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The ϕC31 Integrase System

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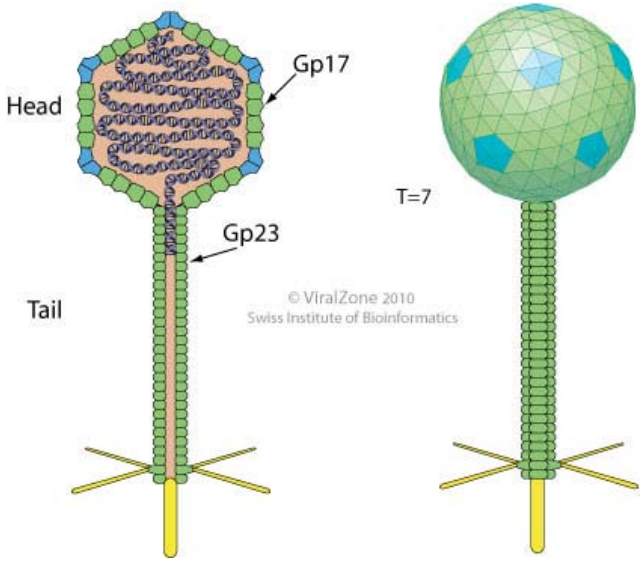
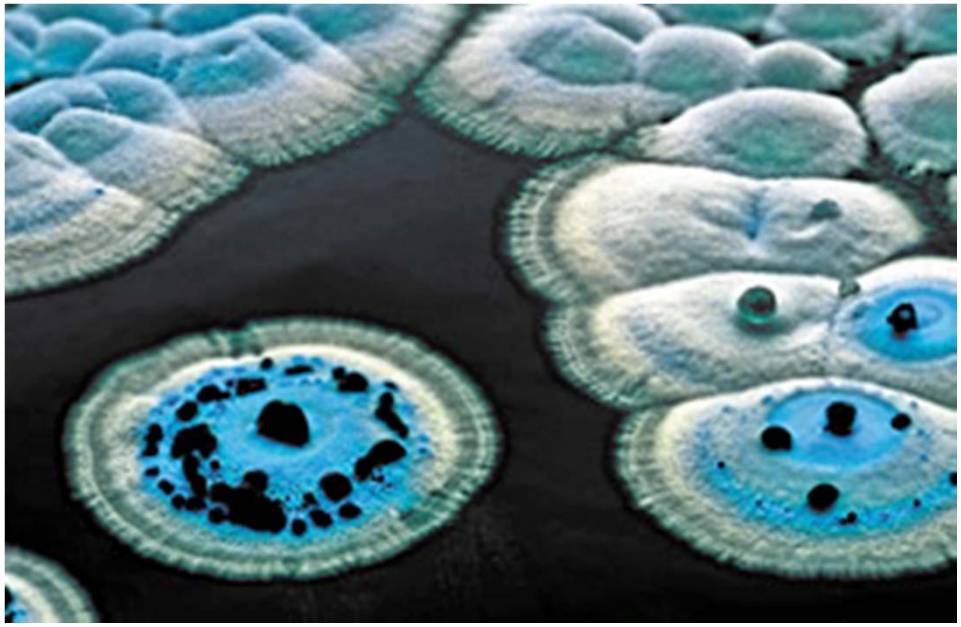
Outline

- What is ϕC31 integrase?
- Where within the human genome does ϕC31 integrase integrate donor vectors?
- Features and Benefits of the system
- How to generate stable cell lines with ϕC31
- iPSC Reprogramming & *in vivo* applications

What is ϕC31 integrase?

