Applied StemCell, Inc.
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Datasheet

TARGATT™ Mouse Tail Genotyping Kit (Rosa26P3 and H11P3)

Product Information

Catalog Number
AST-2005 H11P3
AST-2006 Rosa26P3

Size
100 reactions

Description
The TARGATT™ Mouse Genotyping Kit provides a convenient method to genotype TARGATT™ mice or transgenic mice generated from TARGATT™ H11P3 or Rosa26P3 embryos.

Shipping
Dry ice

Storage and Stability
See table.

Safety Precaution
PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protective Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution.

Limited Use Label License
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Contents

Catalog#	Component	Amount	Storage
AST-2005-a / AST-2006-a	Tail lysis buffer	22 mL	4°C
AST-2005-b / AST-2006-b	Proteinase K (20 mg/ml)	330 µL	-20°C
AST-2005-c / AST-2006-c	Primer set	110 µL	-20°C
AST-2005-d / AST-2006-d	Primer set SSL	110 µL	-20°C
AST-2005-e / AST-2006-e	Primer set SSR	110 µL	-20°C
AST-2005-f / AST-2006-f	Nuclease-free water (2 vials)	1 mL	4°C
AST-2005-g / AST-2006-g	Positive Control (SSL/SSR)	20 µL	-20°C

Reagents Required but not Provided

- Taq DNA polymerase, dNTPs and necessary buffer

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- Agarose gel and DNA standard

Protocol

1. Genomic DNA extraction from tail samples

1.1 Collect approximately 1-2 mm of tail tissue in a 2 mL microcentrifuge tube.
1.2 Add 200 µL Tail lysis buffer and 3 µL Proteinase K solution to each sample.
1.3 Incubate in a 55ºC water bath with occasional inverting until tissue is completely digested (4 hours to overnight).
1.4 Heat the samples at 90ºC for 5 minutes to inactivate Proteinase K.
1.5 Proceed to the PCR reaction or store at -20ºC.

2. Genotyping by PCR

2.1 Set up PCR reaction on ice according to instruction of the Taq polymerase manufacturer. For QIAGEN Taq polymerase use table below.

Component	Amount
DNA – Tail lysis *	1 µL
10X PCR buffer	2 µL
dNTPs (10 mM)	0.4 µL
Primer set (10 µM) **	1 µL
Taq Polymerase (5 U/µL)	0.2 µL
Nuclease-free water	15.4 µL
Total Volume	***20 µL***

* Briefly spin the tube before opening and use 1 µl supernatant for one single PCR reaction.
**Primer sets (See step #2.2)

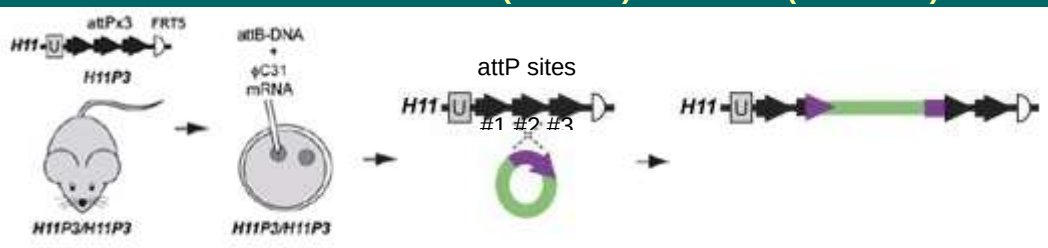
2.2 Primer sets for genotyping:
2.2.1 Use “Primer Set H11P3” (AST-2005) or “Primer Set Rosa26P3” (AST-2006) to examine whether the mouse has *attP* insertion at the correct locus (H11 or Rosa26).
2.2.2 Use “Primer Set SSL” and “Primer Set SSR” to determine site specific gene integration.

2.3 Perform PCR amplification using the following program.

Step	# of Cycles	Temperature	Time
1	1	94ºC	3 min
2	35	94ºC	30 s
3		58ºC	25 s
4		72ºC	45 s
5	1	72ºC	5 min

PCR Result Interpretation

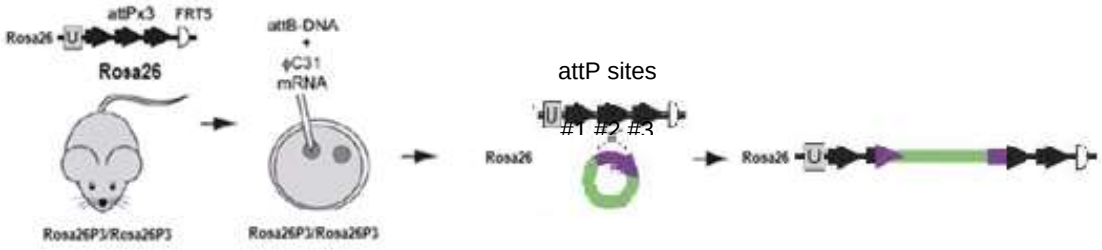
Primer Set H11P3: To detect the *attP3* modified (knockin) H11 locus (AST-2005)



- 690 bp PCR product for attPX3 modified H11 allele.

- 364 bp PCR product for wild type H11 allele.
- You will get both bands for heterozygotes.
- Allele containing transgene will produce a fragment 570bp+transgene or 620bp+transgene in sizes.

Primer Set R26P3: To detect the *attP3* modified (knockin) Rosa26 locus (AST-2006)



- 663 bp PCR product for attPX3 modified Rosa26 allele.
- 278 bp PCR product for wild type Rosa26 allele.
- You will get both bands for heterozygotes.
- Allele containing transgene will produce a fragment 523bp+transgene or 593bp+transgene in sizes.

Primer Set SSL: To detect 5' junction (*attL*) if ϕC31 catalyzed integration occurred.

- 136 bp PCR product for 5'-insertion at attP site 1.
- 206 bp PCR product for 5'-insertion at attP site 2.
- Positive control will show one of the two bands.

Primer Set SSR: To detect 3' Junction (*attR*) if ϕC31 catalyzed integration occurred.

- 225 bp PCR product for insertion at attP site 2.
- 155 bp PCR product for insertion at attP site 3.
- Positive control will show one of the two bands.