LIMSTILL platform

managing resequencing and TILLING projects in model organisms

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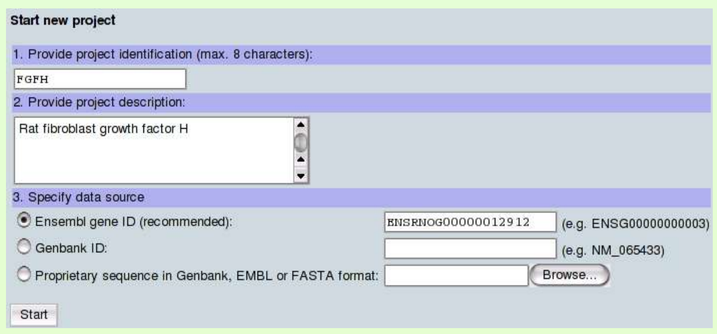
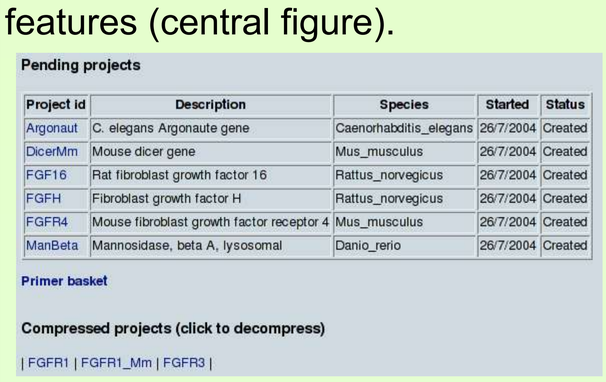
LIMSTILL (**LI**MS for **I**dentification of **M**utations by **S**equencing and **TILL**ING) is an open-source software designed to streamline the informatics and management parts of the Hubrecht Laboratory facility for high-throughput screening for induced mutations in model organisms. It includes steps for amplicon selection, primer design, sequence analysis, and annotation of mutations. The platform is universal and can be used for any resequencing or TILLING (target induced local lesions in genomes) project for any organism of interest.

1. The purpose of LIMSTILL is to maximally automate and efficiently manage all steps of the resequencing and tilling process. The public LIMSTILL web-service is available at **http://limstill.niob.knaw.nl**

Once registered, user can create new project by specifying Ensembl gene ID, Genbank accession or supplying a file with custom sequence in Genbank/EMBL format.

