

## Pronuclei microinjection

- A. Prerequisite: Before placing an order for pronuclei microinjection, the following conditions should be fulfilled.
1. Construct ready upon applying for TCF services.
  2. Method and proof to identify transgenes.
  3. Space to maintain mice once being generated.
  4. Understanding that not every transgenic construct will produce transgenic founders.
- B. Pronuclei microinjection service request form, case evaluation form, agreement form, and pronuclei microinjection checklist should be completely filled and signed by PI. Construct map should be attached on the request form. The request form should be dated and stamped by IMB secretary upon service application.
- C. For optimal process efficiency, a checklist is made based on TCF experiences. Only those requests that fit all items on the checklist can be processed with guarantee. Requests with minor discrepancy from the checklist can still be processed without guarantee. However, requests with major discrepancy from the checklist will be either subjected to committee discussion or be rejected.
- D. TCF routinely uses inbred strain FVB/NJ and C57BL/6J for pronuclei microinjection, however, other mouse strains may be arranged on special request.
- E. The final transgenic fragment should be easily released with as little vector sequences as possible to avoid complication in gene expressions. In addition, the size of the final transgene fragment should be at least 20% different (the size of larger fragment as a denominator and the size difference between transgene and the other fragment(s) as a numerator) from other fragment(s) to avoid contamination.
- F. For best result and to obtain largest number of transgenic founder, construct prep should be fresh and in good quality. TCF staff will inform requester of the scheduled injection date. The digestion of the construct should be as complete as possible at designated quantity and the digestion should be done

shortly before the scheduled date. TCF will elute construct to ensure stable quality for injection.

- G. Upon submitting construct, DNA material condition form should be filled and attached with a gel photo, in which the marker and the target band should be clearly indicated. Construct prep should be freshly digested in described quantity and quality.
- H. Pronuclei microinjection will be performed according to the TCF regulations to provide at least five transgenic founders for each construct. Request for more transgenic founder mice for the same construct will be considered as a new order. If a transgene by nature fails to produce any or expected number of transgenic founders despite of TCF's proper handling and standard microinjection procedures, TCF will terminate injection on the construct after one extra effort.
- I. Tail samples will be delivered approximately five weeks from the scheduled injection day. Upon receiving tail sample, a sample receiving form will be asked by TCF staff to sign. Animals that scored as positive will be released one week later (at weaning). Upon receiving transgenic founder, an animal release form will be asked by TCF staff to sign. The IMB transgenic committee has final right to rule over any dispute that may have arisen.
- J. TCF is not responsible for long-term maintenance of transgenic founder mice.
- K. Specifications/protocols available upon request:
1. DNA preparation for pronuclei microinjection.
  2. Tail DNA extraction for Southern blot or dot blot.
  3. Tail DNA extraction for PCR.
- L. Materials available upon request:
1. Genomic DNA from FVB and C57BL/6 inbred strains.
- M. Charges.
1. NT\$ 30,000 for every request of pronuclei microinjection.
  2. Charge will be billed to the order of requester at the beginning of service.
  3. All service charges for orders from within IMB or other Institutes will be the same. All service charges cover some material cost and animal fee only. However, the IMB transgenic committee will subject price and service type

to change according to the decision.