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## A polymorphic zebrafish line for genetic mapping using SSLPs on high-percentage agarose gels

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## Article Outline



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Simple sequence length polymorphisms (SSLPs) have become an important and powerful genetic tool in constructing linkage maps of vertebrates such as the mouse, rat and human (Ref. 1, 2, 3). These genetic markers consist of two primers flanking a dinucleotide repeat which can be highly variable in length. The zebrafish has become a popular vertebrate model system for studying developmental events at a genetic level (Ref. 4, 5, 6). A newly constructed SSLP map (Ref. 7) demonstrates that SSLP are highly polymorphic, codominant and abundant in zebrafish.

We established a new laboratory line of zebrafish for genetic mapping termed WIK. From this line, which derives from a wild catch in India (H. Pichy and J. Klein, pers. commun.), we gained several sublines from single-pair matings (WIK2, WIK3, WIK4, WIK9, WIK10, WIK11 and WIK20). By screening the progeny of single-pair matings of F1 fish, we found that one of these sublines, WIK11, was free of embryonic and larval lethals with a probability of over 90%.

To test whether WIK11 is a suitable reference line for genetic mapping using SSLPs, we analyzed four individual fish from each of the two different zebrafish lines, Tü and WIK11, using 314 randomly chosen SSLP primer pairs. The Tü line has been used in our laboratory for a large-scale mutagenesis screen (Ref. 8). This line was inbred from a restricted number of founder individuals (Ref. 9), and is thus likely to be very homogeneous. We expect WIK11 to be more heterogeneous since it is a direct derivative of a wild catch. However, because this line was derived from a single pair, we do not expect to find more than four alleles per locus.

Out of 314 SSLPs tested, 136 (43.3%) showed only bands that were polymorphic between Tü and WIK11 and no shared alleles, 148 (47.1%) SSLPs showed only one shared allele and at least one polymorphic allele among the four pairs of fish analyzed and 30 (9.6%) SSLPs showed no polymorphisms between Tü and WIK11. These results demonstrate that the WIK11 line is very polymorphic relative to the Tü line. Within the Tü line, 201 (64%) SSLPs showed one identical allele in all four Tü fish. This confirms the initial assumption that the Tü line is very homogeneous in itself. The number of alleles found in WIK11 does not exceed the expected total of four but shows WIK11 to be less homogeneous than Tü.

We tested two other zebrafish lines, AB (Ref. 10) and TL (Ref. 11), and found a lower rate of polymorphisms than between the Tü and WIK lines. The IN line, which was used in a reference cross with the AB line for the generation of the SSLP map, is probably not free of mutations affecting larval viability (Ref. 7). In our own experience, the IN line also seems harder to breed than the WIK line.

The SSLPs were originally analyzed by using radioactively labeled PCR primers and by separating the PCR products on polyacrylamide sequencing gels (Ref. 7). For the entire analysis described here, we used unlabeled PCR primers and separated the PCR products on high-percentage agarose gels [1-2% Metaphor (FMC) mixed with 1-2% regular agarose (Qualex)] which were stained with ethidium bromide to visualize the PCR products. A representative SSLP amplification product is shown (Fig. 1). This simplified procedure requires less laboratory space, allows direct computer-assisted imaging of the agarose gels and circumvents the problems associated with radioactive waste. We routinely obtained a resolution of at least 10 bp within a fragment size of 100–400 bp. We are planning to map all the mutations identified in the Tübingen screen (Ref. 12) using a bulked segregant analysis strategy (Ref. 13, 14) in which DNA samples from pools of segregating F2 homozygous mutants and siblings are compared using SSLPs. In an artificial DNA mixing experiment, we found that we should be able to detect linked markers in a 25 cM window on either side of the targeted locus.

In summary, the newly established WIK11 line is very well suited as a reference line for mapping mutations induced in the Tü line. In combination with the analysis of PCR products on high-resolution agarose gels, this method should allow simple and efficient mapping of all mutants identified in the Tübingen screen.

