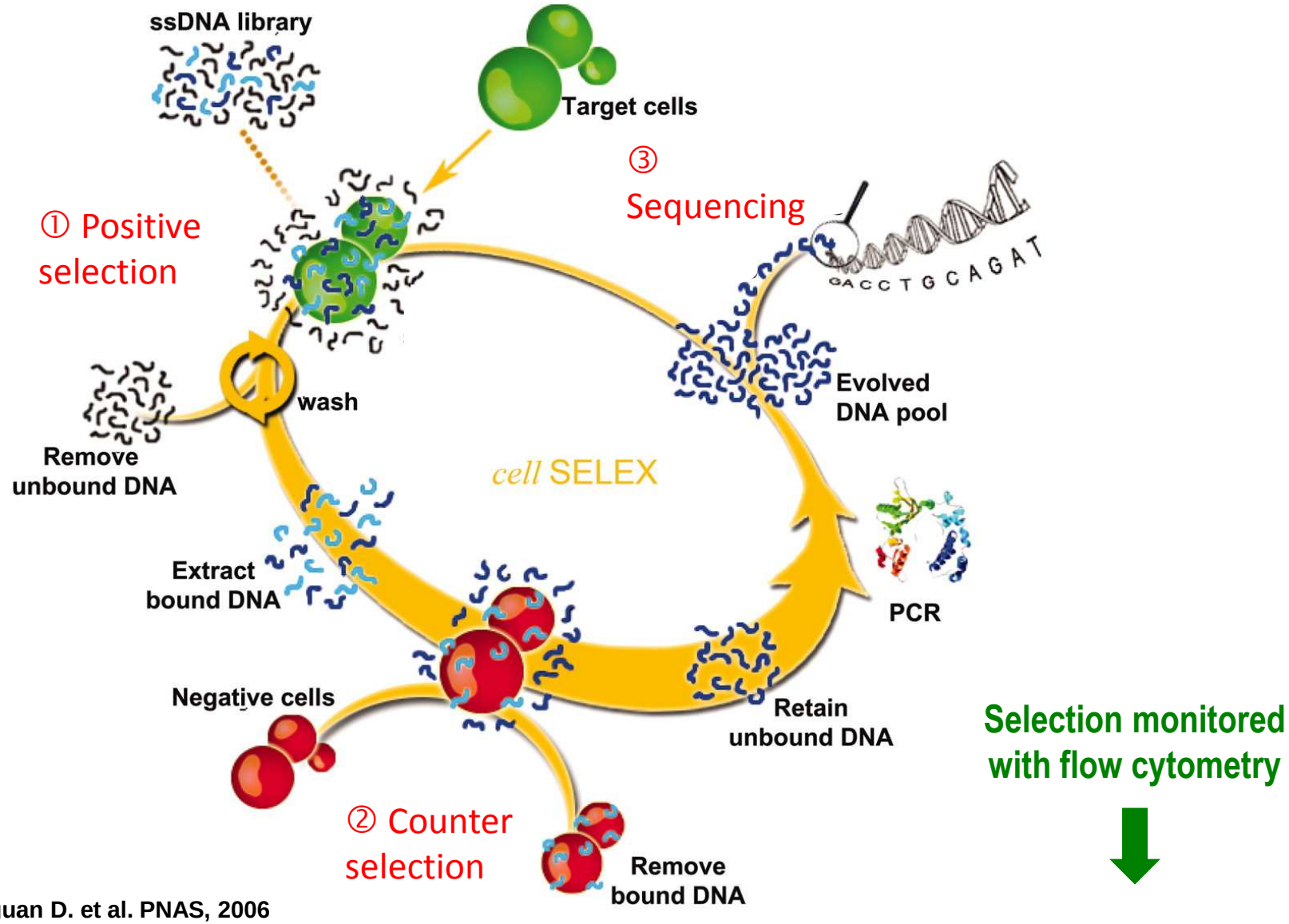
Example of an RNA Aptamer

- Cell-SELEX.

  Repeated competitive binding from an initial DNA or RNA library (random sequences) to the target molecules.

Method & protocols

- Schematic representation of cell-SELEX.

