Transgenic Core Facility

Institute of Molecular Biology, Academia Sinica
2789-9312, 2652-1438

Case #: _____

ES Cell Gene Targeting Application Form

Request date: (IMB secretary)	Submission date: (TCF staff)	Approved date: (TCF manager)

Requester		**Institute**	
PI		**Phone**	

Construct Name	
Preferred ES Cell Genetic Background	☐ **C57BL/6** ☐ **129/SvJ×129/Sv (R1)** ☐ **Other _____**
Genomic Library Origin	☐ **129/SvJ** ☐ **129/SvEv** ☐ **C57BL/6J** ☐ **Other _____**
Genomic Library Format	☐ **BAC** ☐ **λ-library** **Source: _____**
Transgene Origin (knock-in construct)	☐ **Mouse** ☐ **Human** ☐ **Chicken** ☐ **Other _____**
Nature of Construct	☐ **Knock-in** ☐ **Knock-out** ☐ **Conditional** ☐ **Other _____**
Expected Phenotype (Heterozygous)	☐ **Potential lethal** ☐ **Unknown** ☐ **Others _____** **Please check 'Unknown' if no reference!**

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Construct Description	
Gene Location	**Chromosome _____**
Circular Plasmid Preparation Method	**☐ $CsCl_2$ Banding ☐ Qiagen Column** **☐ Other _____**
Construct Size	
Enzyme for Linearization	
Positive Selection	**☐ PGK-neo ☐ Mc1-neo** **☐ Other _____ (include promoter)**
Negative Selection	**☐ PGK-DT ☐ PGK-TK** **☐ Other _____ (include promoter)**
Please paste the gel photo here to proof your construct has been completed	
Note: Please make sure you satisfy the following requirements ☐ Gel electrophoresis should be clear and all the fragments should be fully separated ☐ Gel photo should be large and the following info should be included: a. MW marker b. Uncut circular plasmid c. Linearized fragment (100ng) d. Linearized fragment (500ng) ☐ All fragments should be clearly indicated by size and name	**(Please paste your gel photo here)**

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ES Gene Targeting Construct Map

Note: Please make sure you satisfy the following requirements

- ☐ The map should be large and clear
- ☐ The following info should be included:
  - ☐ construct size
  - ☐ vector location and size
  - ☐ long arm location and size
  - ☐ short arm location and size
  - ☐ positive and negative selection cassette location, including promoter and orientation
  - ☐ size and location of the endogenous fragment to be replaced
  - ☐ linearization enzyme cut site
  - ☐ genotyping primer or probe location

(Please paste you map here)

TCF Notes	

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ES Gene Targeting Genotyping Strategy (5' end)

Note: Please make sure you satisfy the following requirements

1. Genotyping method: ☐ PCR ☐ Southern Blot
2. For PCR: The following info should be included:
   - ☐ estimate positive band size _____ bp
   - ☐ estimate endogeneous band size _____ bp
   - ☐ positive control quantity used in PCR _____ pg
     (Suggested positive control:0.3pg construct plasmid mixed with 0.3μg WT gDNA)
   - ☐ negative control (Suggested negative control: 0.3μg WT gDNA)
   - ☐ confirmation of the DNA sequence from positive PCR product
3. For Southern Blot: The following info should be included:
   - ☐ estimate positive band size _____ bp
   - ☐ estimate endogeneous band size _____ bp
   - ☐ positive control quantity used in Southern blot _____ pg
   - ☐ negative control

(Please paste your test genotyping results here)

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ES Gene Targeting Genotyping Strategy (3' end)

Note: Please make sure you satisfy the following requirements

1. Genotyping method: ☐ PCR ☐ Southern Blot
2. For PCR: The following info should be included:
   - ☐ estimate positive band size _____ bp
   - ☐ estimate endogeneous band size _____ bp
   - ☐ positive control quantity used in PCR _____ pg
     (Suggested positive control:0.3 pg construct plasmid mixed with 0.3μg WT gDNA)
   - ☐ negative control (Suggested negative control:0.3μg WT gDNA)
   - ☐ confirmation of the DNA sequence from positive PCR product
3. For Southern Blot: The following info should be included:
   - ☐ estimate positive band size _____ bp
   - ☐ estimate endogeneous band size _____ bp
   - ☐ positive control quantity used in Southern blot _____ pg
   - ☐ negative control

(Please paste your test genotyping results here)

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ES Cell Gene Targeting Checklist

Special Notice

Please check the following questionnaire according to your construct condition. Faithful answer will help us to precede the case faster and smoother. If any of the condition listed below does not fit with your experimental design, please contact TCF manager or TCF committee. Such case might be either treated as special request or rejected from routine TCF service.

1. Construct Design

- ☐ Construction is based on 129/SvJ or 129/Ola genomic library
- ☐ The total length of the homologous fragments is more than 6 kb.
- ☐ No obviously repetitive sequence is found in the homologous fragments.
- ☐ Construct contains positive selector (neomycin or hygromycin)
- ☐ Construct contains negative selector (TK or DT)
- ☐ Construct is linearized at the junction between homologous fragments and the vector backbone (The negative selector is protected by vector fragment)
- ☐ The size of the region between two arms in construct is similar to the size of replaced endogenous region (size differ within 10kb)

2. Genotyping

- ☐ Short arm genotyping strategy has been checked with wild type isogenic genomic DNA
- ☐ Long arm genotyping strategy has been checked with wild type isogenic genomic DNA
- ☐ Tested genotyping results are attached with this form

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ES Cell Gene Targeting Case Evaluation Form

Please fill up the following questionnaire for case evaluation by the transgenic committee. A briefing maybe asked for the final service approval.

1. Has this animal model been made and/or available elsewhere?

2. Has this requested service been submitted elsewhere?

3. Can products from this service be available for other researchers / institutes?

4. For experience sharing and for teaching purpose, can this service be used as a study case in the TCF monthly discussion meeting?

_____
P.I. name and affiliation

_____ _____
Signature Date

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ES Cell Gene Targeting Agreement

All TCF services require the agreement and signature from service user with full understanding of all the following statements:

1. I have carefully reviewed the TCF guideline and condition for using the service, and I agree to follow completely to the TCF guideline.

2. I acknowledge that TCF reserves rights to reject or stop my service request at any time point, if the guideline and condition are not fully complied.

3. I agree to acknowledge TCF services in the way of using the following statement in publication. "We acknowledged the Transgenic Core Facility of Academia Sinica in consulting and generating the mice. The transgenic core is funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-111-207)".

_____
P.I. name and affiliation

_____ _____
Signature Date