TARGATT™ノックインマウス技術を用いたサイト特異的トランスジェニックマウスの作製

Production of site-specific transgenic mice using the TARGATT™ knock-in technology

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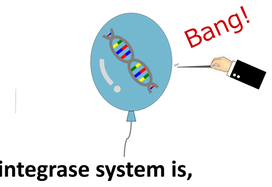
Summary

To produce transgenic (TG) mice, the technique of pronuclear injection of DNA, which results in random integration of the transgene, is commonly used. However, this classical method is unable to regulate the insertion site and copy number of the transgene, which occasionally causes disruption of endogenous genes by transgene insertion and silencing by tandem insertion of transgene. To overcome these problems, an US company, Applied Stem Cell Inc. (ASC), has developed the TARGATT™ TG system, which is based on ϕC31 integrase-based knock-in technique and has a great advantage in highly efficient, single-copy insertion in an active locus. ASC is developing TG mice and rats under the exclusive license from Stanford University. The ϕC31 integrase catalyzes an irreversible recombination event between triple tandem attP sites (attPx3) that have been engineered into a preselected, safe harbor locus (Hipp11) in the mouse genome, and a donor vector containing the gene of interest flanked by attP-recognition (attB) sequences. Co-injection of the donor DNA and ϕC31 integrase RNA into pronuclear stage embryos enables large transgene insertion (up to 22 kb) with very high efficiency (up to 40% or more). Our center has contracted a collaborative research agreement with ASC and are making TG mice using the TARGATT™ KI technology in consultation with ASC. We have already succeeded in the production of CAG-eGFP TG and Cre-ERT2 TG mice

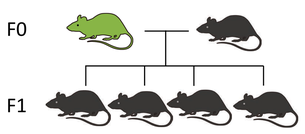
Background & Purpose

➢ **Problems in common TG production**

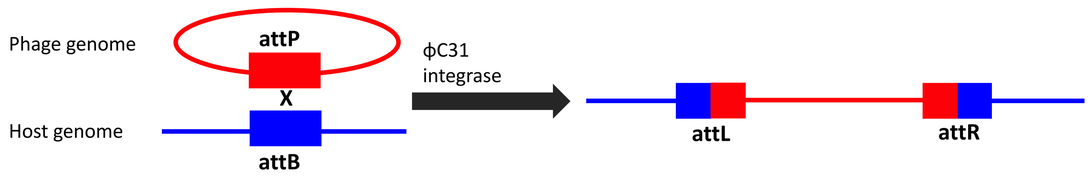
■ Disruption of an endogenous gene by the inserted transgene



■ Gene silencing due to tandem insertion



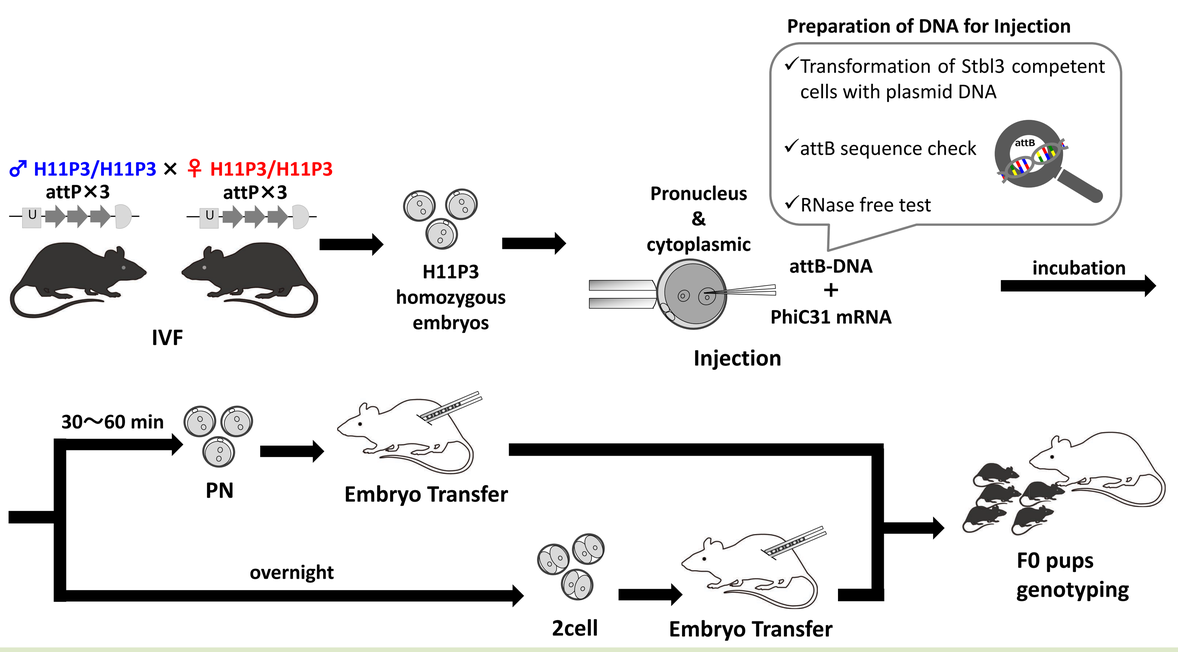
➢ **ϕC31 integrase system is,**
a site-specific gene integration system originally identified as a mechanism of phage DNA insertion into bacteria genome. The system requires no cofactors, therefore, it is active in other organisms, allowing us to use it as a tool for virus-free, one step integration of a gene of interest.