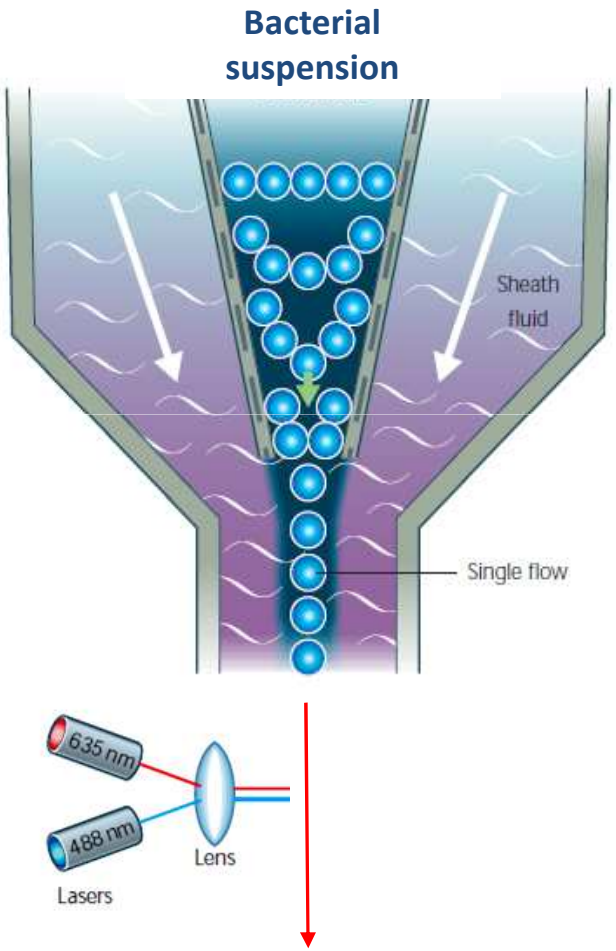


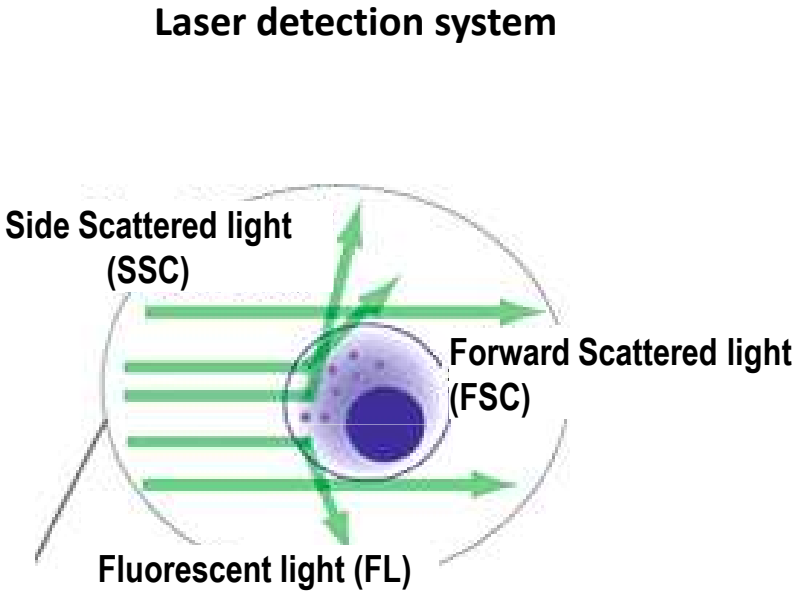
Selection monitored with flow cytometry

From Shangguan D. et al. PNAS, 2006

Method & protocols

- Principles of flow-cytometry





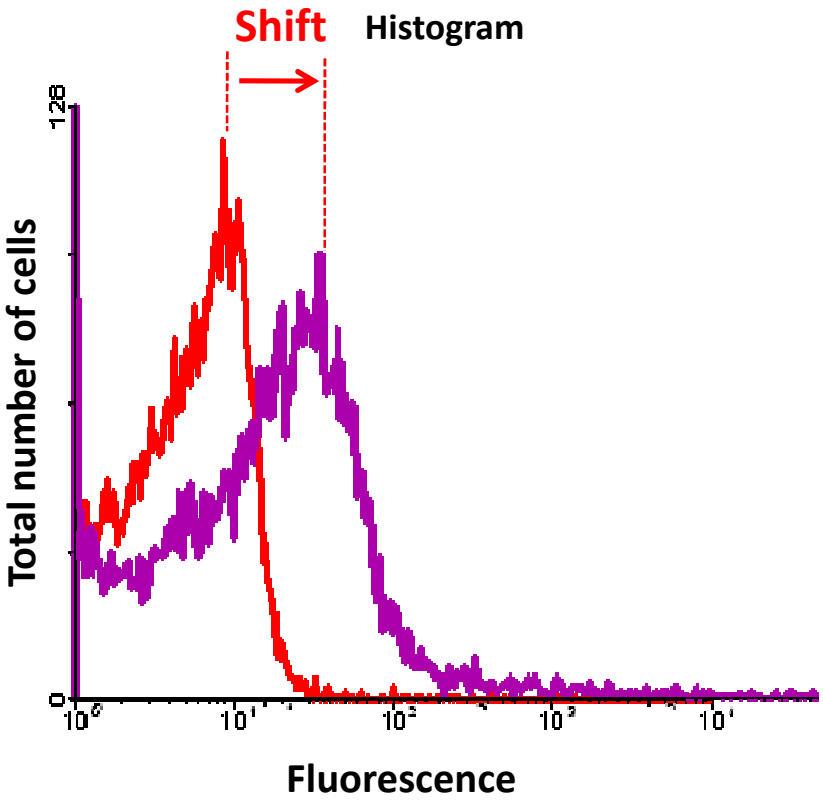
FSC = Indication of particle's size.

SSC = Information about the outer composition of a cell.