Sequence-specific serine recombinase from a phage of *Streptomyces coelicolor*

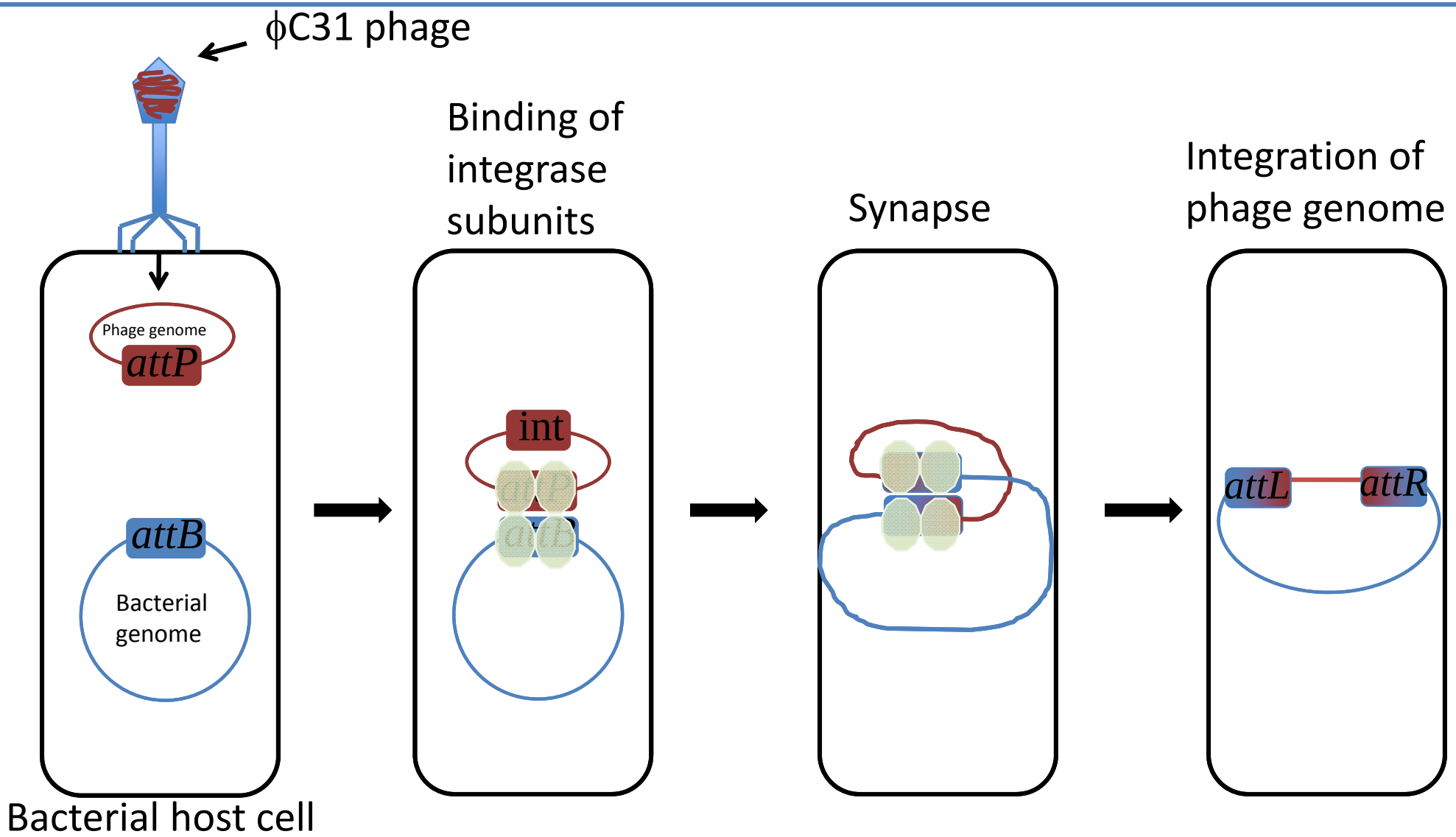




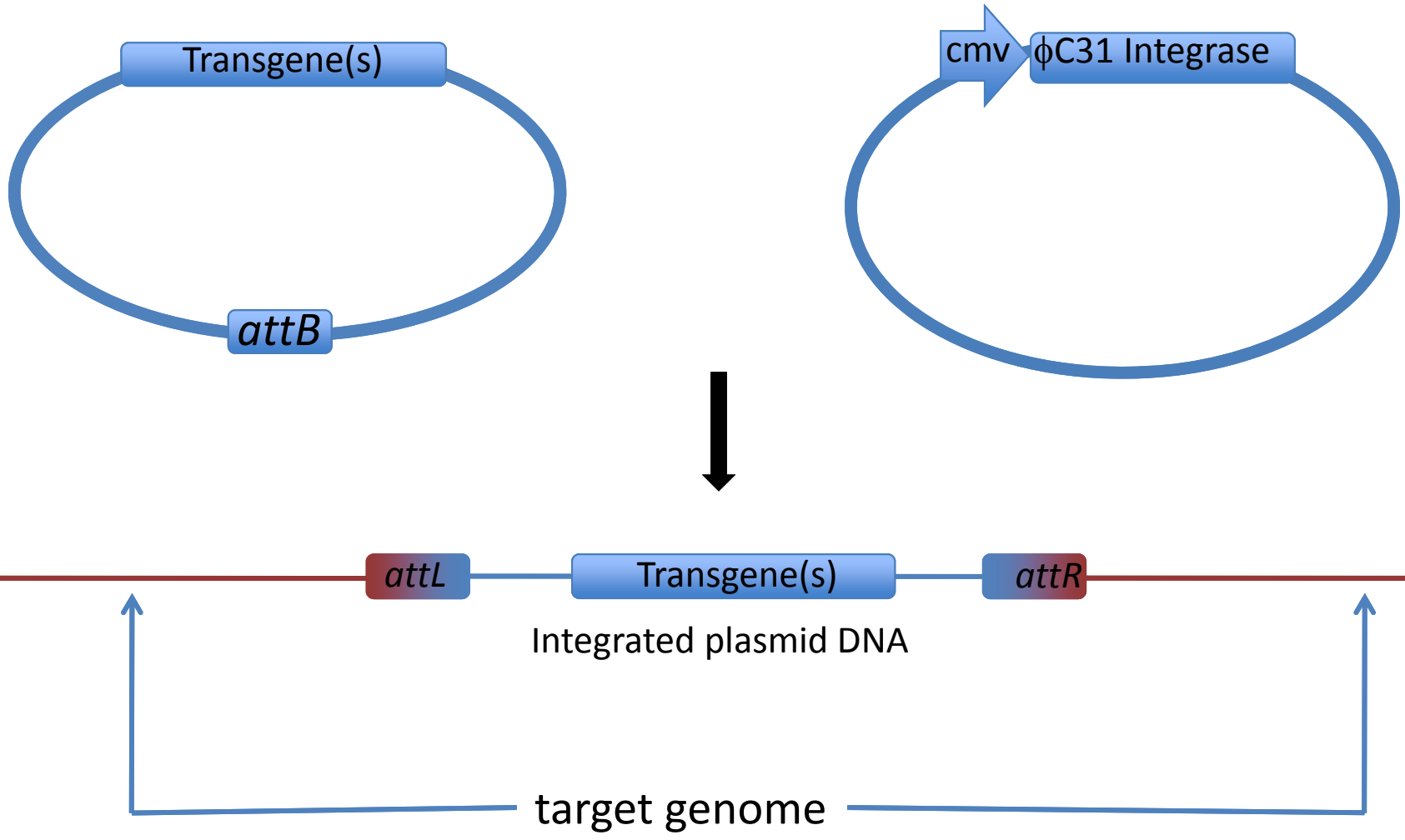
Streptomyces coelicolor colonies. From: Higher Education and Research Opportunitie, the John Innes Centre.

