Generation of Gateway clone library of virulence associated genes of zoonotic buffalopox virus: state-of-the-art resource for proteome analysis

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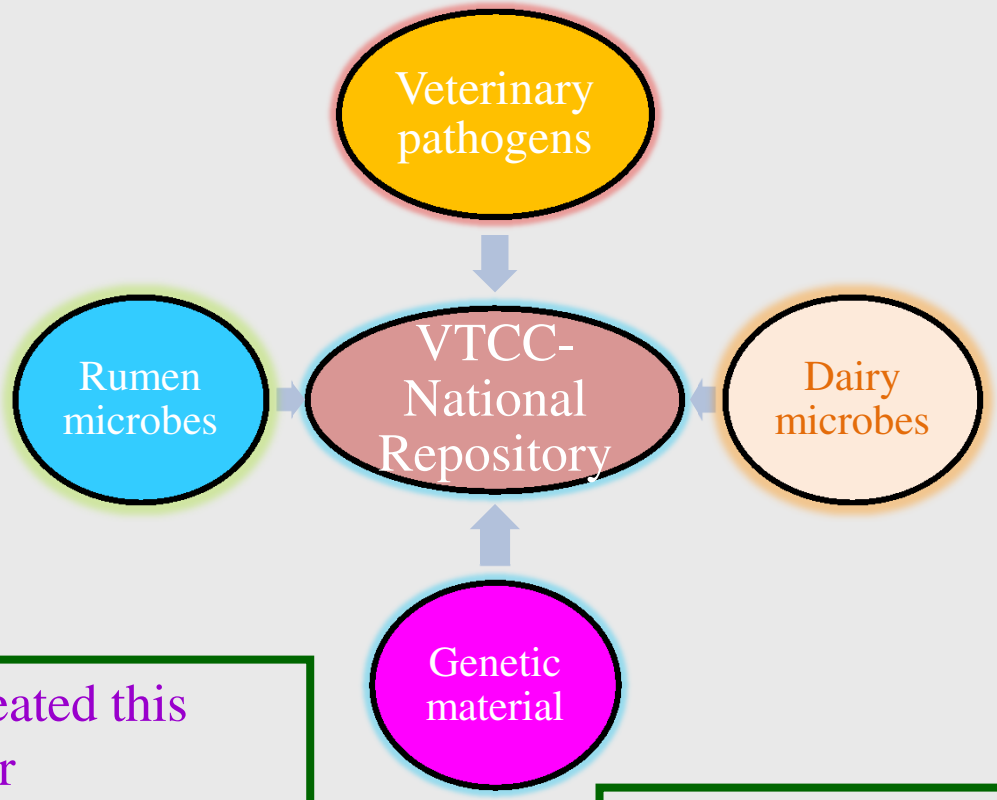
Objective

Generation of repository of Open Reading Frames (ORFs) clones – ORFeome of zoonotic buffalopox virus in Veterinary Type Culture Collection (VTCC) repository

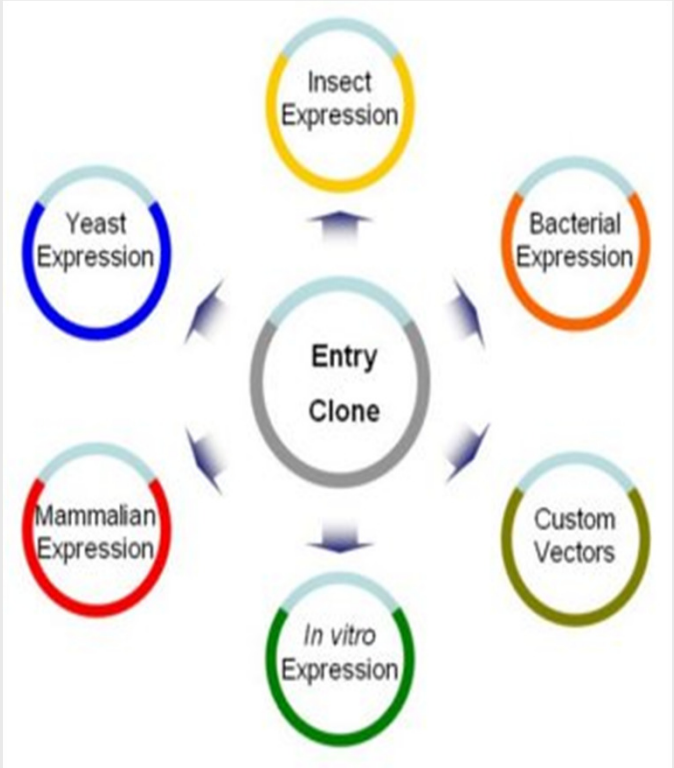


ICAR created this facility for conservation of microbes of veterinary importance

Act as biological resource bank for R&D

What's an ORFeome?

