TECHNISCHE UNIVERSITÄT WIEN
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New avenues for fungal strain improvement towards enzymatic degradation of cellulosic biomass for biofuel production

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Research Area Gene Technology and Applied Biochemistry

Highlights der Bioenergieforschung
Lignocellulosic Biomass and Biofuels
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The fungal genus *Trichoderma*

- Filamentous fungus (mould)
- Genus: *Trichoderma*
- Cosmopolitan in soil and on decaying wood
- Produces large amounts of enzymes (biocatalysts) to degrade carbohydrate biopolymers.

Complete genome sequence of ***Trichoderma reesei*** available

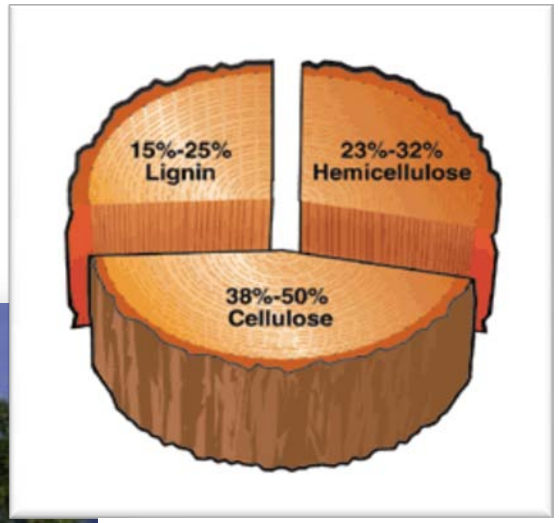
- Genome contains ca. 9200 genes
- Ca. 360 of these genes encode proteins involved in carbohydrate degradation

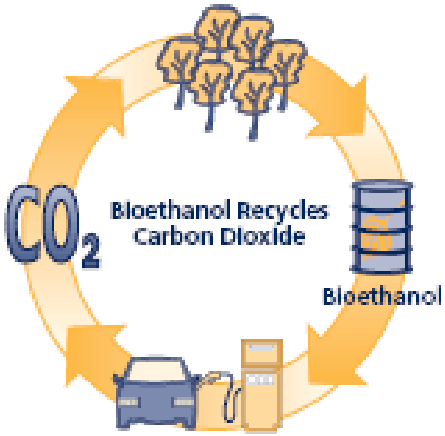
Biotechnological applications of *Trichoderma reesei*

- Produces large quantities (>100g/l) of cellulases and hemicellulases.

