„Mammalia-Chip": Diagnostics of the future?

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What is a Chip?

Microarray technology, originally invented for medical diagnostics, allows the parallel analysis of up to 10´000 separate features on one microscopic glass slide, a so called "chip". To date, applications of diagnostic chips in biology are primarily used for microorganisms. However, this technology has a huge potential of possible experimental designs. For example, a **"Mammalia-Chip"** could include several redundant diagnostic markers to unambiguously identify all European mammal species from tissue, hair of even scat samples. A "**Biodiversity-Chip**" on the other hand could contain diagnostic features to distinguish key species in the taxa of bacteria, lichen, molluscs, insects, fungi, mammals etc.

Major challenges in chip-development are

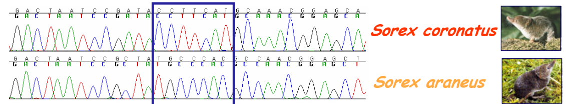
- to get DNA sequences for all species and to include haplotypes from different populations in the geographical regions of interest
- to design species-specific probes (Bioinformatics)

Advantages and drawbacks

- For the design and production of a chip, experience in Microarray technology as well as expensive lab-equipment is necessary. In contrast, chip-application is very easy, reasonable in price and can be done in any lab.
- The higher the feature-density, the more expensive a chip gets, but the larger its application potential will be. This trade-off will be economically relevant for the success of a chip.
- Microarray technology is a serious alternative to Sequencing:
  - Unpurified PCR-products can be hybridized directly ➡ fast and cheap
  - Less than 50ng PCR product are needed ➡ 20µl PCRproduct of 2,5ng/µl Sequencing at this level is only possible with the newest capillary-sequencing instruments
  - Interfering sequences from pseudogenes or other secondary amplification products can be selectively excluded through integration in the primer design

The Making of...

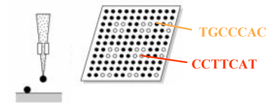
1. Sequence information



2. Probe Design of species-specific oligonucleotide sequences (20-30bp)

3'- CCTTCAT - 5'   3'- TGCCCAC - 5'

3. Spotting of designed probes onto microarray slide



4. Labeling of target DNA

5. Hybridization, reading and identification

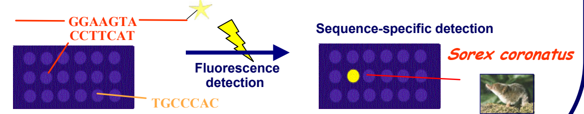


Fig. 1: Chip-preparation and application

Identification of voles and shrews from hairsamples using Microarray technology

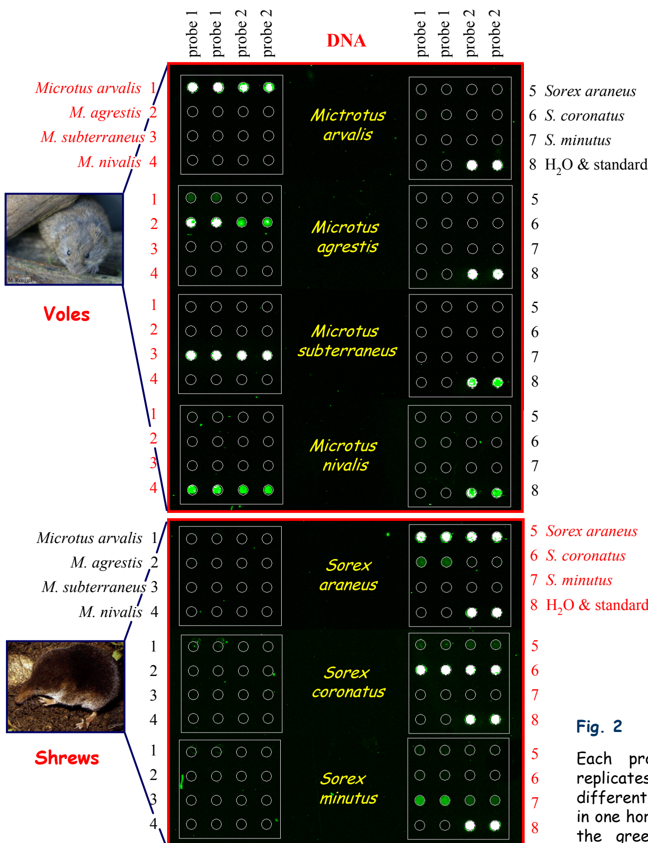


Fig. 2

Each probe is represented by two adjacent replicates, and each species is tested with two different probes (probes 1 and 2) that are arranged in one horizontal line of total 4 spots. The brighter the green of the spots, the better; maximal brightness (overexposure) is shown in white.

Reaching for...

Hair samples were gained from vole and shrew individuals being trapped with life traps during the course of an annual **biodiversity monitoring** schedule for the Biodiversity Monitoring Module Z3 (BUWAL).
From DNA sequence information including different haplotypes we designed two different probes for each of the following seven species:
Sorex araneus, S. coronatus, S. minutus, Microtus arvalis, M. agrestis, M. subterraneus and ***M. nivalis.***

Each of the probes was spotted in duplicates onto aldehyde coated glass slides using a QArray Mini spotter (Genetix).
PCR products were labeled with Cy3-fluorophores and hybridized at low stringency onto the chip. Hybridisation of the labeled PCR product with the probes on the chip can be visualized with a laser scanner which leads to the glowing spots seen in Figure 2.

... the glowing stars

Each of the 14 designed probes hybridized specifically to the DNA of the target species. Only low levels of crosshybridization (=false positives) were observed with some of the probes (Fig. 2).

The closely related and sympatric sistertaxa *Sorex coronatus* and *S. araneus* exhibit uniform external features and can only be differentiated by cranial morphology, chromosome number, enzyme electrophoresis or molecular analysis. The differentiation among *Microtus arvalis* and *M. agrestis* also needs experience and some individuals are hard to identify. The „Mammalia-Chip" presented here has already been successfully applied for the identification and confirmation of hair samples collected from living animals trapped in different Swiss regions.

Using the non-invasive method of hair sampling led to good results. This approach is especially advantageous in biodiversity studies, where certain animals might be rare or where studies are often conducted in nature reserves. The same method can also be used for hair samples from hair traps, which are often used for small carnivores such as weasels, stouts or martens, or for arboreal species such as dormice.