FL = Fluorescence detector (1 to 4).

Method & protocols

- Flow-cytometry data for cell-SELEX



ssDNA Library
(before selection)

Evolved DNA pool
(after *N* rounds of selection)

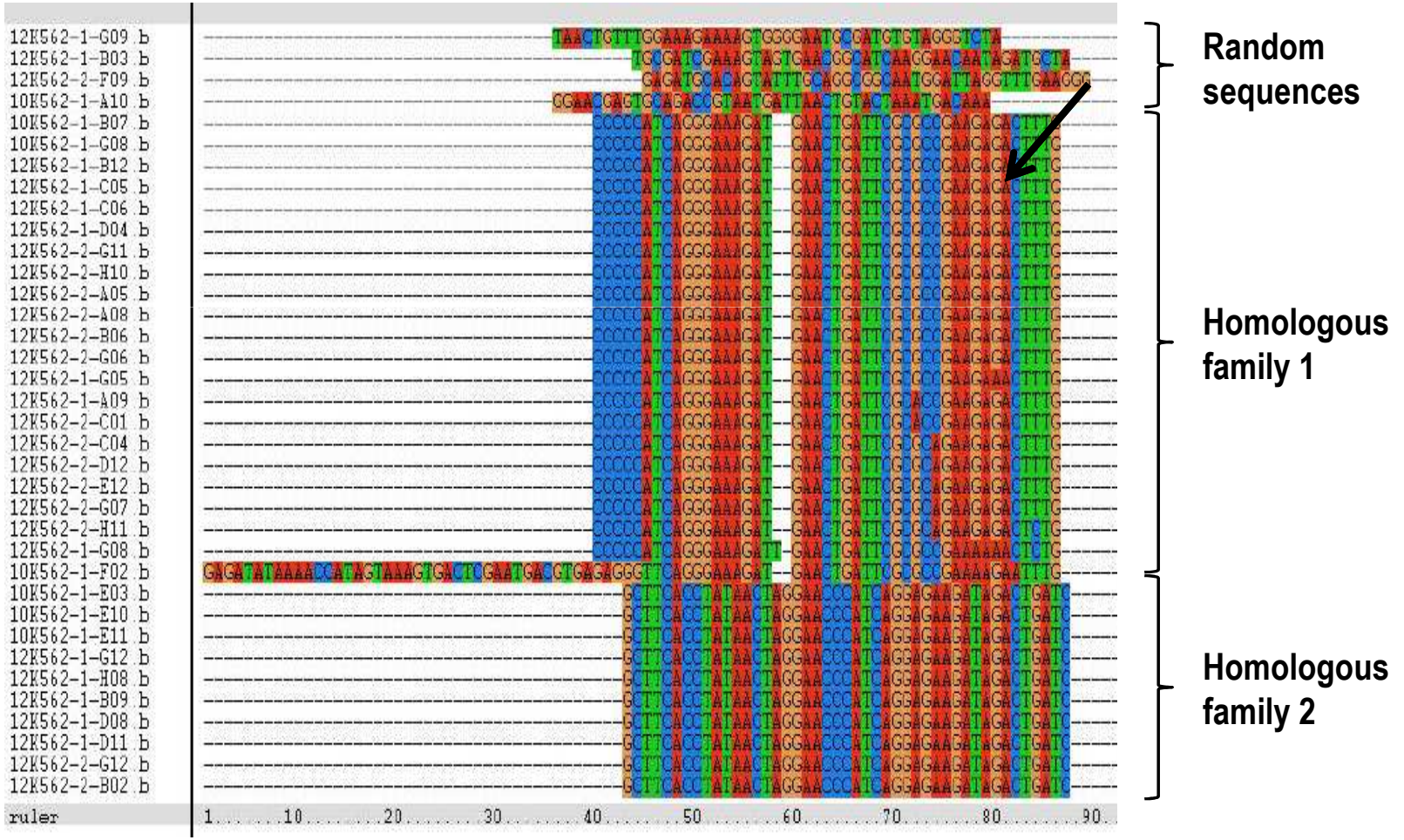
A "shift" of the fluorescence intensity pick is an indication of enrichment of single DNA sequences with high affinity to the target cells (potential aptamers).