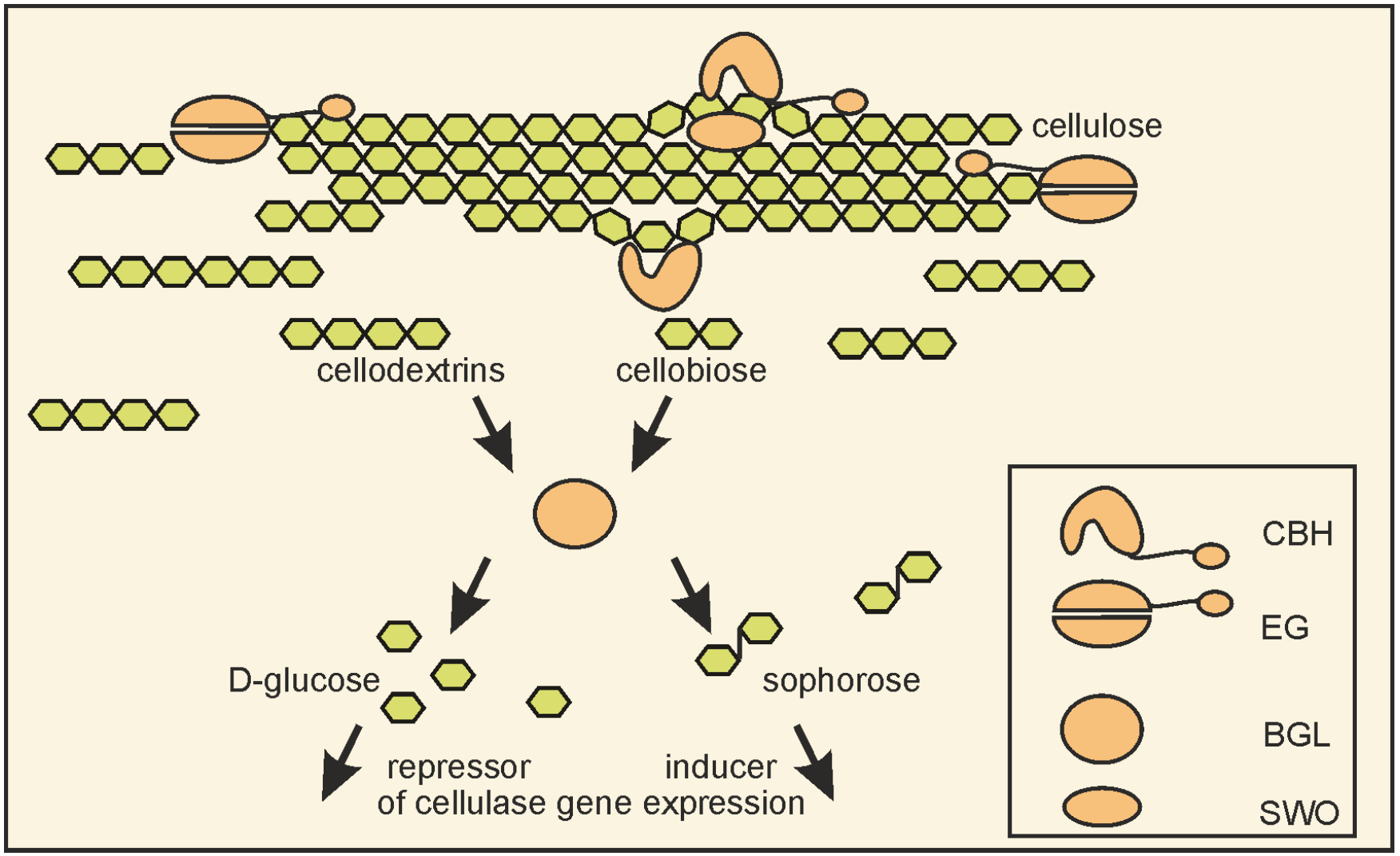
Applications:

- **Second generation biofules from cellulosic biomass**
- Pulp and paper industry
- Textile industry