- Refers to the libraries of complete set of clones of protein-coding open reading frames (ORFs)
- Collection of plasmids containing ORFs of a genome
- Flexible & versatile library allows transfer of ORFs into different destination vectors



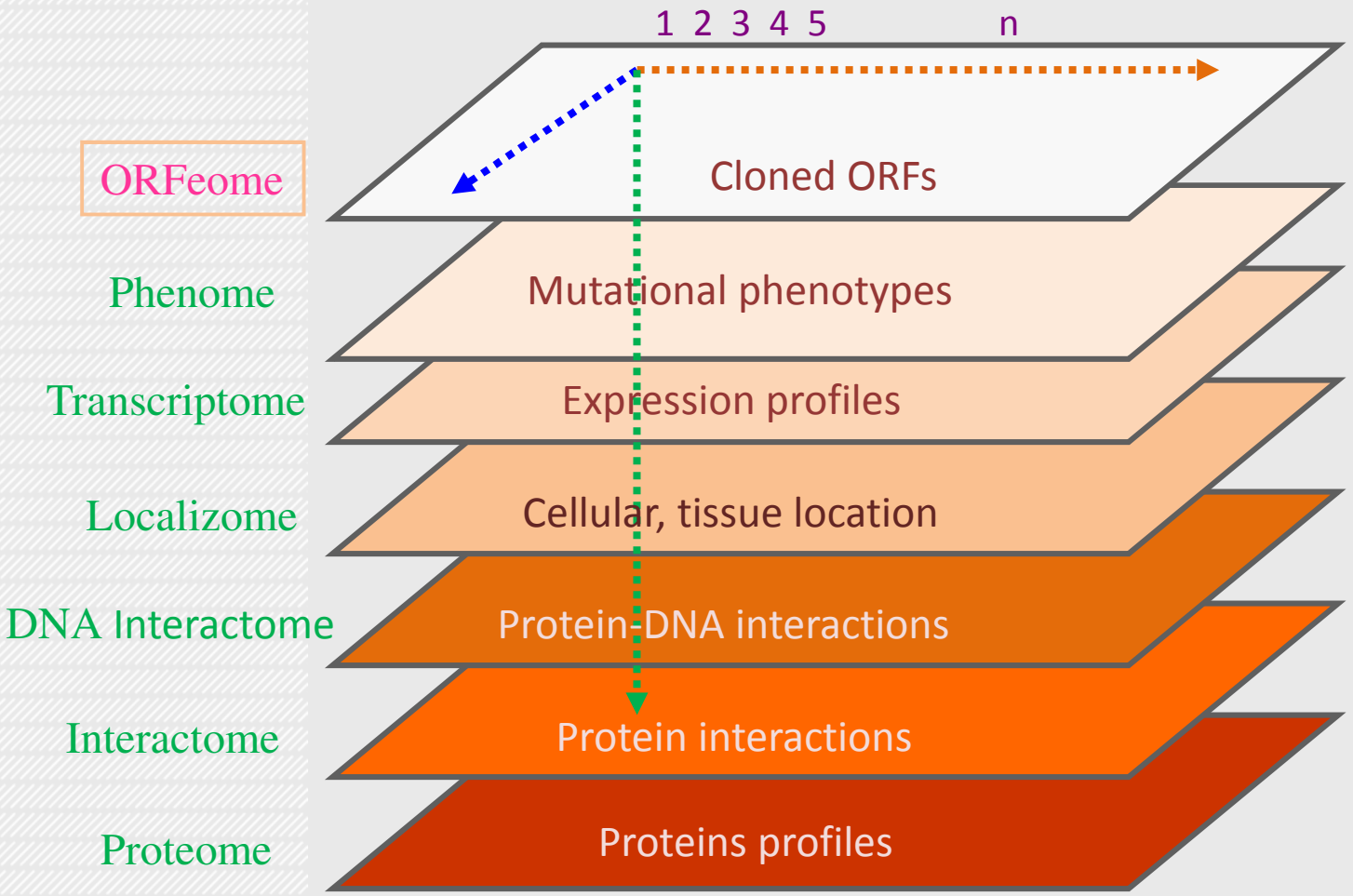
Why ORF clones ?

- Advent of systems biology necessitates the cloning of nearly entire sets of ORFs to allow functional studies of proteomes

- New challenges in post genomic era:
  - ❖ to understand the function of the many genes predicted
  - ❖ Analysis of all genes at a time
  - ❖ high-throughput preparation of versatile resource for the functional and structural studies of proteins

- Resources for functional genomics projects

ORFeome: Gateway b/w Genomics & Omes



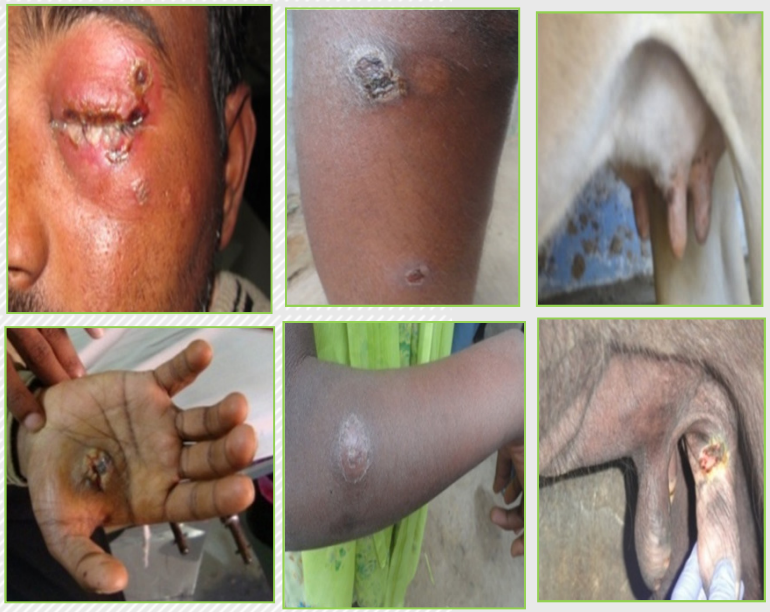
Why ORFeome of animalpox viruses?