Sequencing and analysis

Method & protocols

- Sequence analysis

From around 10^{15} unique random sequences



Method & protocols

- Bacterial strains (cells)

  UW485: Race 3 biovar 2T

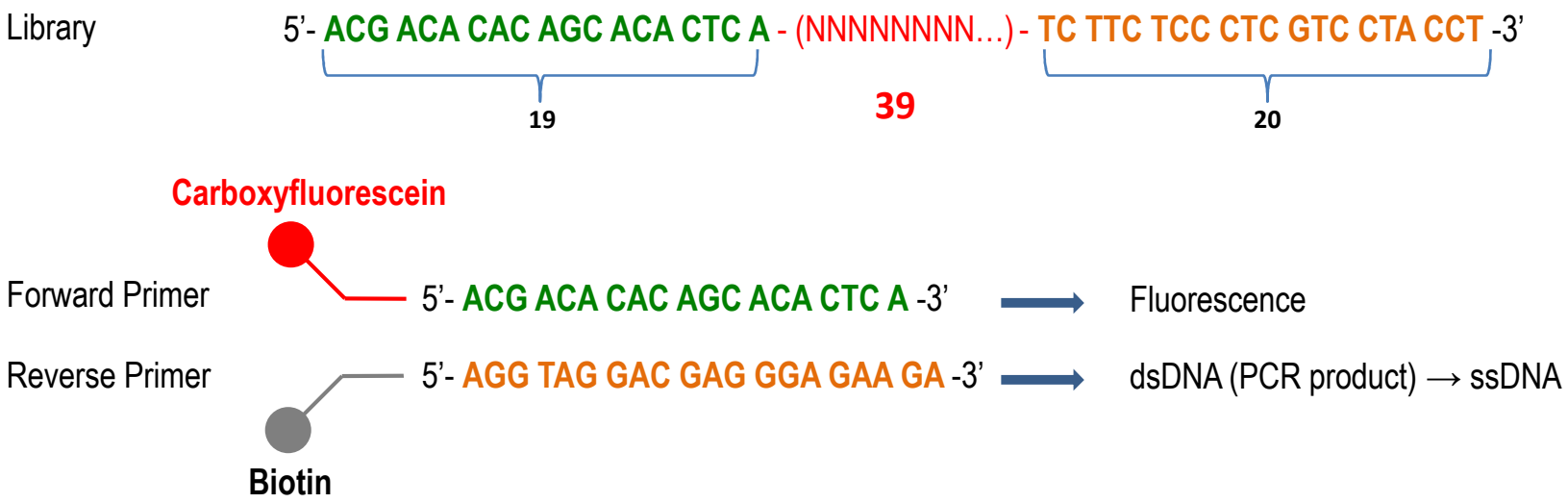
  Rs5: Race 1 biovar 1 (Florida)