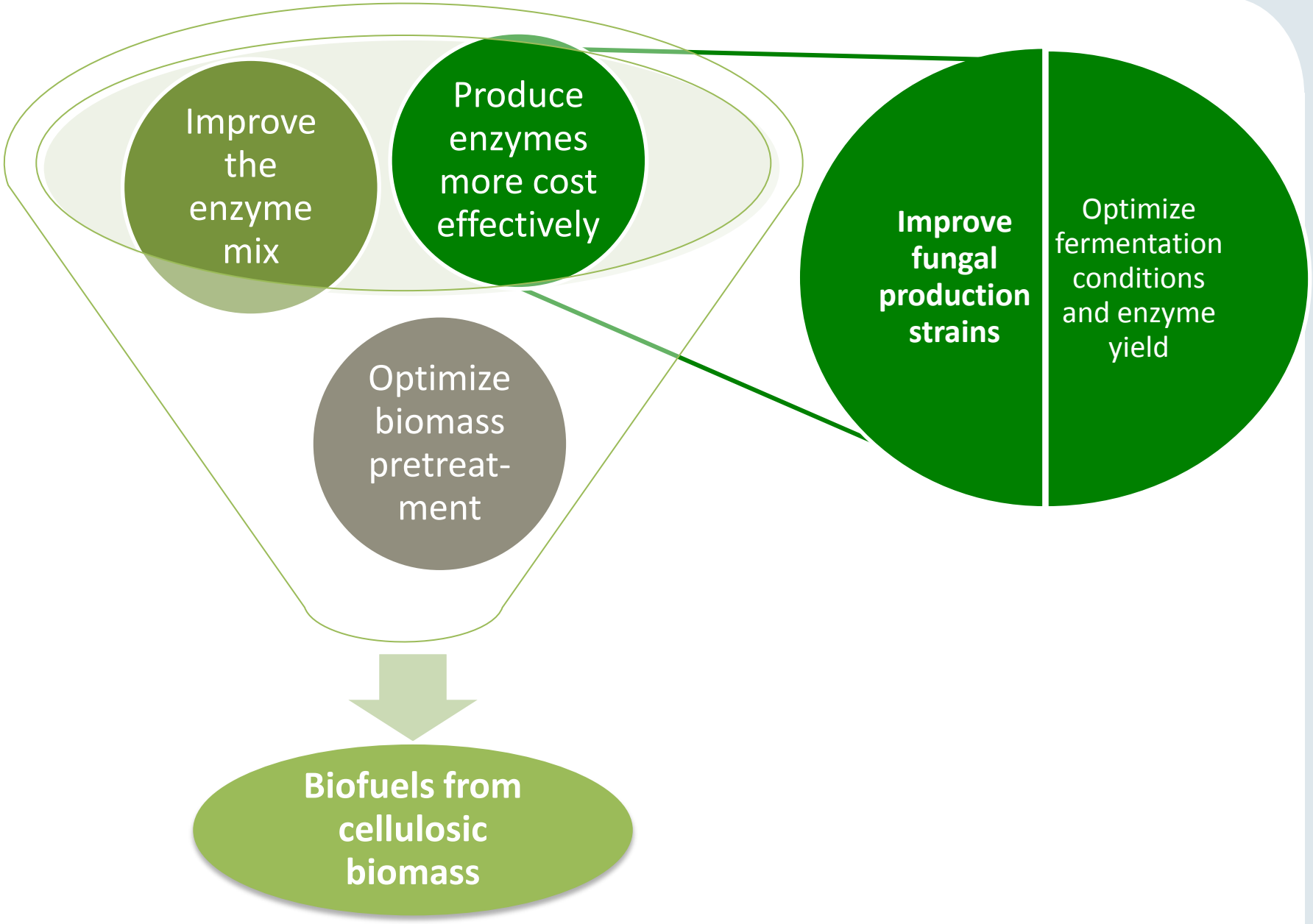
The enzymatic cellulose degradation machinery of *Trichoderma reesei*



CBH: Cellobiohydrolase, EG: Endoglucanase, BGL: beta-Glucosidase, SWO: Swollenin

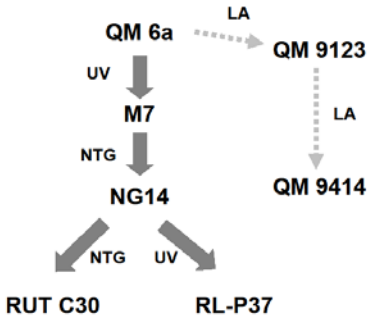
Figure adapted from Seiboth, Ivanova and Seidl-Seiboth, Biofuels, 2011

Requirements for production of cellulosic biofuels



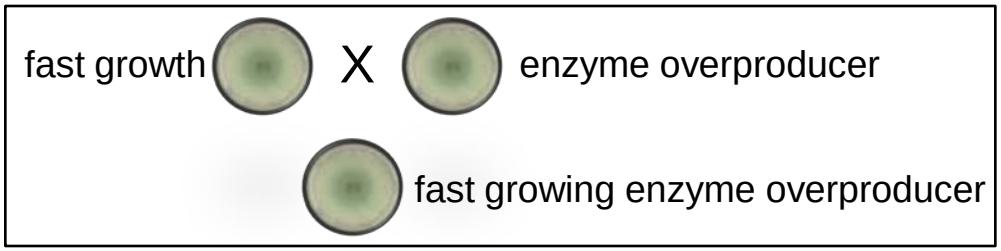
Fungal strain improvement strategies in *Trichoderma reesei*

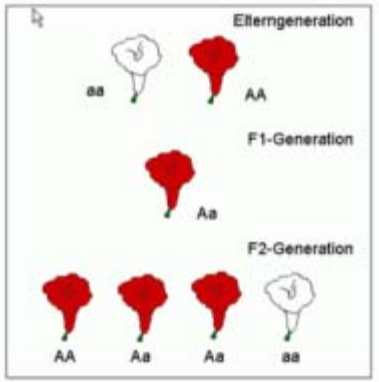
- Mutagenesis
  - UV light
  - mutagenic chemicals