- Functional genomics- Proteomic studies
- To study host-tropism (molecular pathogenesis)
- To develop drugs & vaccines

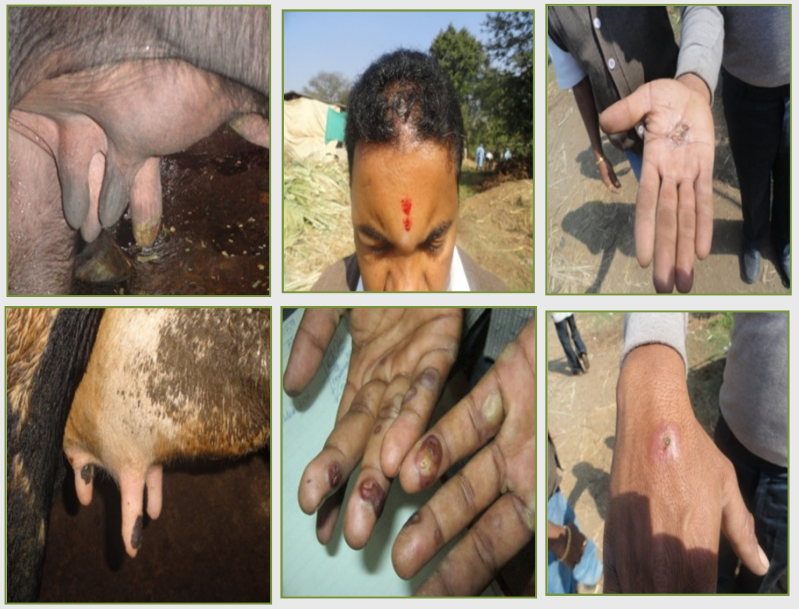
Buffalopox virus

- Zoonotic infections
  - Reduction of cohort- immunity against poxviruses in humans
  - Discontinuation of vaccination against smallpox since 1980
- Change of host tropism (inter-species jumping)
  - BPXV – Human & Cow

Severe cases BPXV Zoonosis



In 2011: Meerut, U.P.