- Cell growth conditions

  Liquid medium: MMP
  Phosphate-based buffer

  36 hours growth

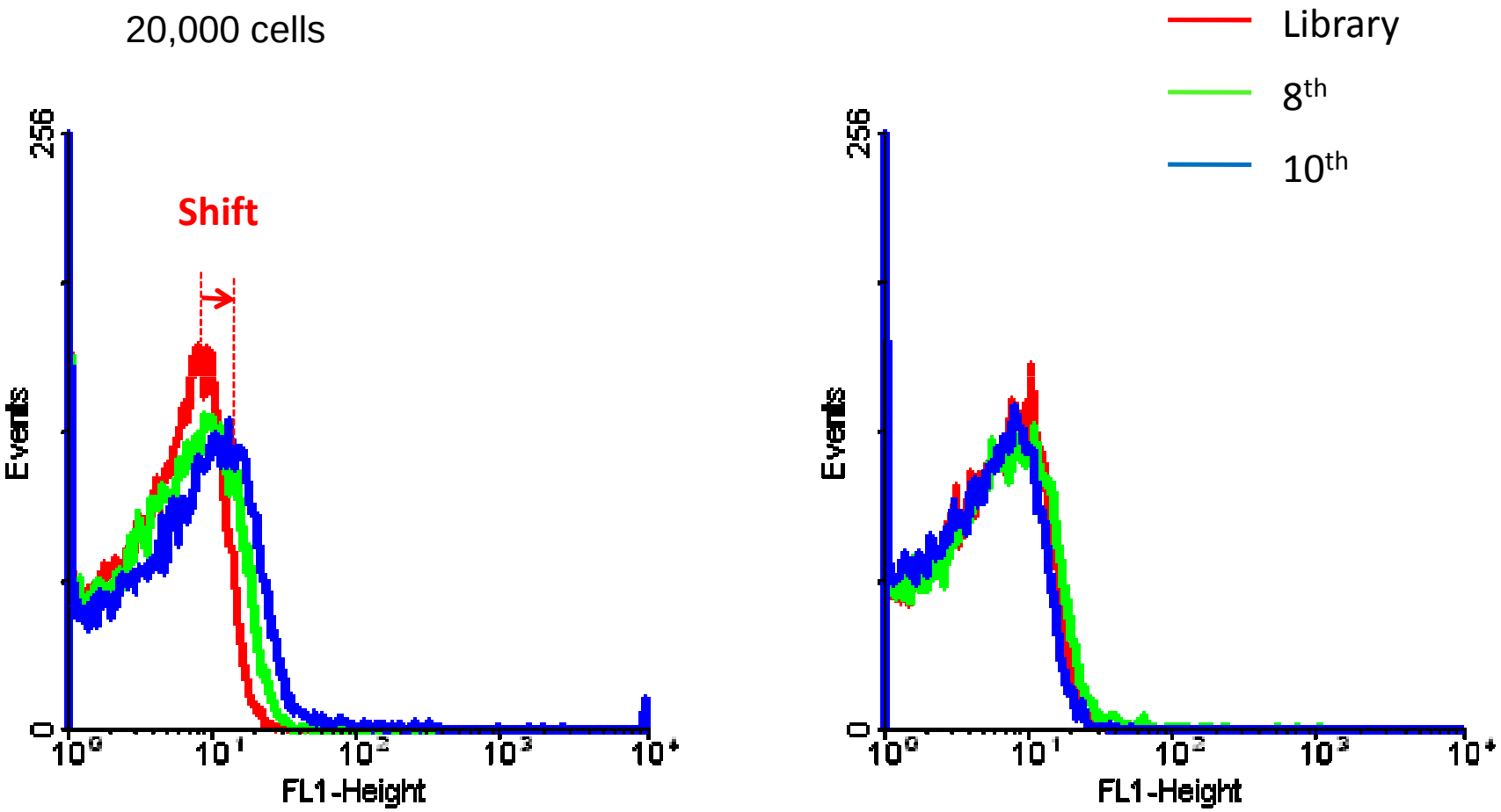
- ssDNA Library (78 nucleotides)



Results

- Flow-cytometry histograms (10 rounds)

  20,000 cells



Target cells (UW485)

