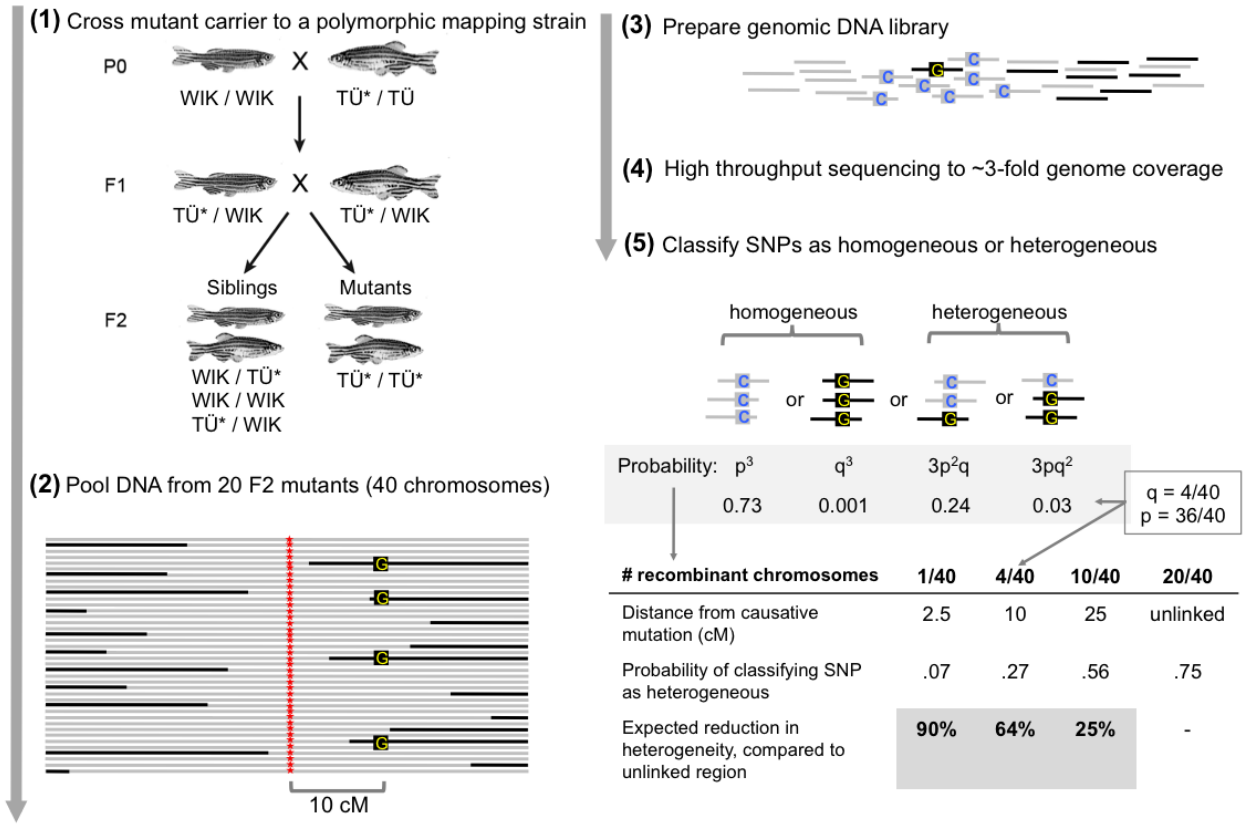

Figure S1 Outline and predicted sensitivity of the approach. (**1**) The generation of fish used in mapping is accomplished by crossing identified mutants carrying a recessive ENU-induced mutation (*) within the Tü background, to a polymorphic mapping strain, (e.g., WIK). Mutant carriers (Tü*/WIK) of the F1 generation are then intercrossed to generate F2 progeny. These F2 fish are sorted based on the presence or absence of the mutant phenotype. (**2**) DNA is prepared from 20 F2 mutant progeny (Tü*/Tü*) and pooled in equal quantities. The diagram depicts the 40 chromosomes containing a phenotype-causing ENU-induced mutation (red asterisk) among the 20 mutant fish. The mutation is linked to genomic sequence originating from the Tü strain used for mutagenesis (grey fragments). Recombinants having sequence originating from the outcross strain (black fragments) can be observed at different distances from the causative mutation as a result of meiotic recombination during meiosis in the F1 generation. In a SNP located ~10 cM from the causative mutation, we expect by definition, 4 of the 40 mutation-containing chromosomes to show a mapping strain allele (G in WIK; black square) as a result of meiotic recombination. (**3**) Physical fractionation of DNA from the 20 mutant fish produces DNA fragments, that contain the aforementioned SNP (boxed C for the Tü and G for the WIK alleles), (**4**) Whole genome sequencing of the fragmented DNA library is performed on a single lane of an Illumina HiSeq platform resulting in ~3x genome coverage. (**5**) Probability for detecting a SNP as being homogenous or heterogeneous in pooled DNA from 20 mutant fish sequenced to 3x coverage. SNPs are classified as homogeneous if all 3 reads covering the SNP represent the same allele (probability = $p^3 + q^3$) and as heterogeneous if both alleles are represented (probability = $3p^2q + 3pq^2$). In an unlinked region, where both alleles are equally represented (q = 0.5; p = 0.5), the probability of a SNP being detected as heterogeneous is 0.75. Likewise, in regions where 10% of the chromosomes are recombinant, as in our example, statistically 4 out of 40 (q = 0.1) reads would show the mapping-strain allele (G), while 36 out of 40 (p = 0.9) would show the reference allele (C; Tü). Thus the probability of detecting the SNP in a heterogeneous state is 0.27. Therefore, the number of heterogeneous SNPs identified in such a region is expected to be ~64% lower than in an unlinked region (0.27/0.75 = 64%). Similarly, a ~90% reduction in heterogeneity is expected for regions containing 1 recombinant chromosome, while a ~25% reduction is expected for regions with 10 recombinant chromosomes. According to this analysis using low genome coverage, it would be of no added benefit to pool larger numbers of fish to increase the resolution of mapping. As the probability of detecting a single recombinant in, for example, 40 fish (80 chromosomes) would be lower than the level of detecting false positive heterogeneous SNPs and thus indistinguishable from noise.