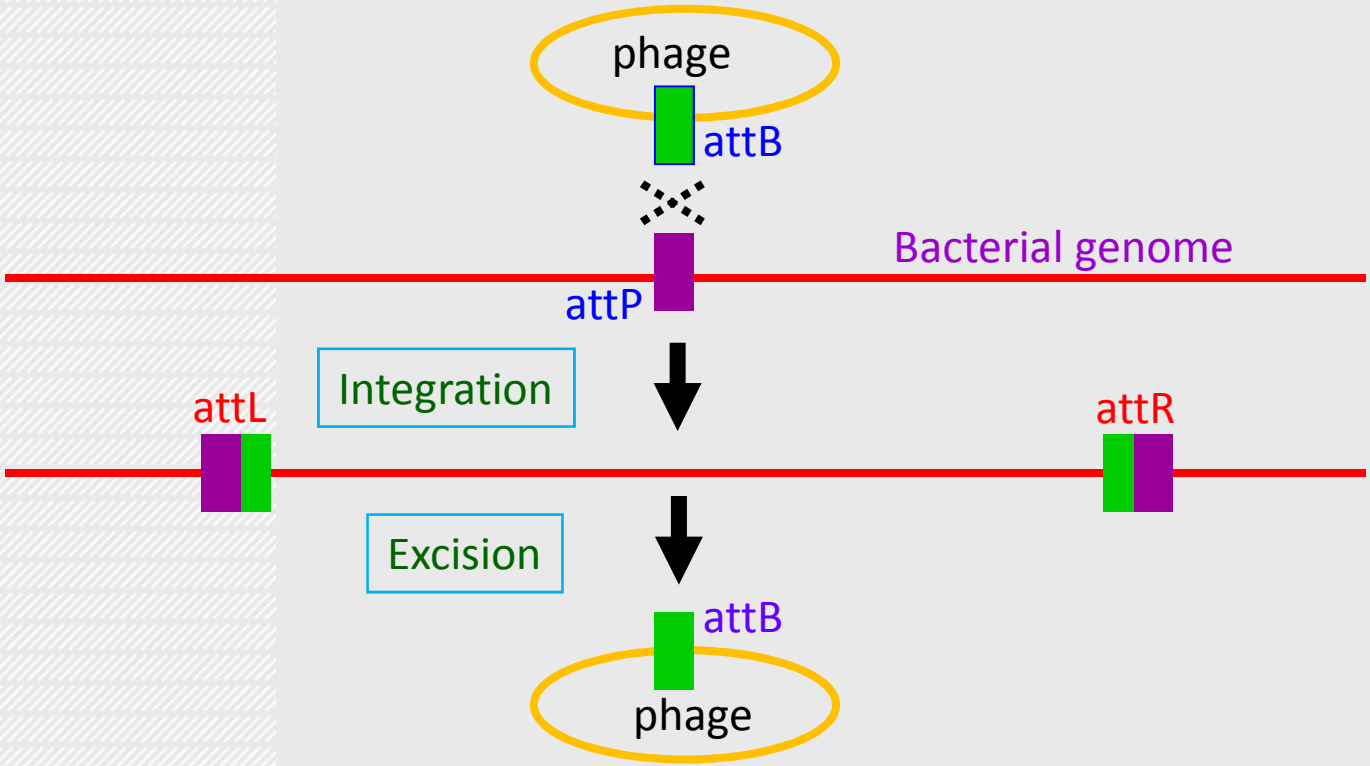


In 2013: BPXV Nashik, M.P.

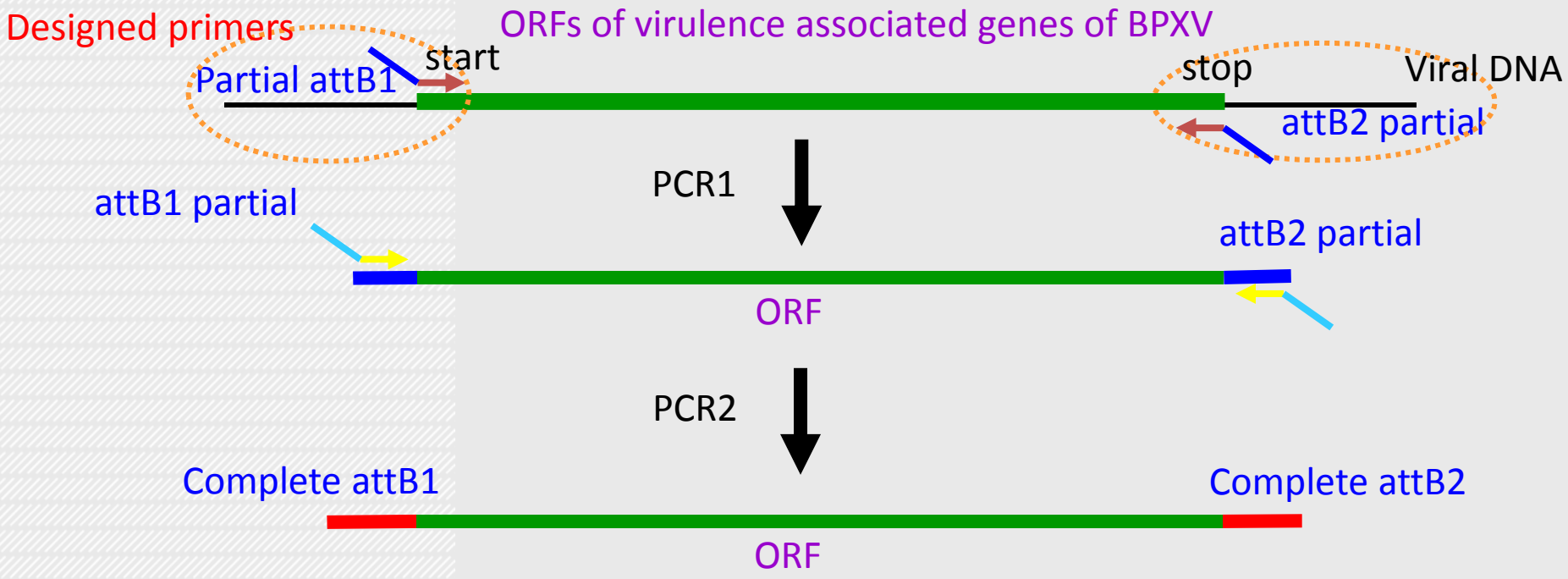
Generation of ORF clones by Recombinational cloning

- One step site specific recombination based cloning technology
- Not dependent on restriction/ligation
- Efficiency: 100% - only one recombinant DNA product without byproducts
- No cloning step needed: no need to assay independent clones
- Very precise recombination system allowing high fidelity DNA engineering
- Versatile cloning technology:
  - ❖ Genes can be easily transferred into a range of vector systems
  - ❖ Expression, Gene fusion, RNAi…
- GATEWAY Recombinational Cloning
  - ❖Based on the bacteriophage lambda integration & excision system

