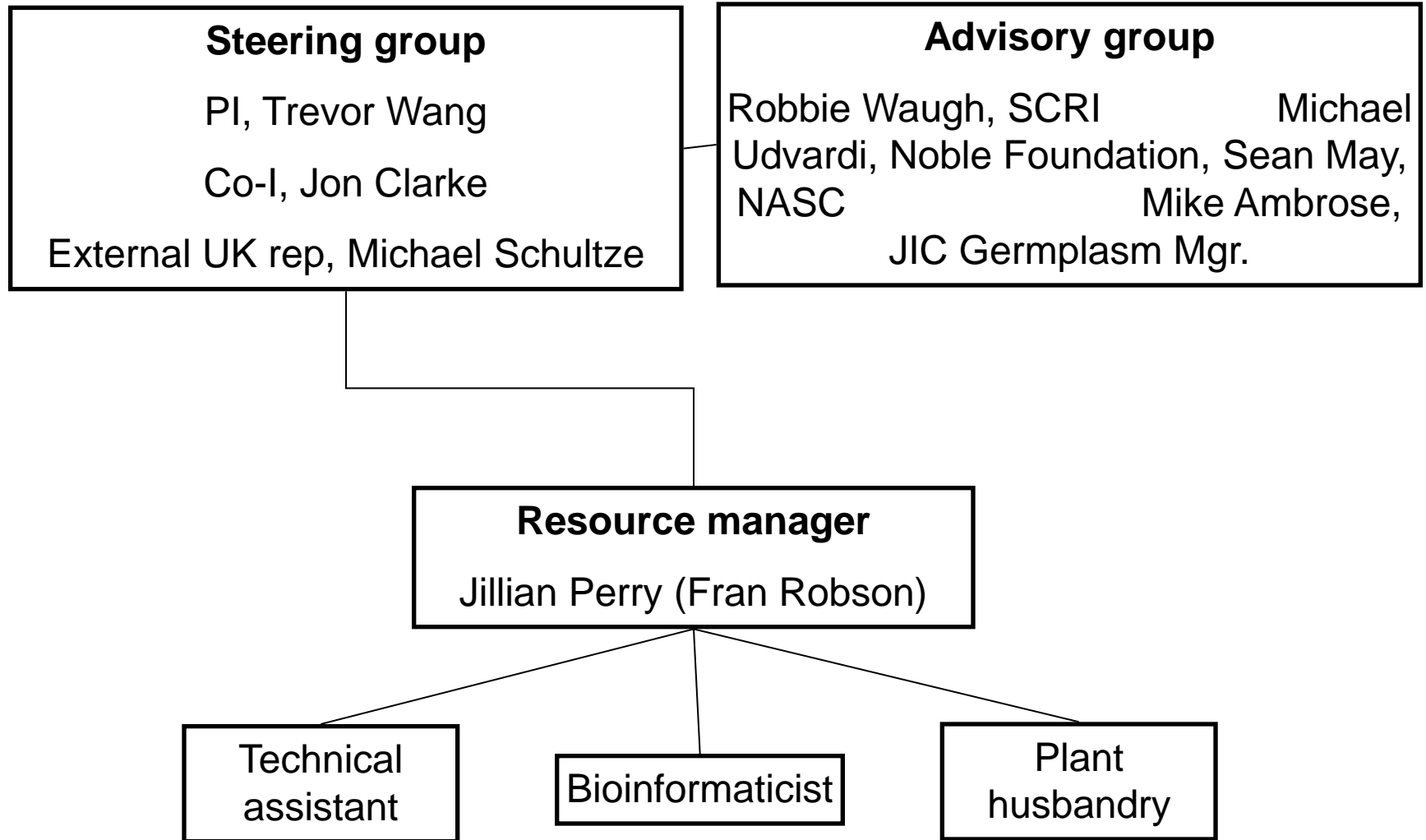


*RevGenUK: An integrated  
informatics and resources platform  
for reverse genetics in dicots*

