

RevGenUK: An integrated informatics and resources platform for reverse genetics in dicots



Medicago truncatula



Brassica rapa



Lotus japonicus

RevGenUK TILLING service

***B. rapa* R-o-18 service:**

- DNA from 3072 M₂ plants, pooled 8-fold
- 1.2kb fragment. Current cost £1500 per fragment
- Use fluorescent primers, CEL1 digest and capillary sequencer, visualise using GeneMapper
- 24 mutations chosen: potential STOPs, splice junctions, regions likely to give deleterious mutations, regions of interest to the client
- De-convolute by conventional PCR and sequencing, visualise using Mutation Surveyor
- Send M₃ seeds harvested from the M₂ plant
- Additional mutants can be ordered if we identify more.

***Brassica rapa* TILLING References:**

Stephenson *et al* (2010) BMC Plant Biology 10: 62.

Girin *et al* (2010) Plant Journal 63: 329-38.

495 *Brassica rapa* mutations confirmed in 21 1.2kb gene fragments

Missense	Nonsense (Stop)	Splice junction	Intron	Silent (no amino acid change)	Regulatory (promoter)
256	18	7	90	111*	13

*(29% of exon mutations)

RevGenUK database: “TILLY”



- **Back Office:**

- Population Manager

- Pedigrees of all populations catalogued by unique plant and seed identifiers and linked through to:

- TILLING Manager

- Job tracking for *RevGenUK* staff
- Database of TILLED and published genes

- **Website/client portal**

- Existing *RevGenUK* website

- New Client Portal for requesting quotes, uploading sequences, tracking TILLING progress and downloading results

- Searchable database of TILLED and published genes

Population and TILLING Manager

The screenshot shows a web browser window with the title "RevGenUK - Population Manager". The browser's address bar is empty. The page has a navigation bar with "Population Manager" and "TILLING Manager" highlighted in red boxes, followed by "Documents | My Account" and a "Logout" link. Below the navigation bar is a "Message" area. The main content area is titled "Population Manager" and includes a search form with a dropdown menu set to "plant" and a "Go" button. Below the search form are two sections: "Population Actions" and "Mutant Population Actions".

Population Actions

- Populations** ■ update basic population details and add new populations.
- Grow Plants** ■ grow new plants from an existing seedbag (transgenes can optionally be applied).
- Grow Cuttings** ■ grow cuttings from an existing plant.
- Harvest Seed** ■ harvest seed from one or more plants.
- Cross Pollinate** ■ create one or more seedbags from two parent plants.

Mutant Population Actions

- Grow M1 Plants** ■ grow plants for M1 generation.
- Batch Harvest** ■ harvest seedbags from a whole generation.
- Batch Grow** ■ grow plants for generations M2 and onwards.

Search and Queries

The browser's status bar at the bottom shows "Local intranet" and a zoom level of "100%".

RevGenUK Website

RevGen UK

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RevGenUK

- Latest News
- About RevGenUK
- Models
- TILLING
- Links
- Publications
- Client Portal**

Coming soon - RevGenUK: the next generation

Welcome to RevGenUK

The John Innes Centre (JIC) is at the forefront of reverse genetics through its establishment of TILLING and de-TILLING technologies in model legumes. Reverse genetics involves making a change to a specific gene, seeing what effect this has, and so working out the gene's normal function. We aim to develop these into a biological and bioinformatics resource for the wider plant scientific community via the RevGenUK project, providing an integrated reverse genetics platform for model plants.

RevGenUK aims to be "A 'single-stop' shop for use in functional genomics research consisting of an efficient, self-sustaining, extendable service for reverse genetics."

It will consist of:

Poor Satisfactory Good **Excellent**

We should be very grateful if you would take a few minutes to fill in our [questionnaire](#). Your answers and opinions will help us to shape and improve the RevGenUK service in the future.

Local intranet 100%

RevGenUK Client Portal

RevGenUK - Client Portal

Home | Page | Safety | Tools

RevGenUK

FAQs | How to Order | Contact Us | Home

- Latest News
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Client Portal

Welcome to the RevGenUK client portal.

In order to take advantage of the services we offer, please [register an account](#) with us. You may then [login](#) at any time, to request a quote, submit a sequence for TILLING, or retrieve results of a job.

If you have any problems using this service, please [contact us](#).