Lambda phage integration & excision system



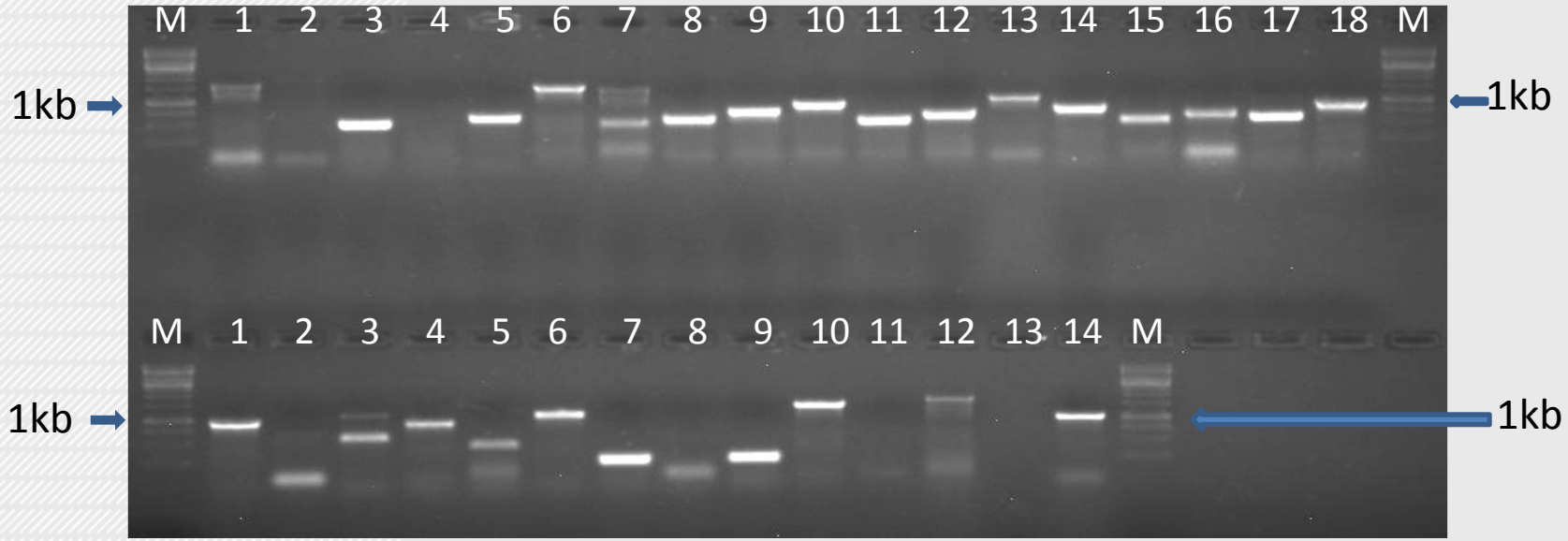
Gateway cloning strategy



Targeted BPXV-ORFs

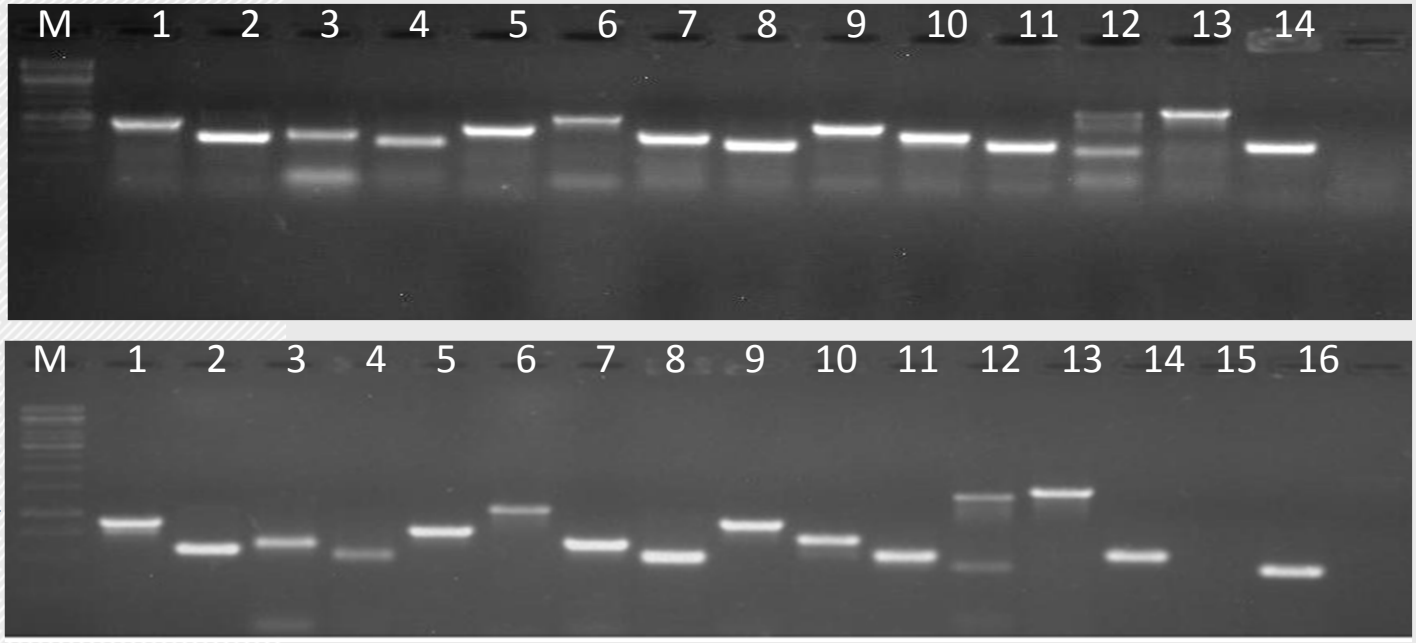
Genes of BPXV	Functions
Vaccinia virus homologue genes: CrmB, CKBP, INFA, IL-18, C7L, C3L, ZFA, N1L, K1L, K2L, K3L, B29R, K7R, A39R, A46R, B5R, VACWR208, L5R, H1L, H2R, H3L, VACWR217, A9L, A17L, A21L, VACWR207, A28L, B1R, N2L, F3L, F10L, F34L, A36L, A38L, A40R, A43R, A44L, A46R, A55R, B4R, B6R, B8R, B12R, B13R, B19R, B25R, C12L, M1L, A56R, B18R	Modulation of host immune defense