Normal role: integrate phage genome into specific ~34-bp sequence (*attB* site) in host genome

The ϕC31 genome integrates into the *Streptomyces* genome via ϕC31 integrase



ϕC31 integrase catalyzes unidirectional chromosomal integration at native sites with partial *attP* identity



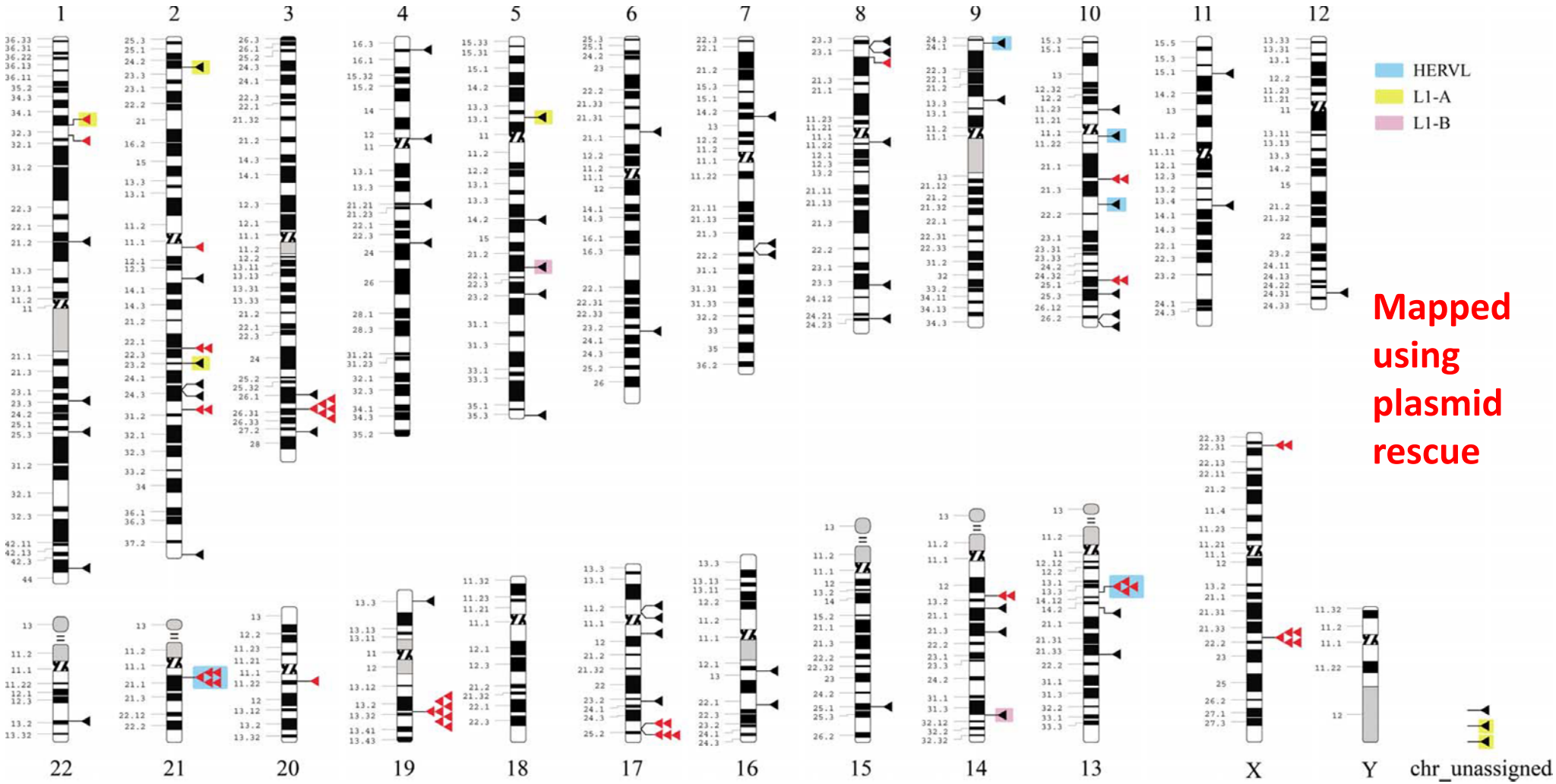
Specificity of ϕC31 integrase

- Retroviral systems often favor integration into actively expressed genes
- Integration is quasi-random with very large numbers (>10^7) of integration sites possible
- Due to the larger sequence recognition of ϕC31 integrase, a more restricted pattern of integration sites have been observed

Where within the human genome does ϕC31 integrase tend to integrate?

