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

Case #: _____

Pronuclei Microinjection Application Form

Genome Editing Approach - CRISPR/Cas9/Cpf1

Request Date: (IMB Secretary)	Submission Date: (TCF Staff)	Approved Date: (TCF Manager)

Requester		**E mail**			
PI		**Lab Tel**		**Institute**	
Gene/Locus Name					
Preferred Mouse Genetic Background	■ C57BL/6J ☐ Other _____ (Special Request)				
Editing Tool	■ $Cas9^{WT}$ ☐ Cpf1 ☐ $Cas9^{D10A}$				
Type of Gene Modification	☐ Indel : Insertion or Deletion ☐ Large Fragment Deletion ☐ Knock-in: _ _ (Please fill in the page of "Design of Donor template") ☐ floxed allele: Donor format: _____				
Expected Phenotype	☐ Potentially Lethal ☐ Unknown ☐ Others _____				

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CRISPR/Cas9 system	■ **Dual guide RNA (crRNA + tracrRNA)** ☐ **Single guide RNA (sgRNA)**			
Guide RNA Design	■ **TCF** ☐ **Others (Tool:)**			
Gene Name	**Target Sequence**	**GC%**	**Off Target Count Total/Exonic**	**Pairing**
Genotyping Primer Design and Test	■ **TCF** ☐ **User (Please Paste or Attach the *In-Vitro* Test and Genotyping Results)**			

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The *in-vitro* TEST of gRNA

■ **TCF**

☐ **User** (Please Paste or Attach the *In-Vitro* Test and Genotyping Results)

1. The gRNA 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. ☐ Others: (please specify)

(Please paste and explain the result of gRNA *in-vitro* test)

TCF Note	

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Genotyping Strategy & Test Results

Please use a diagram to describe the genomic structure of the target locus, and indicate the locations of primers and gRNAs. Explain your genotyping strategy and provide the test results

TCF Note	

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Design of Donor template (If Applicable)

Please provide the design of donor template and indicate the sequence of gRNA, PAM, intended mutation, as well as the homologous arm.

TCF Note	

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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.

- **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