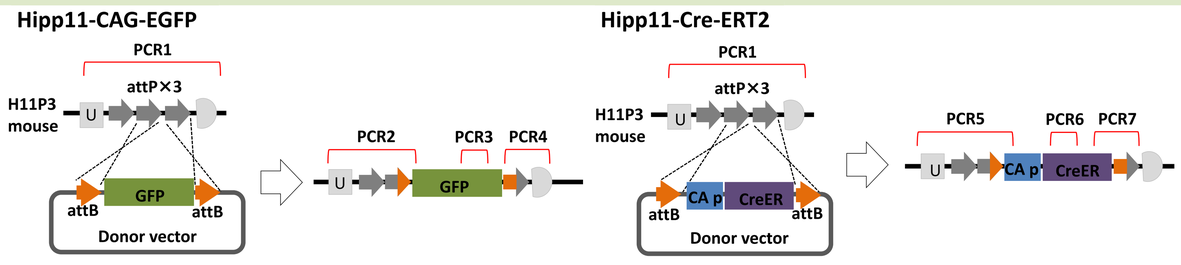


ϕC31 integrase,
is a sequence-specific serine recombinase encoded in bacteriophage ϕC31 genome. It catalyzes recombination between attP sequence in the phage genome and attB sequence in the host genome, which results in integration of attL-attR in the host genome.

➢ **TG mice production using ϕC31 integrase system**
(TARGATT™ knock-in system)
The ϕC31 integrase catalyzes an irreversible recombination event between triple tandem attP sites (attPx3) that have been engineered into a preselected, safe harbor locus (Hipp11) in the mouse genome, and a donor vector containing the gene of interest flanked by attP-recognition (attB) sequences. Co-injection of the donor DNA and ϕC31 integrase RNA into pronuclear stage embryos enables large transgene insertion (up to 22 kb) with very high efficiency (up to 40% or more). Hipp11 is a locus in chromosome 11, which stably induce the integrated gene like ROSA26 locus.

Method





Results

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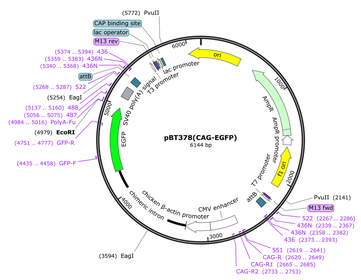

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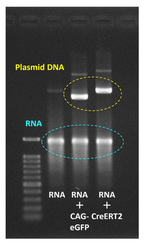

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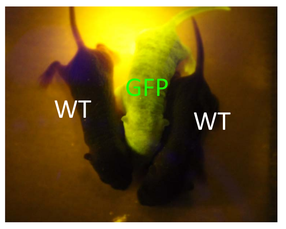

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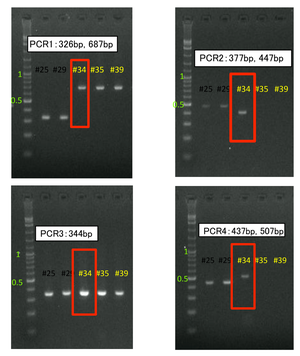
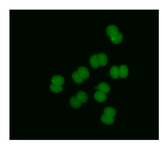
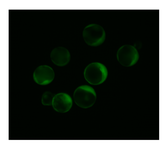
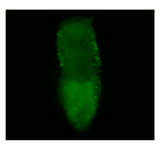
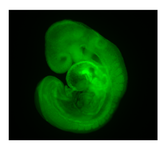
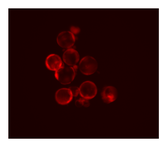
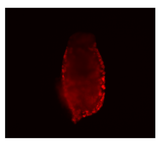
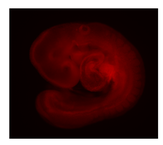