- Logout
- My Account
- Your Quotes
- Search TILLED Genes

John Innes Centre

RevGenUK, John Innes Centre
Norwich Research Park, Norwich, NR4 7UH, UK
E: revgenuk@bbsrc.ac.uk

BBSRC
bioscience for the future

Reverse Genetics UK

Local intranet | 100%

RevGenUK Client Portal

The screenshot displays the RevGenUK Client Portal interface. At the top, the browser address bar shows 'RevGenUK - Client Portal'. The main header features the RevGenUK logo and navigation links: 'FAQs', 'How to Order', 'Contact Us', and 'Home'. A vertical sidebar on the right reads 'Reverse Genetics UK'. The central content area is titled 'Request Quote' and includes a dropdown menu for 'Population' with options: 'Brassica rapa', 'Lotus japonicus', and 'Medicago truncatula'. Below this is a 'No. of Fragments' field and a 'Your Message' text area. A 'Submit' button is located at the bottom right of the form. The footer contains contact information for the John Innes Centre and BBSRC - Biotechnology and Biological Sciences Research Council.

RevGenUK - Client Portal

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RevGenUK

Reverse Genetics UK

Request Quote

Population

No. of Fragments

Your Message

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John Innes Centre

RevGenUK, John Innes Centre
Norwich Research Park, Norwich, NR4 7UH, UK
E: revgenuk@bbsrc.ac.uk

BBSRC - Biotechnology and Biological Sciences Research Council

Local intranet 100%

RevGenUK Client Portal

The screenshot shows a web browser window displaying the RevGenUK Client Portal. The browser's address bar shows the URL <http://revgenuk.jic.ac.uk/>. The page features a green and white color scheme. At the top, there is a navigation menu with links for 'FAQs', 'How to Order', 'Contact Us', and 'Home'. The main header displays the 'RevGenUK' logo, where 'RevGen' is in green and 'UK' is in black with a green leaf icon above the 'U'. A vertical banner on the right side reads 'Reverse Genetics UK' with a green leaf icon. The central content area is titled 'Quote Confirmation' and contains the message: 'Quote request rguk60029 successfully submitted. We will get back to you as soon as possible with a price, any information you have requested and instructions on how to proceed.' Below this message are two buttons: 'Exit' and 'Return to List'. To the left of the main content is a sidebar menu with items: 'Latest News', 'About RevGenUK', 'Models', 'TILLING', 'Links', 'Publications', and 'Client Portal'. To the right of the main content is a sidebar with a list of links: 'Logout', 'My Account', 'Your Quotes', and 'Search TILLED Genes'. The footer of the page is dark green and contains contact information for the John Innes Centre and BBSRC - Biotechnology and Biological Sciences Research Council. The browser's taskbar at the bottom shows the system tray with icons for 'Local intranet' and '100%' zoom level.

John Innes Centre

RevGenUK, John Innes Centre
Norwich Research Park, Norwich, NR4 7UH, UK
E: revgenuk@bbsrc.ac.uk

BBSRC -
Biotechnology and
Biological
Sciences
Research Council

Reverse Genetics UK

RevGenUK Client Portal

RevGenUK - Client Portal

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Add Job to Quote: rguk60029

Gene Name

Genomic Sequence

```
tattgtatagcaattggtagatagattaaagcatggccaaacatat
gagataacaaaatagaagaatctttctttctttctttctttcttt
tttggatctagctatttgataaaatgtagactcattatggtggt
gtggttggtgattgtatagatgaattgagaattgaaatagttt
gatgaaaacotagatttgaatcagcaagaaaaaattggtattaga
tagcaattggaagatagattaaagcaggaccaaagattgtcacata
atagaggattatctttatctttatctttatctttatctttatctt
ttagctatttgataaaatgtagactaatgatggtggtgatgtga
gtgcagTAGTGCAGCAAGCCAGCACATCTGTTCTTGAATGAGCTTG
GAGTGGATTATGATGAGCAGGACAACATATGTGGTGATCAAGCATGC
```

Coding Sequence

```
GGCTCACCTGCGCCTACGAGCTCAGTAAGAACCCCAAGTCCAGGT
TGCTATCATTGAGCAATCTGTTAGCCCCGGTGGCGGCGCGTGGCTC
GGTGGCCAACCTCTTCTCTGCCATGGTAGTGCAGCAAGCCAGCACATC
TGTTCTTGAATGAGCTTGGAGTGGATTATGATGAGCAGGACAACATA
TGTGGTGATCAAGCATGCTGCTCTCTTCACTTCCACAATCATGAGC
TAGCTGCTGGCTAGGCTAATGTGMAAGCTTTTCAMTACTGTGGCC
```