PCR amplifications of BPXV-ORFs



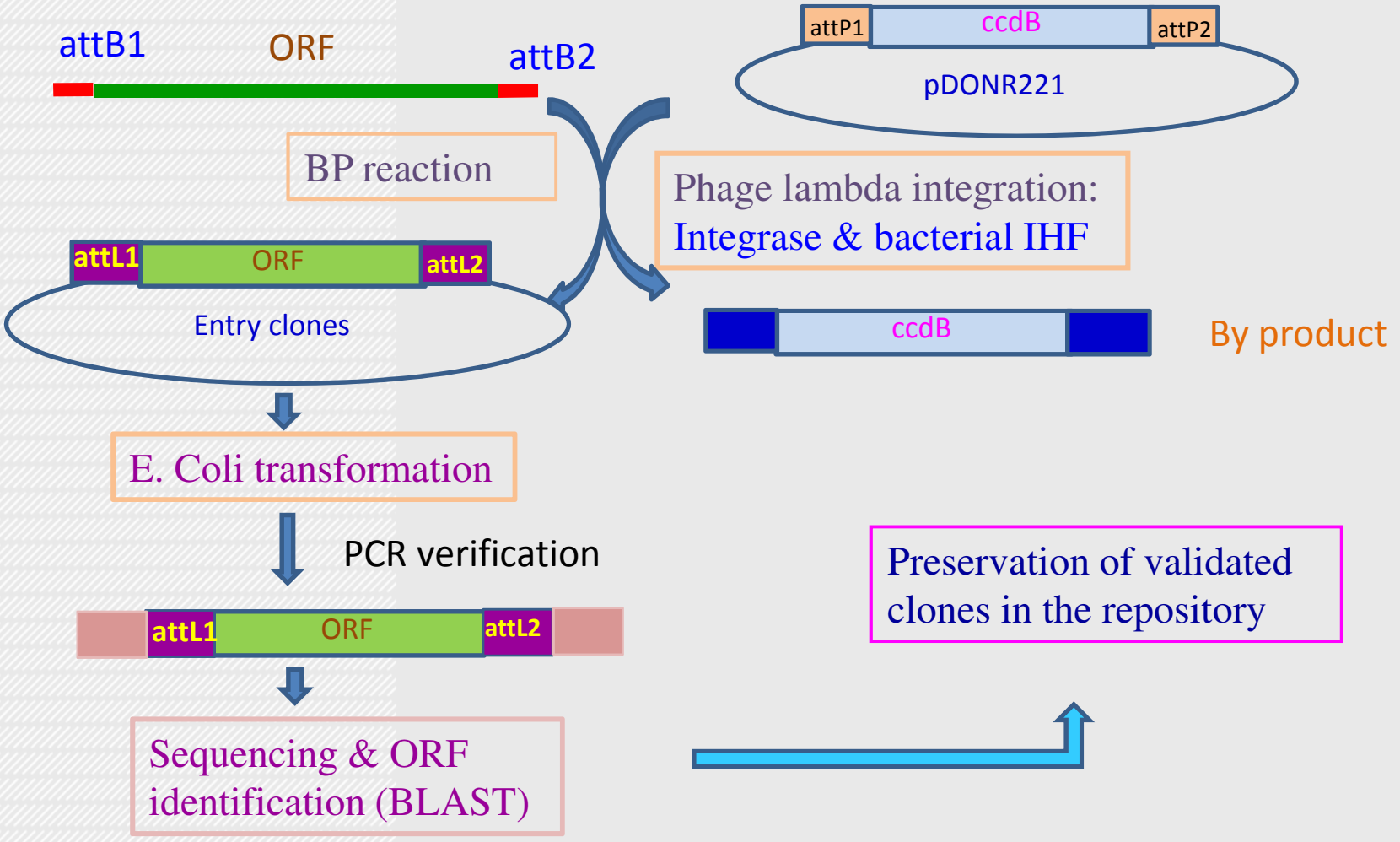
M = 1kb DNA marker,
L1-18 & L1-14 = amplicons of ORFs of BPXV