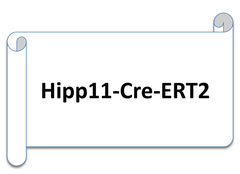
<Summary table>

No.of trials	Conc. of nucleic acids		total no. of injected oocytes	total no. of oocytes survived (%/injected)	total no. of transferred		total no. of pups		
	DNA [ng/uL]	RNA [ng/uL]			stage	embryos	no. of recovered pups (%/embryos)	no. of survived pups	no. of transgene positive pups (%/survivrd pups)
1	3	100	262	161 (61.5)	2cell	113 (70.2)	37 (32.7)	37	0
2	3	100	256	174 (68.0)	2cell	99 (56.9)	10 (10.1)	9	0
3	3	100	288	276 (95.8)	2cell	111 (40.2)	14 (12.6)	13	1 (7.7)
4	5＊	50	205	124 (60.5)	2cell	80 (64.5)	6 (7.5)	6	0
5	5＊	50	194	110 (56.7)	2cell	94 (85.5)	13 (13.8)	12	0
6	5＊	50	389	263 (67.6)	2cell	180 (68.4)	44 (24.4)	43	1 (2.3)

＊ Purifed at TMDU

<Embryos>

	2 cell	Blastocyst	E7.5	E9.5
Hipp11-CAG-eGFP				
ROSA-tdTomato	—			



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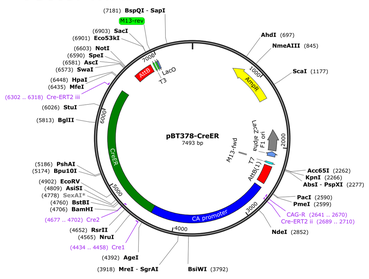

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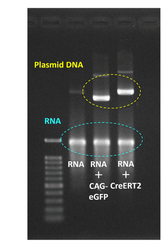

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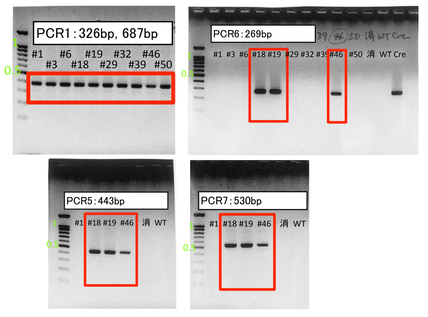

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	DNA [ng/uL]	RNA [ng/uL]			stage	embryos	recovered pups (%/embryos)	survived pups	transgene positive pups (%/survivrd pups)
1	5	50	229	155 (67.7)	2cell	102	16 (15.7)	15	0
2	5	50	384	278 (72.4)	2cell	210	33 (15.7)	32	0
3	5	50	147	92 (62.6)	PN	92	17 (18.5)	5	0
4	5	50	366	241 (65.8)	PN	238	23 (9.7)	19	3 (15.8)
5	5	50	317	214 (67.5)	PN	214	31 (14.5)	27	4 (14.8)

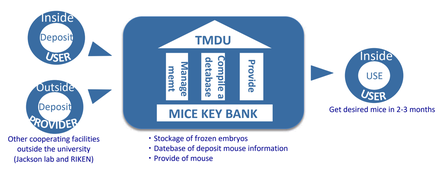
Discussion

1. Advantages in TARGATT™ TG mice production

- Do not disrupt endogenous gene expression
- Time-saving and cost-effective gene integration using highly efficient ϕC31 integrase system
- Single copy knock in is free from repeat-induced gene silencing and genomic instability
- No need of large-scale clone screening
- Variety of donor plasmid vectors
  TARGATT™2: CAG-PolyA, TARGATT™3: no promoter-MCS,
  TARGATT™6.1: CAG-L4SL-MCS-PolyA, TARGATT™7: PGK-MCS-PolyA,
  TARGATT™8: PCA-MCS-PolyA, TARGATT™9.1: PCA-L4SL-MCS-polyA

2. The Mice Keybank system at TMDU: Storage and resource system of TARGATT ™ strains for internal

Our Center is planning to provide the produced TARGATT TGs to the institutional internal users via the Mice Keybank system, which was founded to collect, store and share useful mouse strains among internal users.



Acknowledgment

We thank Prof. Hiroshi Asahara and Dr. Yoshiaki Ito (Dept. of Systems BioMedicine, TMDU) for construction of Cre-ERT2 plasmid.