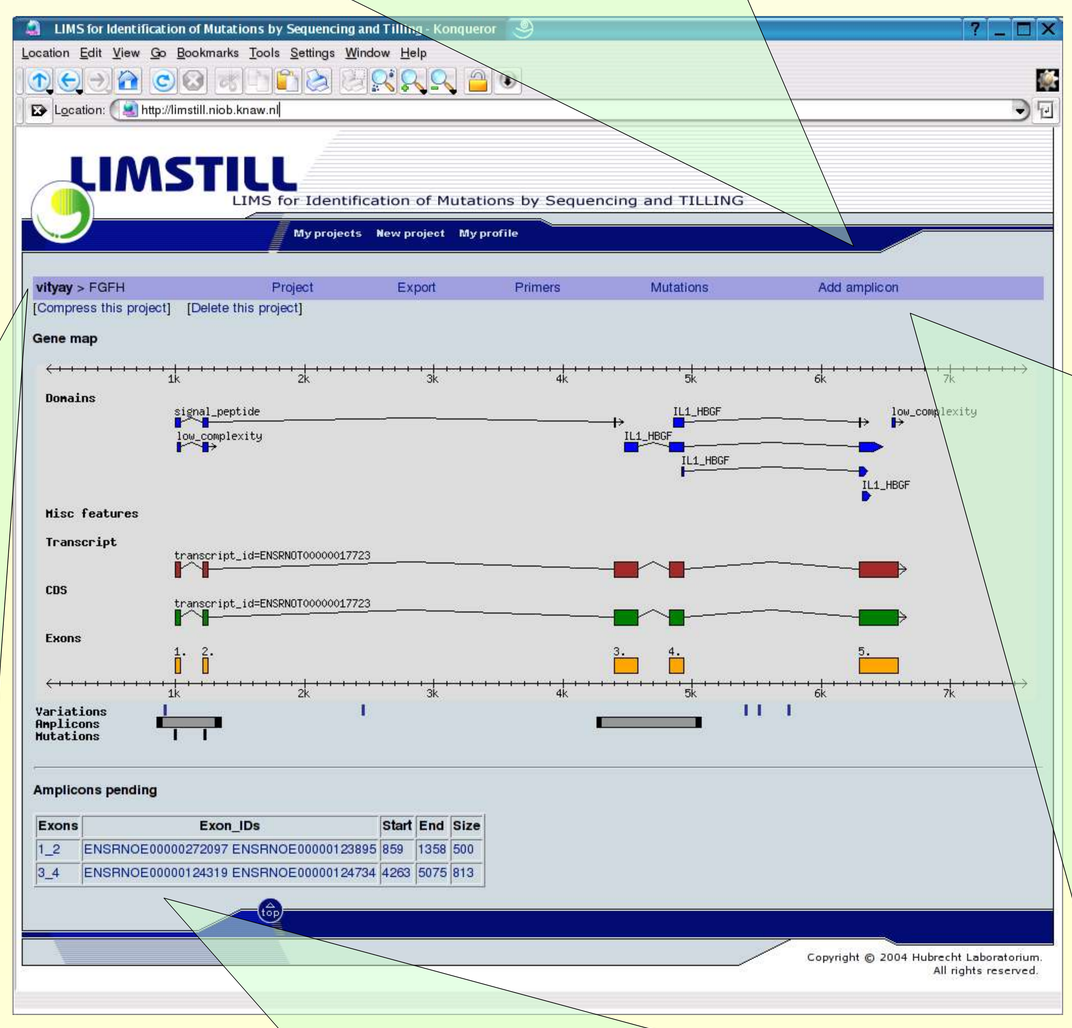


LIMSTILL fetches information for the gene from Ensembl database or parses provided annotation and generates a graphical representation of the gene that includes intron/exon structure, alternative transcripts and location of annotated protein domains/ features (central figure).

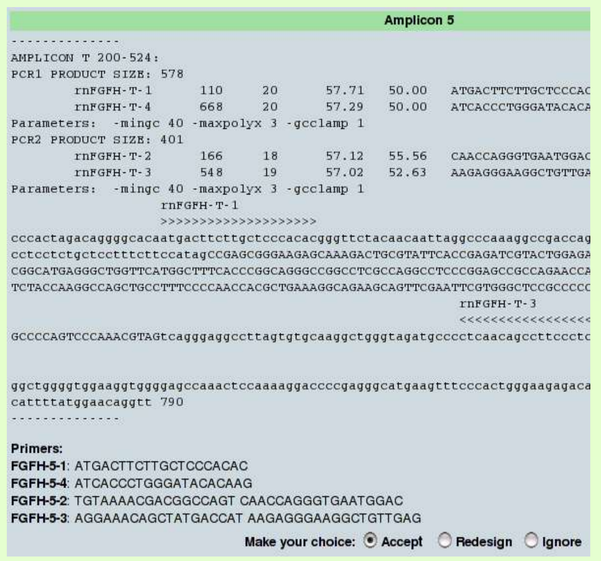


2. Amplicon selection. Based on structural gene information, LIMSTILL proposes a list of possible amplicons to be selected for further analysis. The graphical representation of the gene structure and amplicon ratings reflecting the chance to identify a knockout based on the mutation spectrum and amplicon characteristics, assist the researcher to select gene regions to be targeted. User can also specify an amplicon with custom coordinates.

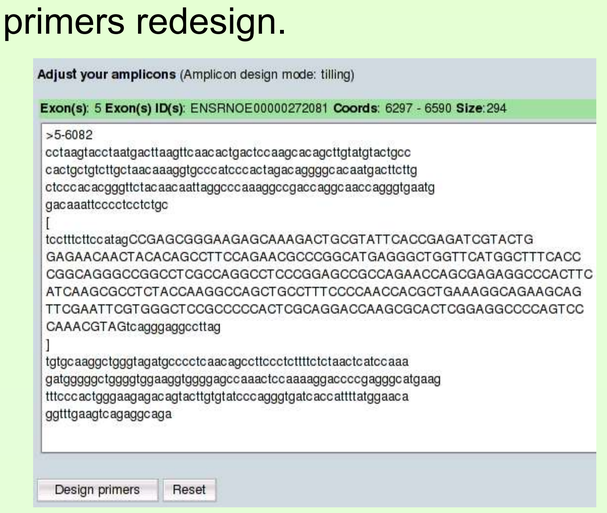
For resequencing projects, for scanning genes for SNPs/mutations, all exons can be selected. There are three options for mutation detection method (resequencing using either a single or nested PCR or using TILLING) available in this section.