## Protocol

For PCR amplification, 10 µl genomic fish DNA (2 ng/µl) was combined with 10 µl reaction mixture. The reaction mixture is made by mixing 3.9 µl $H_2O$, 3.5 µl dNTPs (Pharmacia, 1.25 mM), 2.2 µl 10× PCR buffer (Pharmacia), 0.5 µl forward primer (6 µM), 0.5 µl reverse primer (Research Genetics, 6 µM) and 0.4 µl Taq polymerase (Pharmacia, 2U/µl). The DNA was denatured for 2 min at 95°C, then amplified for 35 cycles ( 30 s at 94°C, 30 s at 60°C and 1 min at 73°C) followed by extension for 10 min at 73°C.

Gel electrophoresis was carried out on 2–3% agarose gels [QualexGold (AGS)]. In some cases, regular agarose was mixed with Metaphor (FMC) high-resolution agarose at a ratio of 1:1. The electrophoresis conditions were 5V/cm for 2 h and we used 1× TBE (18 mM Tris–Borate pH 8.0, 0.4 mM EDTA) for gels and running buffer.

All zebrafish lines described in this paper are available through the zebrafish stockcenter at the Max-Planck-Institut für Entwicklungsbiologie in Tübingen (hgf@mailer1.mpib-tuebingen.mpg.de).

Figure 1

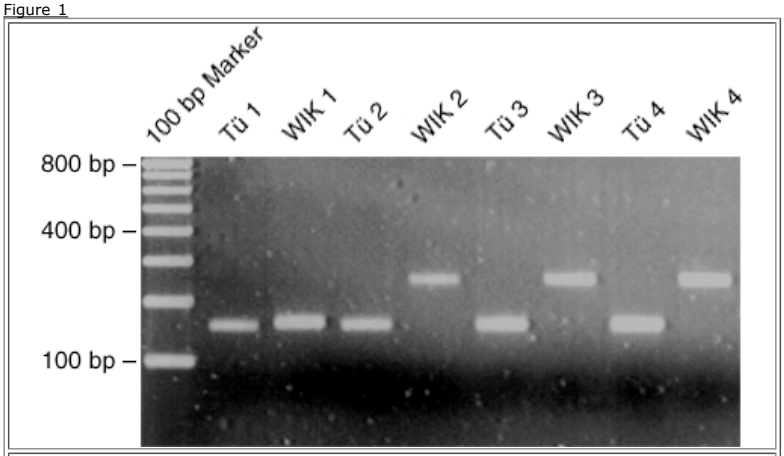


**FIGURE 1.**
Characterization of one SSLP Marker (z1295) using genomic DNA of four different pairs of Tü and WIK fish. The PCR products were run on a 2% agarose gel. The polymorphism between the two lines can clearly be resolved in pairs 2, 3 and 4, whereas pair 1 shows shared alleles.

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## Glossary

**Products Used**

**Metaphor**
Metaphor from FMC BioProducts
**agarose gel**
agarose gel from Sigma
**agar**
agar from Difco
**dNTP**
dNTP from Promega Corporation
**dNTP**
dNTP from PE Applied Biosystems
**dNTP**
dNTP from Pharmacia
**dNTP**
dNTP from Promega Corporation
**dNTP**
dNTP from Boehringer Mannheim

**PCR buffer**
PCR buffer from Pharmacia
**PCR buffer**
PCR buffer from PE Applied Biosystems
**Taq DNA polymerase**
Taq DNA polymerase from PE Applied Biosystems
**Taq DNA polymerase**
Taq DNA polymerase from Life Technologies (Gibco BRL)
**Taq DNA polymerase**
Taq DNA polymerase from Life Technologies (Gibco BRL)
**Taq DNA polymerase**
Taq DNA polymerase from Promega Corporation
**Taq polymerase**
Taq polymerase from Boehringer Mannheim
**Taq polymerase**
Taq polymerase from Pharmacia
**Taq polymerase**
Taq polymerase from Bioline
**Taq polymerase**
Taq polymerase from Advanced Biotechnologies
**Taq polymerase**
Taq polymerase from Boehringer Mannheim
**Taq polymerase**
Taq polymerase from Bioline
**Metaphor**
Metaphor from FMC BioProducts

**PII:** S016895259601208