

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

Case #: _____

Pronuclei Microinjection Application Form

Genome Editing Approach

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)			
Type of Genome Editing Tool		<input type="checkbox"/> TALENs (_____ of <i>in-vitro</i> transcription) <input type="checkbox"/> CRISPR/Cas9 (_____ of <i>in-vitro</i> transcription)			
Off-Target counts		_____			
Nature of Construct		<input type="checkbox"/> Homologous Recombination (HR, (co-injected with donor DNA)			
Expected Phenotype		<input type="checkbox"/> Potentially lethal <input type="checkbox"/> Unknown <input type="checkbox"/> Others _____			

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Construct Description	
Promoter for <i>in-vitro</i> Transcription	<input type="checkbox"/> T7 <input type="checkbox"/> SP6
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
Enzyme for Linearization	
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 (please specify) <input type="checkbox"/> Uncut circular plasmid <input type="checkbox"/> linearized plasmid <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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Results of *in-vitro* Tests

Note: Please test the efficiency and genotyping approach of the TALENs/CRIPR-Cas9 *in-vitro* in advance

1. The TALENs/CRISPR-Cas9 was tested in _____ (cell line name)
2. The screening was performed using
 - a. PCR and Restriction Enzyme(RE) Digestion: _____ (RE name)
 - b. PCR and mismatch-specific nuclease: _____ (i.e. T7E1, Surveyor assay...etc.)
 - c. SSA (single strand annealing) assay, tested in _____ (cell line name)
 - d. Others: (Please specify)

(Please use a diagram to explain your screening strategy and paste or attach the *in-vitro* test results)

TCF Note

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Donor DNA information

Type of Donor DNA	<input type="checkbox"/> ss ODN (oligo-deoxynucleotide) : _____ bases, concentration: _____ng/ μ l <input type="checkbox"/> dsDNA , concentration: _____ng/ μ l length of homologous arms: Long arm _____ kb, short arm _____ kb
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(Please use a diagram to explain your design, expected result after HR and genotyping strategies)

TCF Note

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TALEN, CRISPR/Cas9 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 and Genotyping

- In-vitro test and genotyping strategies have 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