Aquatic Vertebrates Platform Services

(See services description below, page 3)

Services

1. Micro-injection of DNA, morpholino or mRNA in zebrafish and medaka embryos.

2. Generation of stable zebrafish and medaka transgenic lines.

3. Maintenance of transgenic and mutant lines of zebrafish and medaka.

4. Tanks renting.

5. Delivery of WT and transgenic embryos.

6. Delivery of fixed embryos and larvae for in situ hybridization and inmunohistochemistry.

7. Mosaic analysis by blastula transplantation.

8. Capture of quality images with a fluorescent steromicroscope.

9. Time-lapse confocal imaging.

10. Introduction to zebrafish as model system with Hands-on-training.

11. Whole mount *in situ* hybridizations and inmunohistochemistry.

12. Enhancer activity assessment.

13. Analysis of the insulating capacity of genetic elements.

14. ChIP-seq and ChIP-PCR techniques.

15. Chromosome Conformation Capture Technology: 3C.

16. Chromosome Conformation Capture Technology: 4C-seq + data analysis

17. RNA-seq + data analysis

18. CRISPR/Cas9 technology.

20. ATAC –seq.

21. Experiment “à la carta”.

22. Do it yourself in our Guest Laboratory

Workshops to learn some of the previous techniques will be organized on a regular basis. The 1st and 2nd CABD Workshop: Applied Techonologies on Aquatic Vertebrates have been successfully held in June 2013 and July 2014 respectively.

For more information, please visit the Aquatic Vertebrate Platform:

http://www.upo.es/CABD/AquaticVertebratesPlatform

or contact us:

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Description of the services

1. Micro-injection of DNA, morpholino or mRNA in zebrafish and medaka embryos.

A clutch of 300 newly fertilized embryos will be injected per condition. DNA, morpholino or mRNA are to be provided by the customer.

Comment: Additional services such as fixation, embryo delivery, embryo imaging... can be performed to the injected embryos.

2. Generation of stable zebrafish and medaka transgenic lines

- Micro-injection into one-cell stage embryos.
- Raising of the embryos to adulthood (3 months).
- Screening of the founders (3 founders are provided/line).

Note: Constructs are to be provided by the customer. Please, contact the AVP staff for advice and/or available vectors.

3. Maintenance of transgenic and mutant lines of zebrafish and medaka.

We maintain two generations per line.

4. Tanks renting.

There is the possibility to manage your lines yourself having the support of the AVP staff.

5. Delivery of WT and transgenic embryos.

We provide a clutch of 200 fertilized and bleached wild-type or transgenic embryos.

6. Delivery of fixed embryos and larvae for *in situ* hybridization and immunohistochemistry.

We provide a clutch of 200 dechorionated wild type or transgenic embryos fixed for *in situ* or for inmunostaining at the desired stage.

7. Mosaic analysis by blastula transplantation.

Cell transplants are performed at blastula stage. The service also includes micro-injection with fluorescent markers/morpholino…, at one-cell stage of donor or host embryos if required.
Note: Additional services such as fixation, delivery, mosaic embryos imaging, etc can be performed upon request.

8. Analysis and capturing of quality images of transgenic or injected embryos with a fluorescent steromicroscope.

The AVP has two stations with Olympus SZX16 fluorescent stereomicroscopes coupled to CCD cameras.
We provide pictures at different developmental stages of transgenic or injected/mosaic embryos.

9. Analysis and capturing of quality images of transgenic or injected embryos with confocal microscopes.

Available confocal microscopes: Leica SP2, Leica SP5, Nikon A1R.
We provide Z-stacks or time-lapse movies at different developmental stages of transgenic/injected/mosaic zebrafish embryos. For time-lapse movies embryos are mounted in low melting agarose in a tissue culture dish with glass bottom cover.

10. Introduction to zebrafish as model system with Hands-on-training.**

The user will gain expertise on the following techniques:
- Transgenesis.
- Morpholino and mRNA micro-injection.
- Blastula transplantation.
- Time-lapse confocal imaging in zebrafish.

Note: The users have the opportunity to perform their own experiments while learning the techniques.

11. Whole mount in situ hybridizations and inmunohistochemistry.

We perform protein localization and gene expression studies in zebrafish embryos. We will provide 30 embryos at 4 developmental stages per target gene.
Note: Antibodies, antisense and sense probes are to be provided by the customer.

12. Enhancer activity assessment.

- Cloning of the putative enhancer region into the Zebrafish Enhancer Detector (ZED) Vector using the Gateway Technology.
- Micro-injection into one-cell stage embryos.
- Raising of the embryos to adulthood (3 months).
- Screening of the founders (3 founders are provided/line).

13. Analysis of the insulating capacity of genetic elements.

- Cloning of putative insulators into the Z48-minitol vector using the Gateway Technology.
- Micro-injection in one-cell stage embryos

- Analysis of insulator capacity in 30hpf F0 embryos. We provide a negative control.

14. ChIP-seq and ChIP-PCR techniques

- Sample processing (from zebrafish, medaka, mouse, cell culture and chicken)
- Sample measurement with Qbit.
- qPCR Primer design and validation by real-time qPCR
- Sequencing and data analysis.

15. Chromosome Conformation Capture Technology: 3C.

- Experiment and 3C primers design.
- Sample processing (from zebrafish, medaka, mouse, cell culture and chicken).
- BAC processing
- Real-time qPCR and analysis of the results.

16. Chromosome Conformation Capture Technology: 4C-seq + data analysis

- Experiment and primers design.
- Sample processing (from zebrafish, medaka, mouse, cell culture and chicken).
- High complexity PCR (Library for Illumina sequencing).
- Sequencing and data analysis

17. RNAseq

- Sample processing
- Sequencing and data analysis

18. CRISPR/Cas9 technology

Each service includes:
1. Generation of two sgRNA/experiment.
2. Co-injection of the two sgRNA into one-cell stage embryos to increase efficiency.
3. Test for indel (insertions/deletions) mutations in the F0 injected embryos using the T7 Endonuclease I protocol.
4. Confirmation of indel in F0 injected embryos by sequencing.
5. Raising of the injected embryos (3 months).
6. F0 adults genotyping.
7. We provide F1 embryos from 3 adults carrying indel mutations.

19. ATAC-seq

20. Experiment "á la carta"

Contat us to discuss about your specific experiment.

21. Do it yourself in our AVP Guest Laboratory

The Aquatic Vertebrate Platform also offers a Guest Laboratory where to develop your projects under the technical advice from the scientific manager of the facility.

(*) When requiring multiple services, please contact the AVP staff to get an offer.
(**) The AVP organizes workshops on the mentioned techniques. Please, check the AVP webpage for more information on the next workshop .