PCR amplifications of BPXV-ORFs



M = 1kb DNA marker,
L1-14 & L1-16 = amplicons of ORFs of BPXV

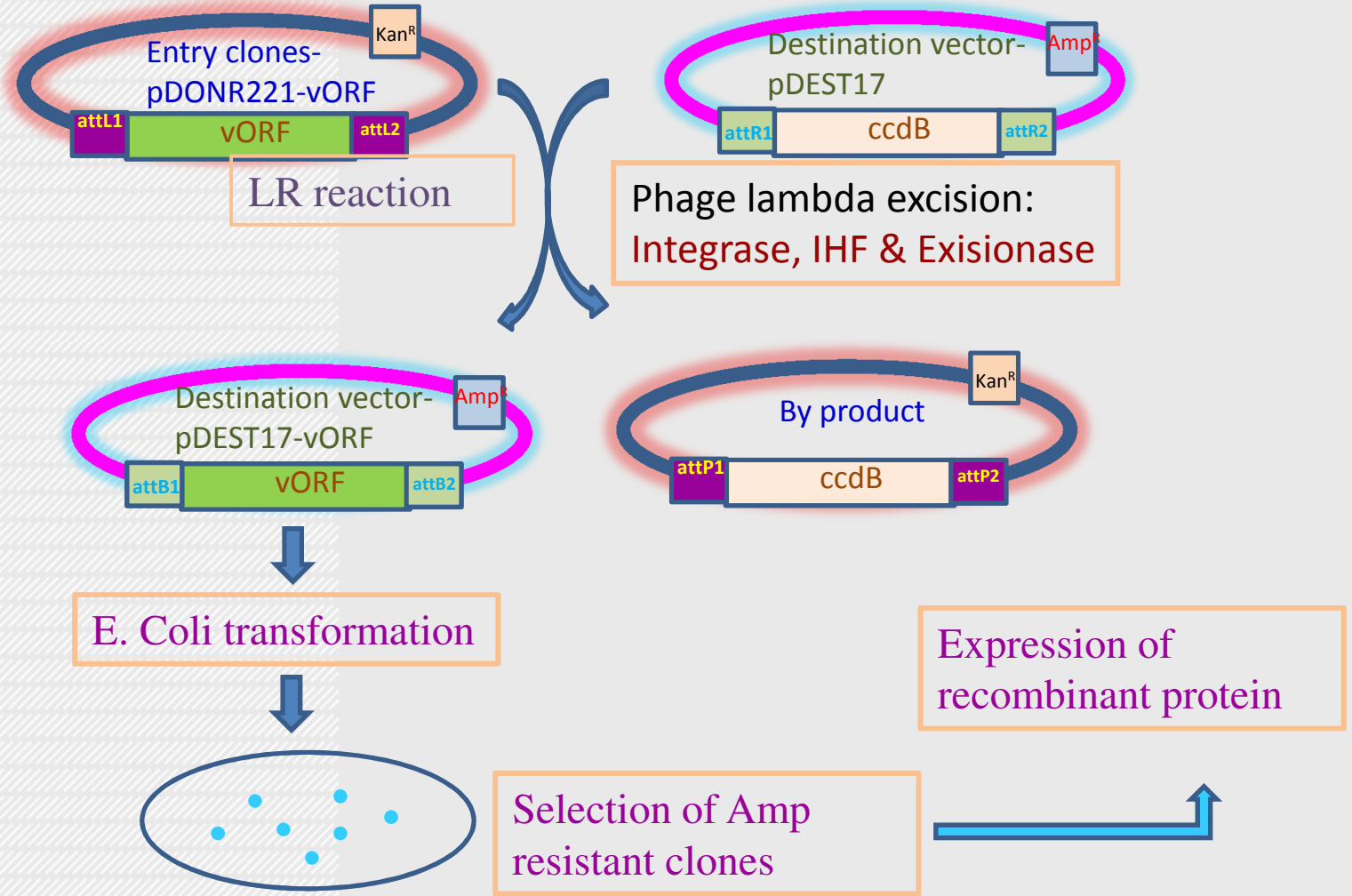
Construction of Entry clones



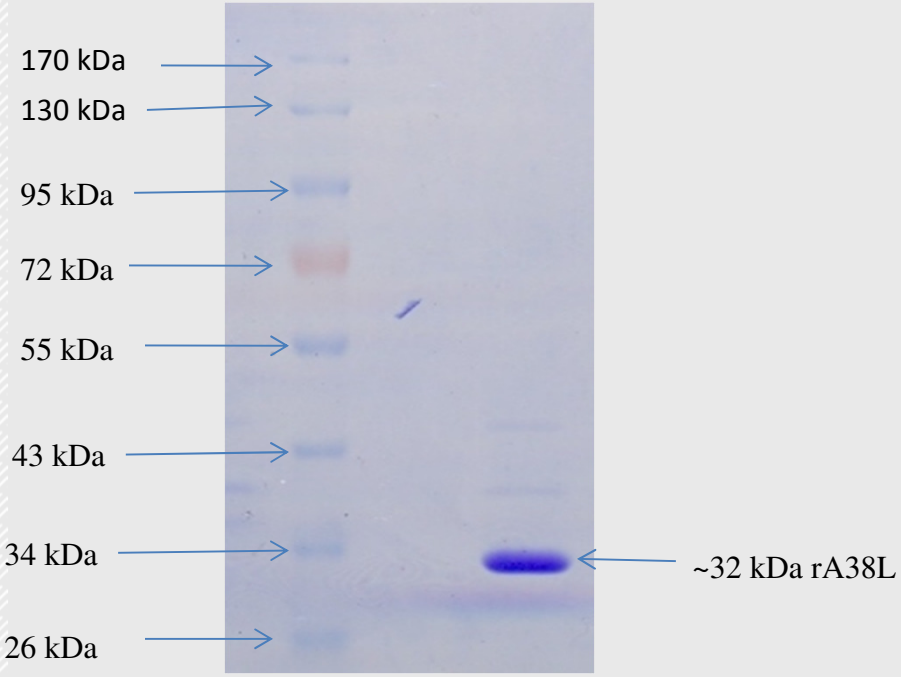
Generated gateway entry clones

- 50 gateway entry clones of BPXV-ORFs generated
- All clones validated by sequencing and BLAST analysis
- Clones preserved in the VTCC repository:
  - 5 clones (recombinant *E.coli*) of each ORF stored as glycerol stock at -80^{o}C
  - Purified recombinant plasmids stored at -80^{o}C as ethanol precipitate