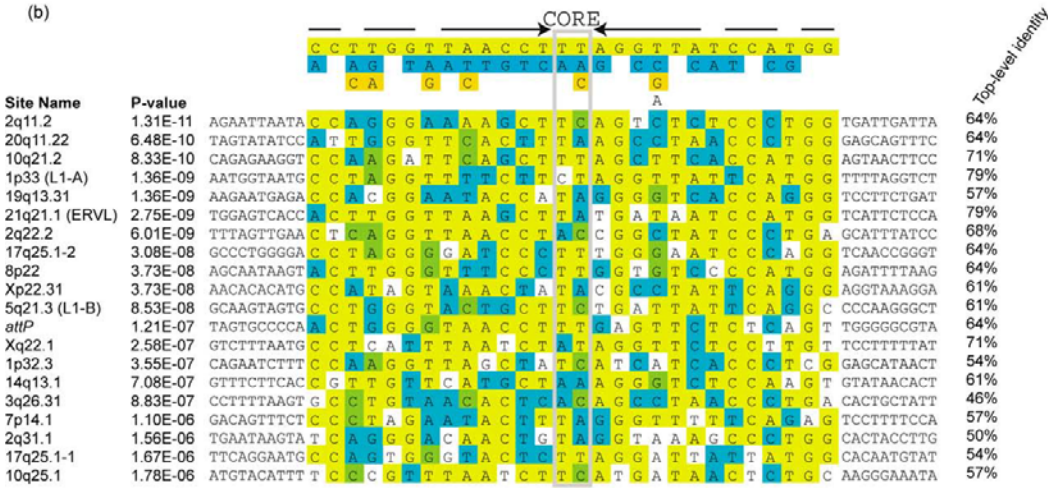
- 117 independent integration events have been identified in tissue culture cells
- 106 of which were mappable to the human genome
- 56% of all integrations occurred at 19 loci
- With 19q13.31 being the most frequent
- Number of pseudo-*attP* sites estimated within the human genome is 370

ϕC31 integration sites across the human genome



ϕC31 integration sites share a DNA consensus sequence

- The consensus is symmetrical around the core
- Contains inverted repeats extending over the length of the consensus
- Identity to *attP* ranges from 46-79%



Within what genomic elements does ϕC31 tend to integrate?

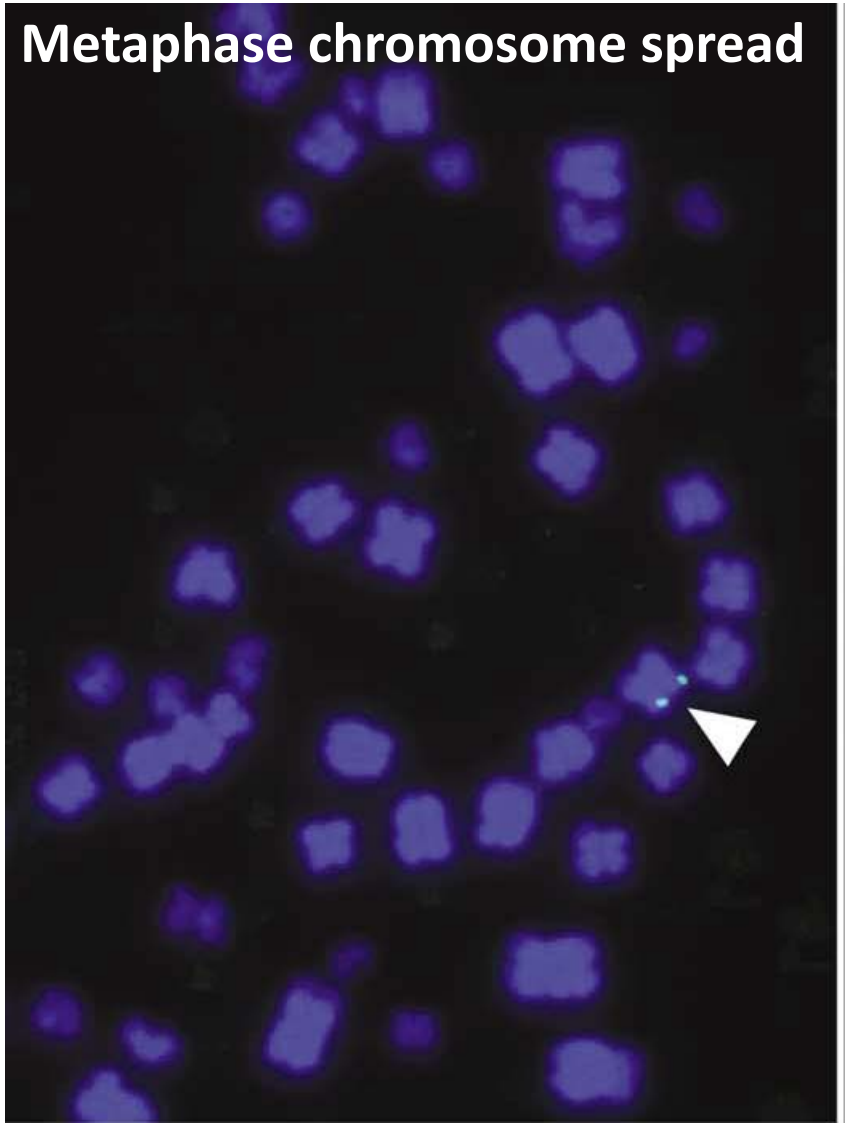
- Of the 106 mapped integration sites, 39 (36.8%) were in introns, 2 (1.9%) within exons, and 65 (61.3%) were within intergenic regions
- Approximately 24.4% of the human genome is intronic, 1.1% exonic, and 75.5% intergenic
- ϕC31 integrase is slightly skewed towards transcriptional units