- **Genetic engineering**
  - gene knockout strains
  - overexpression strains

- **Classical crossing techniques (sexual recombination)**

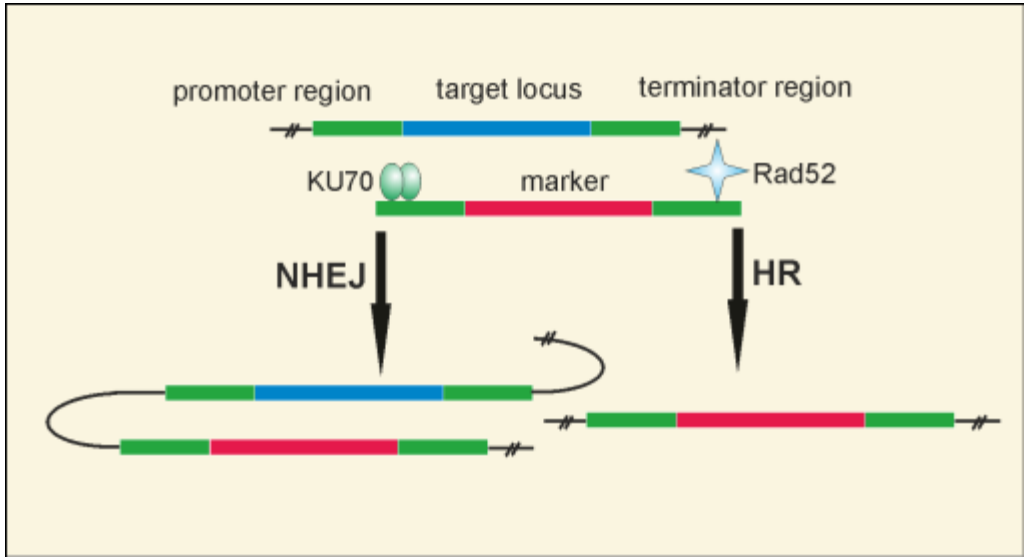




Gene targeting in *Trichoderma reesei*

Genes of interest need to be replaced by other genes to improve the production capacity of *T. reesei* for more cost-efficient cellulase production:

- Introduce new genes that encode cellulases with improved properties (e.g. protein stability, enzyme performance, etc.)
- Alter the regulatory regions of cellulases to produce more enzymes.



NHEJ: non-homologous end joining, HR: homologous recombination

Figure adapted from Seiboth, Ivanova and Seidl-Seiboth, Biofuels, 2011

Gene targeting in *Trichoderma reesei*

A *T. reesei tku70 gene*-knockout strain resulted in > 95 % gene-targeting efficiency in comparison to 5-10 % in the partental – *tku70* non-deleted strain.

This enables the development of high-throughput approaches on the genomic level to improve the biotechnological potential of *T. reesei*.

Publication: Guangtao et al, 2009, Journal of Biotechnology.