Recombinational cloning into destination vector



Expression of A39R protein

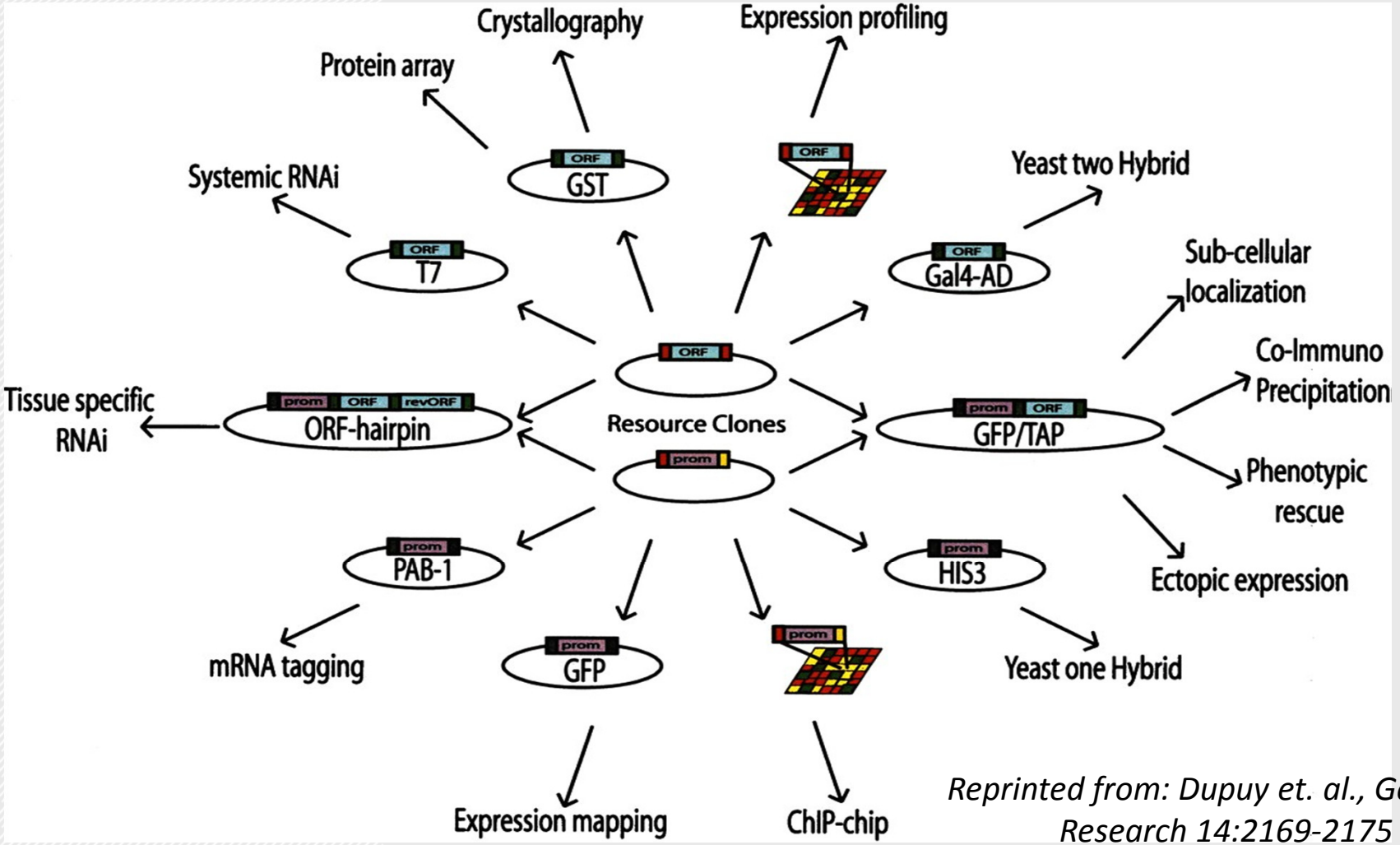


Conclusion

- Generated entry clone resource of 50 ORFs of virulence associated genes of buffalopox virus -
  - Platform for functional genomics
  - Basic biology: molecular networks, structural & functional analysis
  - Understanding pathogenesis: virus–host interaction
  - Identifying vaccine candidates : reverse vaccinology

Thank you for kind attention

APPLICATIONS OF RECOMBINATIONAL CLONING



Reprinted from: Dupuy et. al., Genome Research 14:2169-2175 (2004)