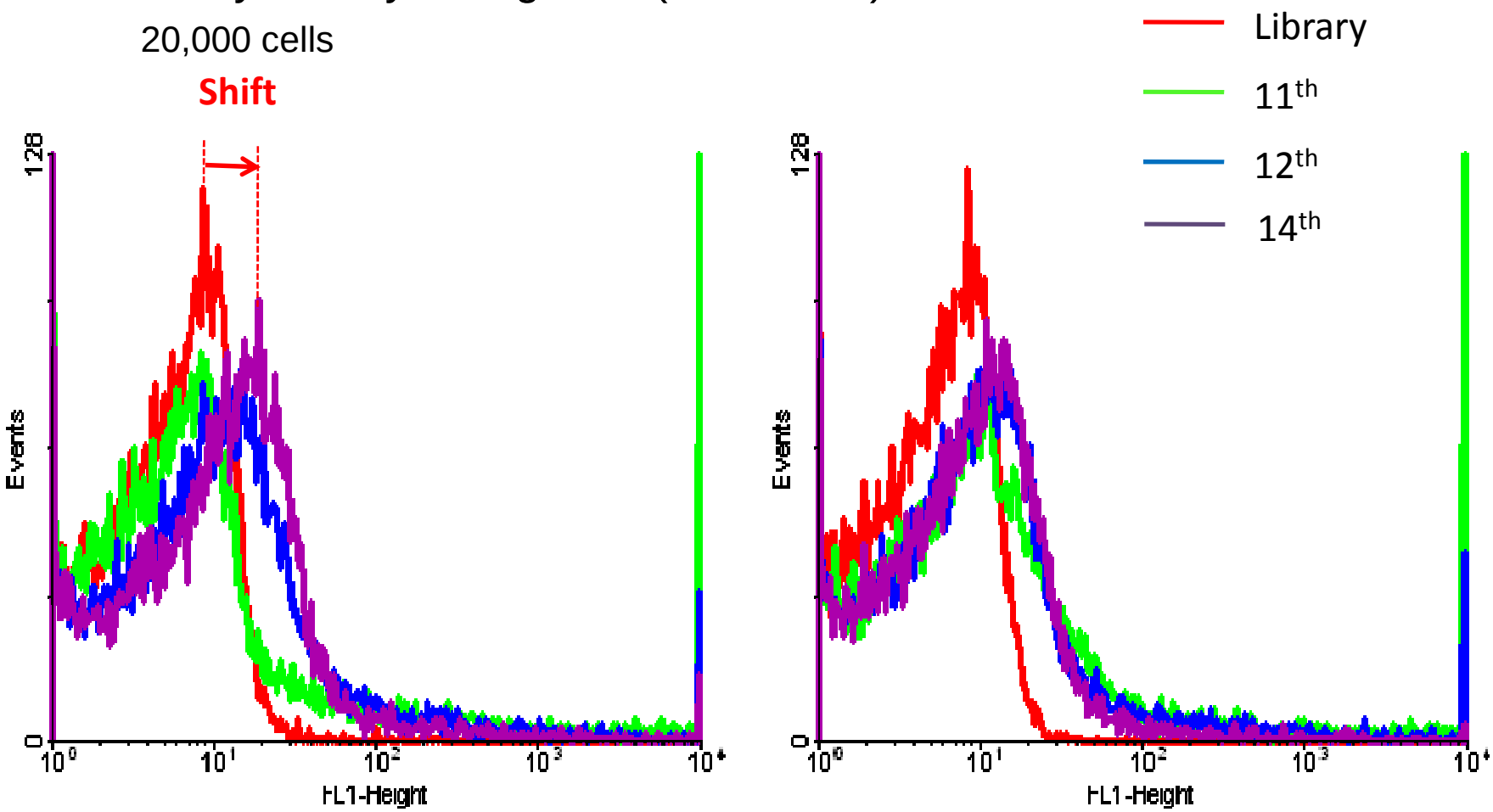
Negative cells (Rs5)

Results

- Flow-cytometry histograms (14 rounds)

