

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

Case #: _____

Pronuclei Microinjection Application Form

Request date: (IMB secretary)	Submission date: (TCF staff)	Approved date: (TCF manager)	
Requester		Institute	
PI		Phone	
Construct Name			
Preferred Mouse Genetic Background	<input type="checkbox"/> FVB/NJ <input type="checkbox"/> C57BL/6J <input type="checkbox"/> Other _____ (Special request)		
Preferred Transgene Copy Number	<input type="checkbox"/> Low (<10 copies) <input type="checkbox"/> High (>10 copies)		
Transgene Origin	<input type="checkbox"/> Mouse <input type="checkbox"/> Human <input type="checkbox"/> Other _____		
Nature of Construct	<input type="checkbox"/> Reporter <input type="checkbox"/> Over-expression <input type="checkbox"/> Anti-sense <input type="checkbox"/> Other _____		
Expected Phenotype	<input type="checkbox"/> Potentially lethal <input type="checkbox"/> Unknown <input type="checkbox"/> Others _____		

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Construct Description	
Promoter	
Circular Plasmid Preparation Method	<input type="checkbox"/> CsCl₂ Banding <input type="checkbox"/> Qiagen Column <input type="checkbox"/> Other _____
Construct Size	<input type="checkbox"/> Total Size _____ KB <input type="checkbox"/> Transgene _____ KB
Enzyme for Releasing Transgene	<input type="checkbox"/> 5' end _____ <input type="checkbox"/> 3' end _____
Gel Photo	
<p>Note: Please make sure you satisfy the following requirements.</p> <p><input type="checkbox"/> Gel electrophoresis should be clear and all the fragments are fully separated</p> <p><input type="checkbox"/> Gel photo should be large and the following info should be included:</p> <ul style="list-style-type: none"> <input type="checkbox"/> MW marker <input type="checkbox"/> Uncut circular plasmid <input type="checkbox"/> Transgene fragment released by the enzyme indicated above <p><input type="checkbox"/> All fragments should be clearly indicated by size and name</p>	<p>(Please paste the gel photo here to proof your transgenic construct has been completed)</p>

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Map

Note: Please make sure you satisfy the following requirements.

1. The map should be large and clear
2. The following info should be included:
 - a. Construct size
 - b. Vector location and size
 - c. Transgene location (include promoter and polyA) and size
 - d. Enzyme cut sites for transgene releasing

(Please paste or attach the map of your transgenic construct here)

TCF Note

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Transgenic Mice Genotyping Strategy Confirmation

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 transgene size _____ bp
 - PCR cycle number _____ (should be less than 35 cycles)
 - positive control quantity used in PCR _____ pg
Suggested positive control: 0.3 pg construct mixed with 0.3µg WT gDNA
 - negative control
Suggested negative control: 0.3µg WT gDNA
3. For Southern Blot: The following info should be included:
 - estimate transgene size _____ bp
 - positive control quantity used in Southern blot _____ pg
 - negative control

(Please paste your test genotyping results here)

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Pronuclei Microinjection Checklist

Special Notice

Please check the following questionnaire according to your construct conditions.

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 services.

1. Construct Design

- Promoter: non-viral/ eukaryotic promoter**
- Gene of interest is not biohazardous**
- Construct is not mobile**
- Transgene expression does not cause lethality**
- Vector sequence has been removed as much as possible**
- Released transgene is bigger than 1kb and smaller than 15kb**
- The size of released transgene fragment differs from other fragment(s) by at least 1kb**
- Use FVB/NJ or C57BL/6J as embryo donor strain**

2. Genotyping

- Genotyping strategy has been tested successfully**
- Tested genotyping result(s) is attached with this form**

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Pronuclear Microinjection 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?

5. Is the production of this transgenic mouse approved by IACUC? Please specify the IACUC protocol number below.

Yes. IACUC protocol No.: _____

(Please note that if IACUC protocol hasn't been submitted or approved, TCF will hold the process until it is approved.)

P.I. name and affiliation

Signature

Date

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Pronuclear Microinjection 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