How does ϕC31 integration compare to retroviral systems?

- Retroviral systems, by contrast, integrate into transcriptional units with a frequency as high as 67% for lentivirus vectors
- Can cause insertional mutagenesis, oncogene activation and tumor suppressor dysregulation
- Virally integrated transgenes are subject to silencing

ϕC31 integrase mediates integration in a single copy fashion

- Fluorescence *in situ* hybridization (FISH) was used to characterize integrations
- Probe was to the integrated *attB* plasmid
- Cell was known to contain an integration at 8p22 by PCR



Why use ϕC31 integrase?

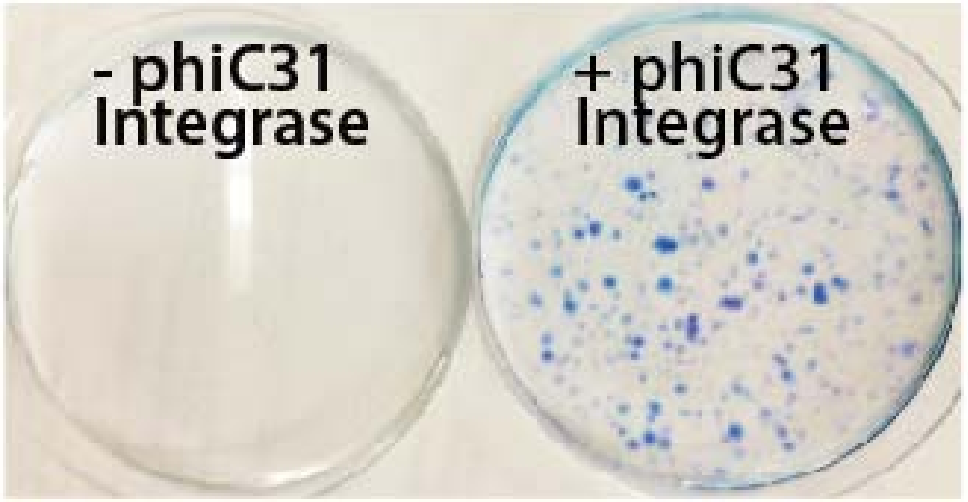
- Single-copy, non-viral delivery system
- Simple and effective way to generate stable cell lines and to genetically modify animals
- Single transfection
- Safe for the user, plasmid DNA
- Works in humans, mice, rats, pigs, chickens, cows, flies, frogs, bacteria, plants, and yeast

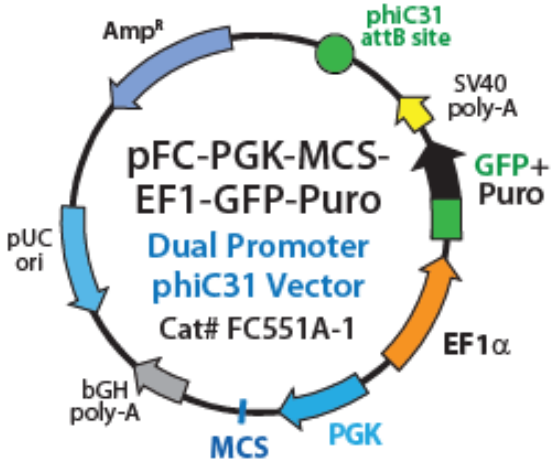
Advantages of ϕC31 integrase

- Sustainable transgene expression
- Low risk of insertional mutagenesis (which can often lead to oncogenic phenotype or chromosomal abnormalities)
- Ideal for stable transgene delivery to primary cells (which are often much more sensitive to random viral integration effects)
- Optimal for translating to *in vivo* applications (including gene therapy and regenerative medicine)

Generation of stable cell lines using the ϕC31 integrase system

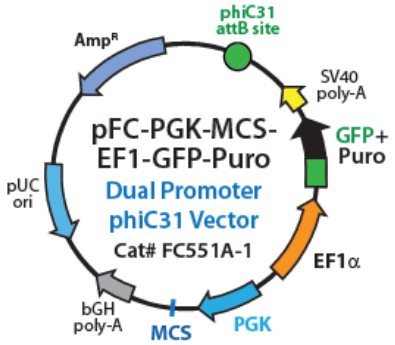
- 293 cells were transfected with an attB plasmid and +/- ϕC31 int
- The following day cells were split 1: 20 onto 10 cm dishes
- Puro selection was carried out for 11 days