3. Primer design is based on a custom interface to primer3 program to design primers for nested PCR under universal conditions, allowing easy automated processing in robotic setups. The primer design step performs iterative primer search and masking for repetitive sequences.



The process of primer design can be repeated until satisfactory results are obtained. The suggested boundaries of amplicon can be interactively altered (specified by square brackets) followed by primers redesign.

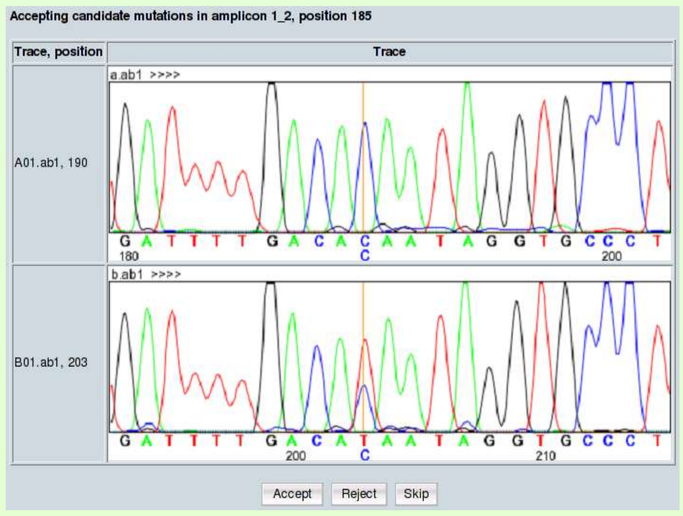


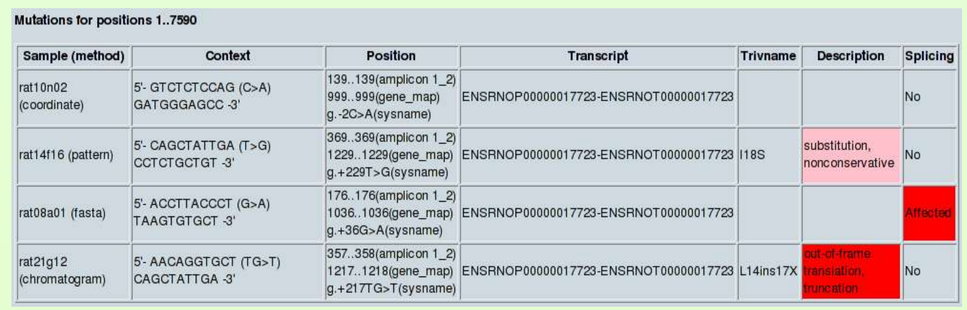
4. Amplicon view provides the interactive graphical representation of information on intron/exon structure of an amplicon, coding frames, primer positions and mapped mutations.

5. Mapping and annotation of mutations is accessible from amplicon view and can be done in four ways:

1) by providing coordinates in the amplicon and mutant allele
2) by specifying nucleotide context of a mutation for pattern search
3) by supplying preformatted fasta file
4) by submitting sequencing chromatograms of samples from a screen for mutation detection

In the latter case phred/phrap/polyphred package is used for sequences analysis and identification of potential homo- and heterozygous mutations. The analysis is done in the background and the researcher is presented with chromatograms of potential mutation-harboring regions for manual inspection and approval.





Submitted/discovered mutations are then annotated for their impact on gene structure (silent, AA-change, stop-codon, splice-site) and added to the graphical interface. The summary on mutations obtained in the project can be viewed in the mutations view.

LIMSTILL is a LAMP-type (Linux, Apache, MySQL, Perl) open-source project that uses HTML templates and extensively utilizes open-source software like BioPerl, Ensembl, Emboss, Staden, primer3, phred/phrap/ polyphred.

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