- Logout
- My Account
- Your Quotes
- Search TILLED Genes

Genetics UK

RevGenUK Client Portal

The screenshot shows a web browser window with the title "RevGenUK - Client Portal". The main content area is divided into three vertical panels. The left panel is green. The middle panel contains a text area with a DNA sequence: `atagaggattatTTTTATTTTTATTTTTATTTTTGCTTTTGTGAT` followed by `TTAGCTATTTGATAAAAATGTGACACTAATGATGTGTGGATATGTGAGTGGATTATGATGAGCAGGACAACCTATGTGGTGATCAAGCATGC`. Below this is a section titled "Coding Sequence" containing a longer DNA sequence: `GGCTCACCTGCGCCTACGAGCTCAGTAAGAACCCCAACGTCCAGGT` followed by `TGCTATCATTGAGCAATCTGTTAGCCCCGGTGGCGGCGCGTGGCTC`, `GGTGGCCAACCTCTTCTCTGCCATGGTAGTGCGCAAGCCAGCACATC`, `TGTTCTTGAATGAGCTTGGAGTGGATTATGATGAGCAGGACAACCTA`, `TGTGGTGATCAAGCATGCTGCTCTTCACTTCCACAATCATGAGC`, `AAGCTGCTGGCTAGGCCTAATGTGAAGCTTTTCAATGCTGTGGCCG`, `CTGAGGATTTGATTGTAAAAAATGGGAGAGTTGGTGGGGTTGTCAC`, `CAACTGGGCTTTGGTTTCCATGAACCATGACACCCCAATCCTGCATG`, `GATCCTAATGTCATGGAGGCCAAGGTGGTGGTAAGCTCTTGTGGCC`, and `ATGATGGGCCCTTTTGGAGCCACTGGGGTGAAGAGGCTTAAGAGCAT`. Below the coding sequence is a text box with the text "There are three exons but I would prefer to target exon 1 if possible". A "Submit" button is located at the bottom right of the middle panel. The right panel is yellow and contains the text "Reverse Genetics UK" written vertically. The footer of the page is green and contains the logos for John Innes Centre, BBSRC - Biotechnology and Biological Sciences Research Council, and RevGenUK. The browser's address bar shows "http://revgenuk.jic.ac.uk/".

RevGenUK Client Portal

The screenshot shows a web browser window with the address bar displaying "RevGenUK - Client Portal". The browser's address bar also shows navigation icons for home, RSS, mail, print, and page settings, along with "Page", "Safety", and "Tools" menus. The main content area features the RevGenUK logo at the top left, a navigation menu on the left, and a central message titled "Job Confirmation". The message states: "Your job will now enter the work queue. We will design primers and carry out TILLING for you. We will contact you when you will be able to download information about the mutations we find and then you can request the appropriate seed bearing your mutation from which you can isolate your mutant. TILLING cannot guarantee to find deleterious mutations." Below the message are two buttons: "Exit" and "Return to List". On the right side, there is a list of links: "Logout", "My Account", "Your Quotes", and "Search TILLED Genes". The "My Account" and "Your Quotes" links are highlighted with a red box. At the bottom of the page, there is a green footer containing contact information for the John Innes Centre and BBSRC - Biotechnology and Biological Sciences Research Council. The browser's status bar at the bottom shows "Done", "Local intranet", and "100%".

RevGenUK - Client Portal

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BBSRC -
Biotechnology and
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Research Council

Done Local intranet 100%

RevGenUK: the next generation

- BBR (Bioinformatics and Biological Resources) grant application November 2010:
 - Continue offering TILLING in Model Legumes and *Brassica rapa*
 - TILLING in cereals including development of a *Brachypodium* population at JIC and populations of Oat and Barley at IBERS and SCRI
 - Develop TILLING by Sequencing in collaboration with TGAC
 - Bespoke TILLING service for clients who wish to develop their own populations

RevGenUK TILLING team

Trevor Wang (PI)
Fran Robson (Manager)
Beth McCullagh (Technician)
Rob Cook (Database)

Thanks to:

Lars Østergaard (Co-PI) and Pauline Stephenson
JIC Horticultural staff
Summer Casual Assistants

RevGenUK TILLING service

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