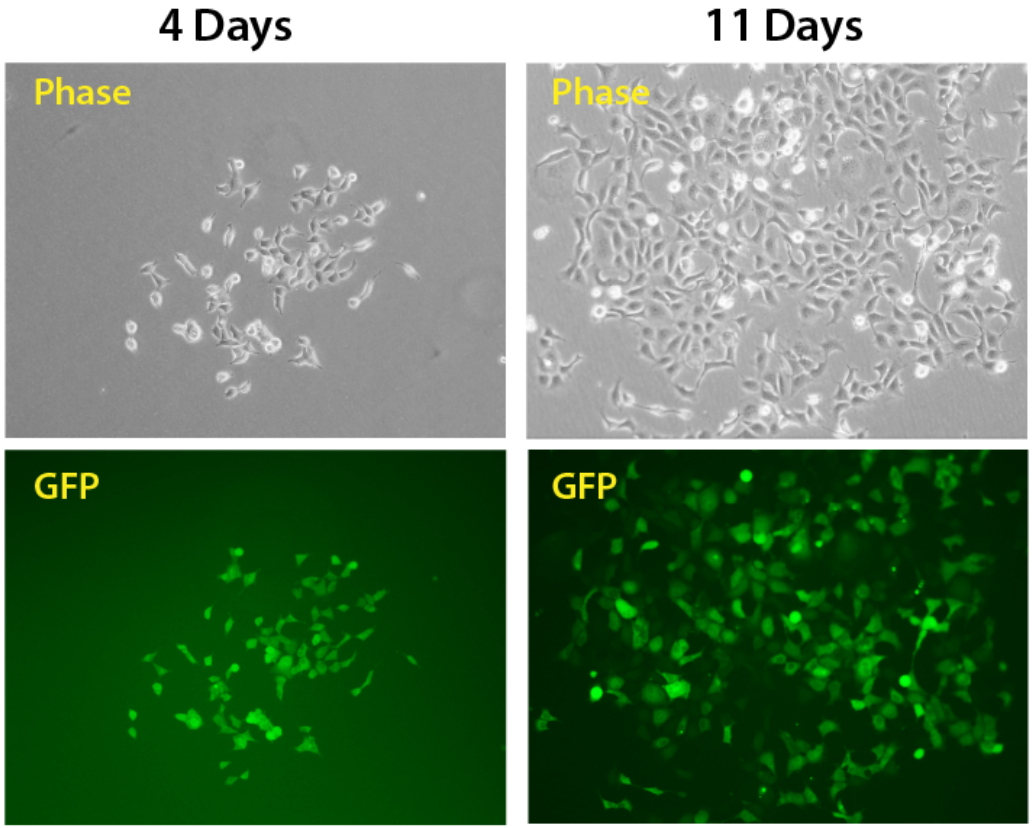




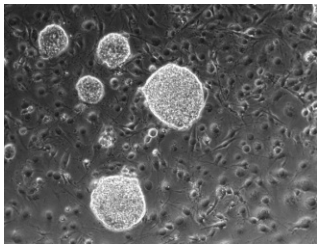
Generation of stable cell lines using the ϕC31 integrase system

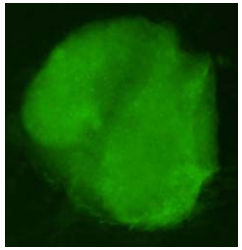
- 293 cells transfected with a GFP/puromycin expressing *attB* plasmid
- After puromycin selection colonies are formed
- Colonies can be picked and expanded
- Integration sites can be determined by plasmid rescue or LM-PCR