20,000 cells

Shift



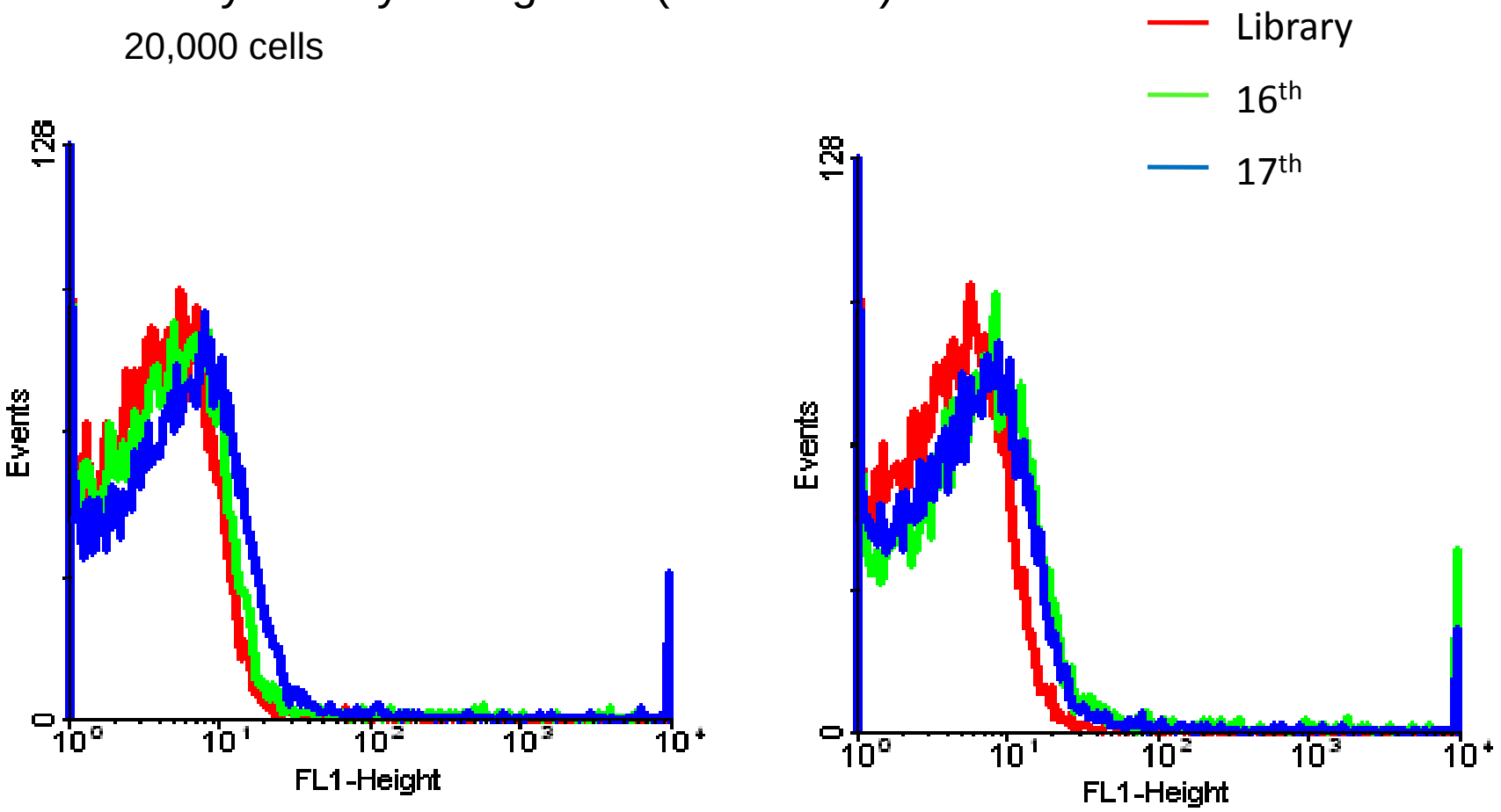
Target cells (UW485)

Negative cells (Rs5)

Results

- Flow-cytometry histograms (17 rounds)

  20,000 cells



Target cells (UW485)

Negative cells (Rs5)

Results

- Sequencing of evolved DNA pools (454)

SP10

353 repeated sequences (out of 3263)

One homologous family : **12 repeated sequences (0.4% total)**

SP16-17

769 repeated sequences (out of 8044)

Several homologous family : **22 repeated sequences (0.3% total)**

Only one conserved DNA family

For Primer – **cagggctcgagatgtttgcgatctgttgtccgttggga** - Rev Primer

Conclusion and perspectives

Conclusion

- Efficiency of flow-cytometry to differentiate cells of *R. solanacearum* after 10 rounds of selection.
- Enrichment of DNA sequences with cell-SELEX.
- Low enrichment due to variability in target molecules (could be EPS ?).
- Amplification of potential aptamers not shown.

Perspective

- Increase the number of repeated sequences.

More rounds of selection.

- Test the affinity of the repeated sequences.

Amplification and testing these sequences as potential aptamers.

- Control the specificity of the repeated sequences.

Test with target cells and heterologous bacteria.

Acknowledgements

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