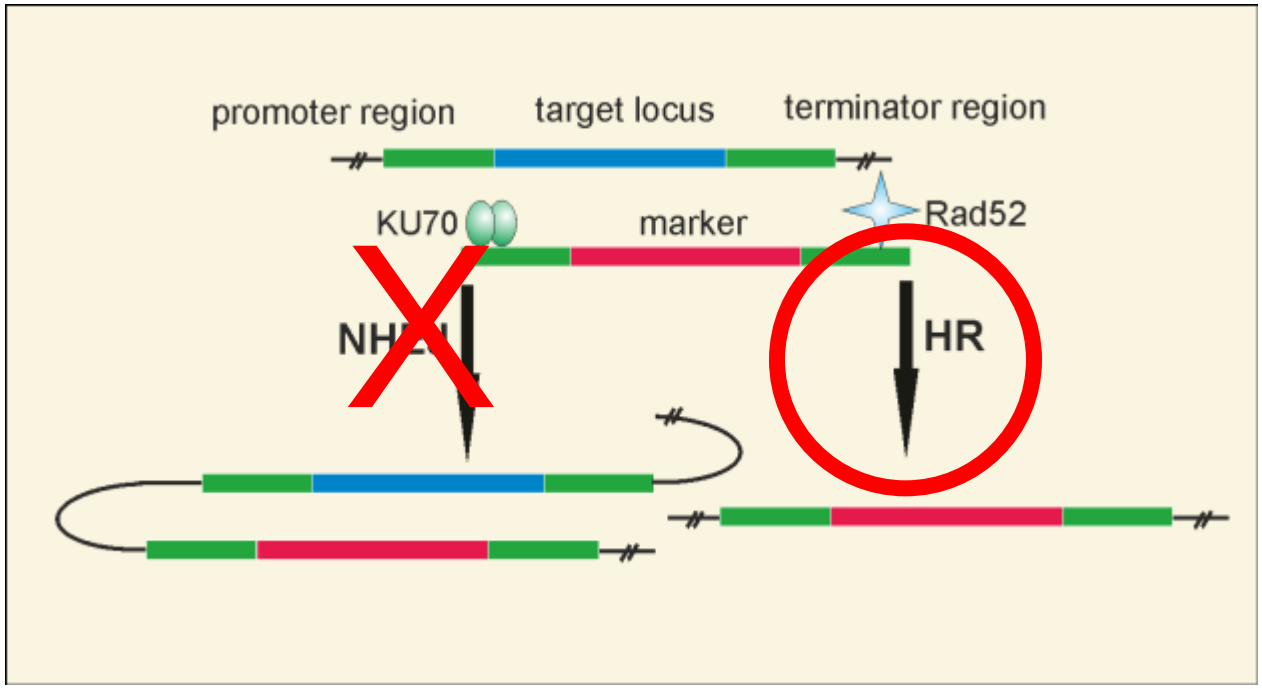


Figure adapted from Seiboth, Ivanova and Seidl-Seiboth, Biofuels, 2011

Sexual recombination in *Trichoderma reesei*

All industrial mutants are derived from one isolate, *T. reesei* QM6a.

- ***Hypocrea jecorina* is the sexual form of *T. reesei* .**

  (Kuhls et al, 1996; PNAS)

- *T. reesei* QM6a was repeatedly reported to be an asexual clonal line.

- Few examples of *Hypocrea* spp. Undergoing sexual reproduction *in vitro*.

  (Samuels, 2006; Phytopathology)

Sexual recombination in *Trichoderma reesei*

Genome analysis revealed that *T. reesei* is a heterothallic species and that for sexual recombination a fungal strain with the opposite mating type is required.

Using appropriate wild-type strains from a fungal culture collection, strain QM6a, the ancestor of ALL industrial *T. reesei* strains could be sexually crossed for the first time in 2008, more than 50 years after its discovery.

The crossing technique was already sucessfully applied to currently biotechnologically applied *T. reesei* strains.

T. reesei on agar plate

T. reesei strains with opposite mating types produce fruiting bodies.

No production of fruiting bodies in *T. reesei* strains with the same mating type.

Seidl et al, PNAS, 2009

Applied Research Award (VWR Forschungspreis) of the ÖGMBT 2010

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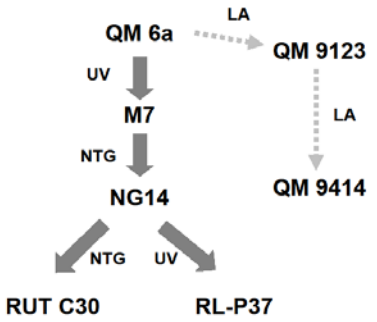
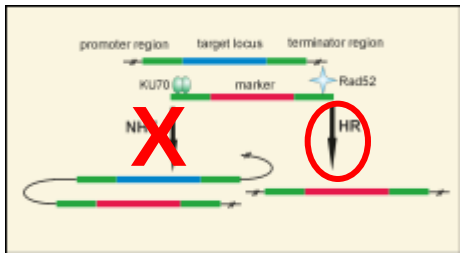


✓ **Genetic engineering**

  - gene knockout strains
  - overexpression strains



✓ **Classical crossing techniques (sexual recombination)**

Affiliation and further information

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Related publications:

D. Martinez et al, 2008, Nature Biotechnology

Z. Guangtao et al, 2009 J. of Biotechnology

V. Seidl et al 2009, PNAS

Recent reviews:

V. Seidl and B. Seiboth, 2010, Biofuels

B. Seiboth et al 2011, Biofuels 2 (book chapter, in press)