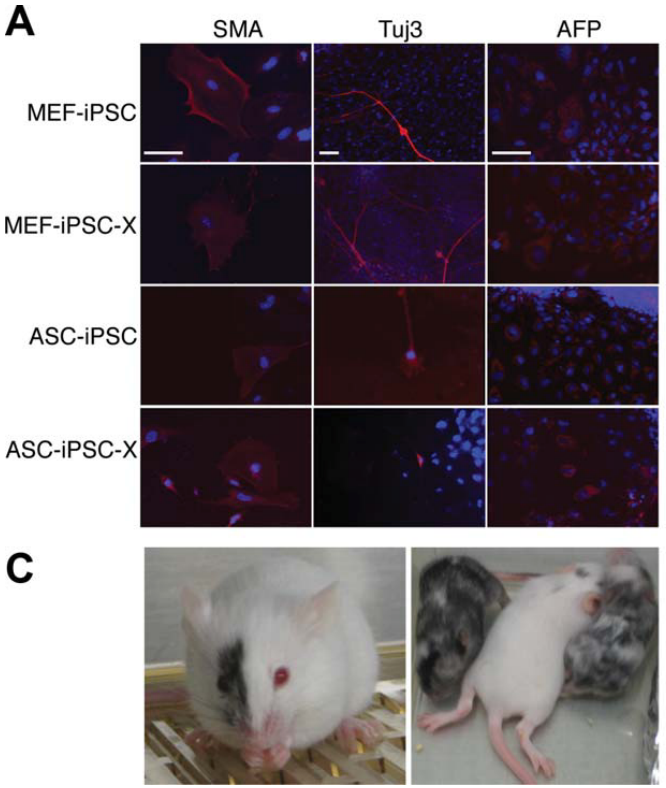




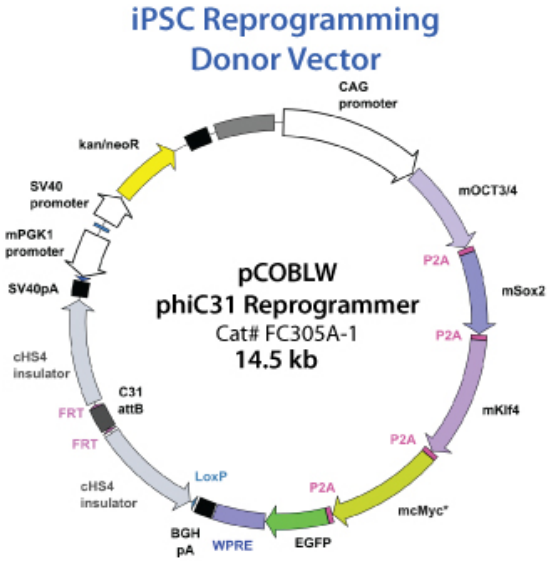
Generation of mouse induced pluripotent stem cells (iPSC)

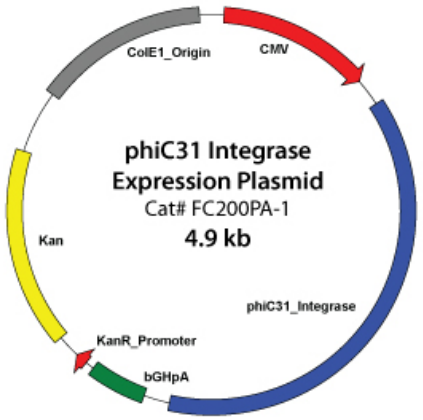




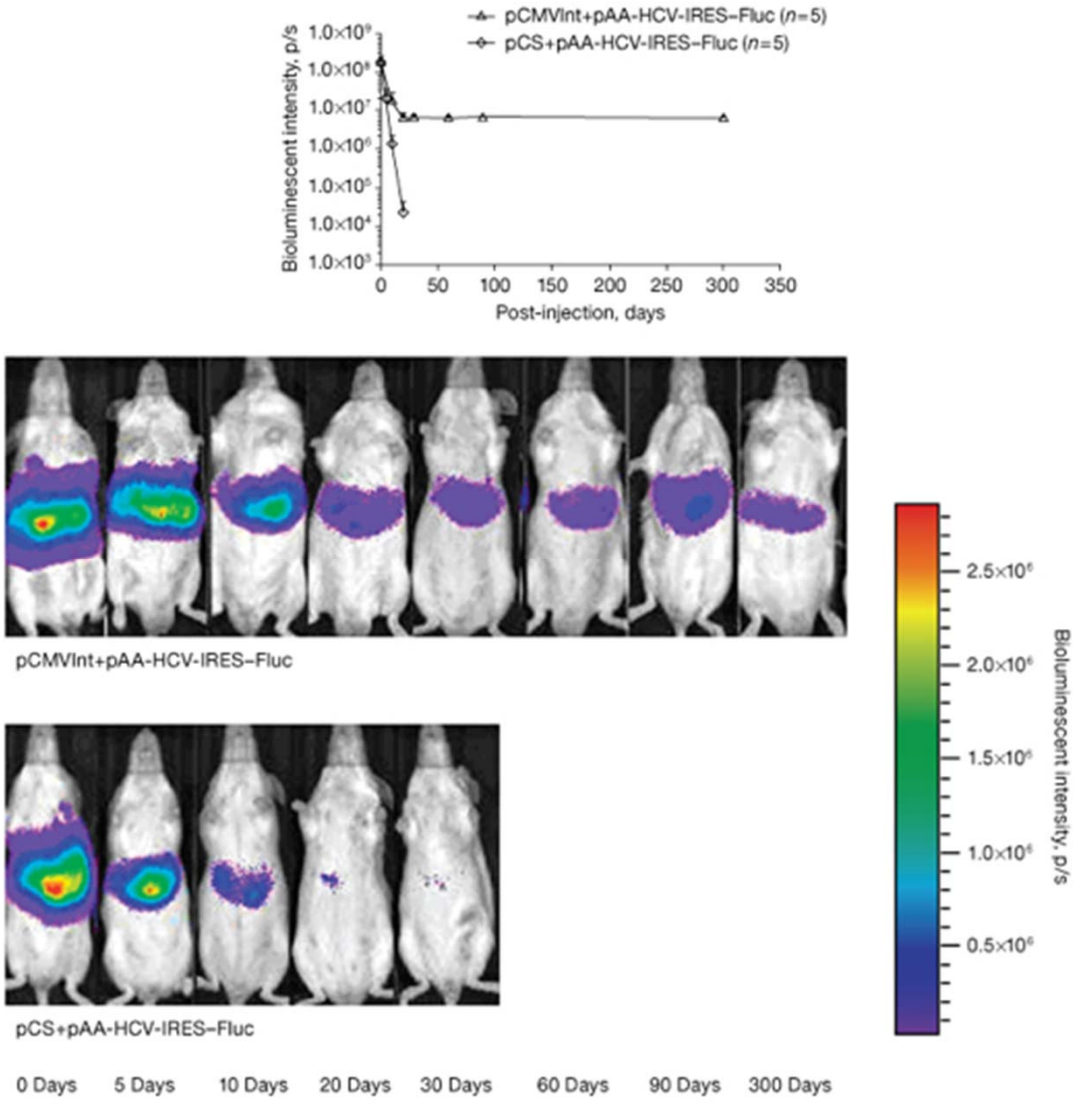


Karow, Chavez, Farruggio et al. 2006



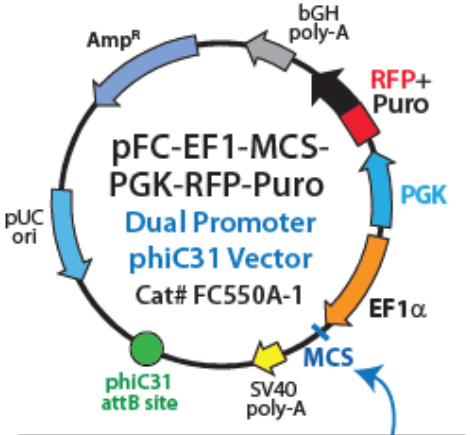


The ϕC31 integrase system can generate long-term transgene expression in mouse liver



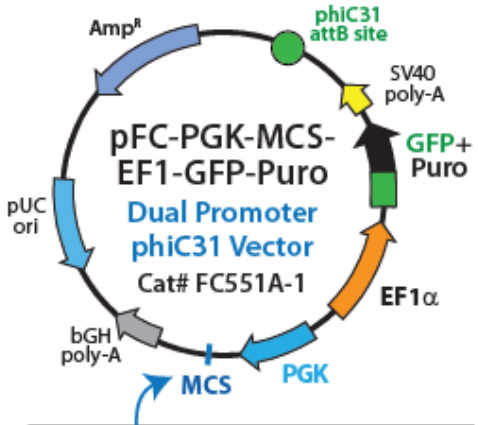
Sun, Z *et al*. 2008

Vectors currently available at SBI



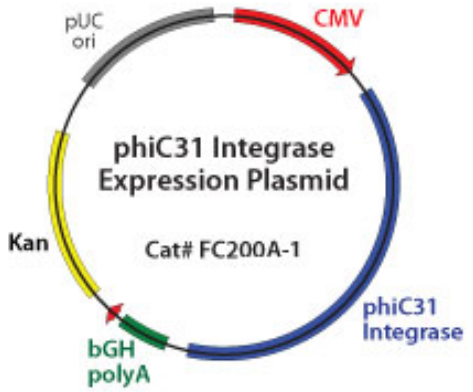
MCS

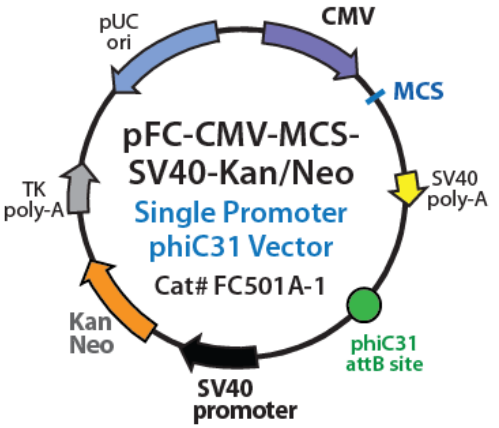
5′- EcoRI-EcoRV-Acc65I-KpnI-NheI-AvrII-MluI-3′



MCS

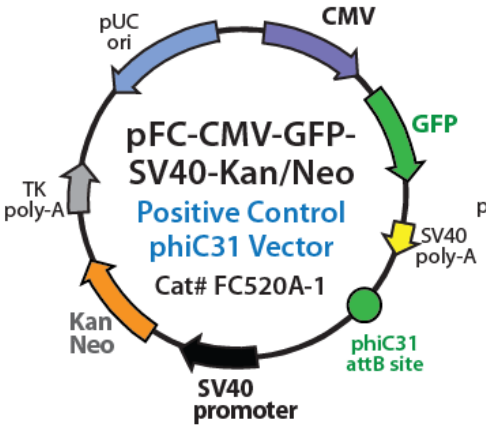
5′- ClaI-NheI-Acc65I-KpnI-EcoRV-BamHI-3′

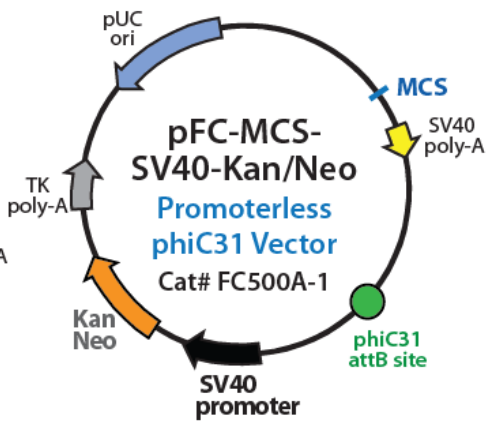




FC501A-1 MCS

5′- NheI-Eco47III-AgeI-XhoI-XbaI-SpeI-NotI-
-SbfI-PstI-BamHI-BglII-EcoRV-HindIII-MluI-3′





FC500A-1 MCS

5′- XhoI-XbaI-SpeI-NotI-NheI-
-BamHI-BglII-EcoRVHindIII-NdeI-3′

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