Fran Robson  
Metabolic Biology  
John Innes Centre  
Norwich  
UK

# Structure



# RevGenUK populations

<http://revgenuk.jic.ac.uk/>

Based on 3 species currently:

1) *Lotus japonicus*

- 1) *Gifu* (population first TILLED in 2001; Perry *et al*, Plant Physiol, 131:866-71, 2003)
- 2) *MG20* (new population currently under development)

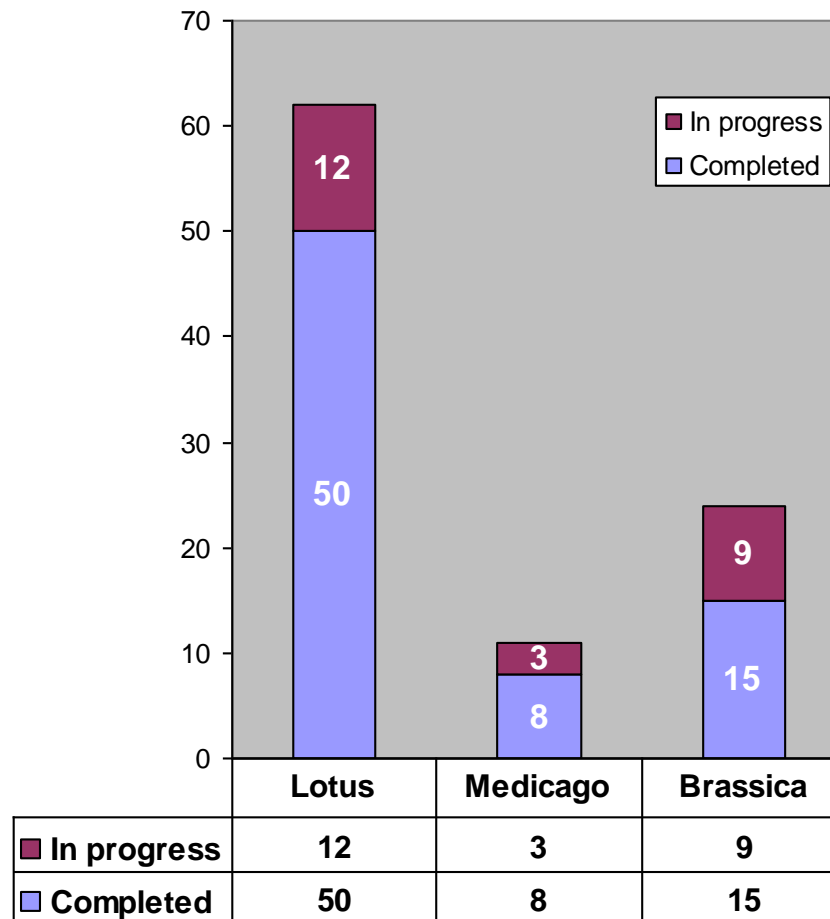
2) *Medicago truncatula* 'jemalong'

- 1) *Dijon* (Le Signor *et al*, Plant Biotechnology J 7:430, 2009)
- 2) *Norwich* (6000 M1 plants currently growing)

3) *Brassica rapa* 'R-o-18' (Stephenson *et al*. BMC Plant Biology, 10:62 2010)

9,216 M2 plants in the population, we routinely TILL 3072 plants

# RevGenUK TILLs



# 342 Brassica rapa mutations confirmed in 15 1kb TILLs

Missense	Nonsense (Stop)	Splice junction	Intron	Silent (no amino acid change)	Regulatory
165	12	6	81	65 (27% of exon mutations)	13

# RevGenUK

- Latest News
- About RevGenUK
- Crops
- TILLING
- de-TILLING
- Links
- Publications

## 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:

1. EMS, fast neutron and ecotype populations, with their corresponding DNA libraries, of *Lotus japonicus*, *Medicago truncatula* and *Brassica rapa* for TILLING, de-TILLING and Eco-TILLING
2. A robust user-friendly plant, seed and mutant database as a web-accessible information source
3. Currently (April 2008), TILLING is available for all three target species.

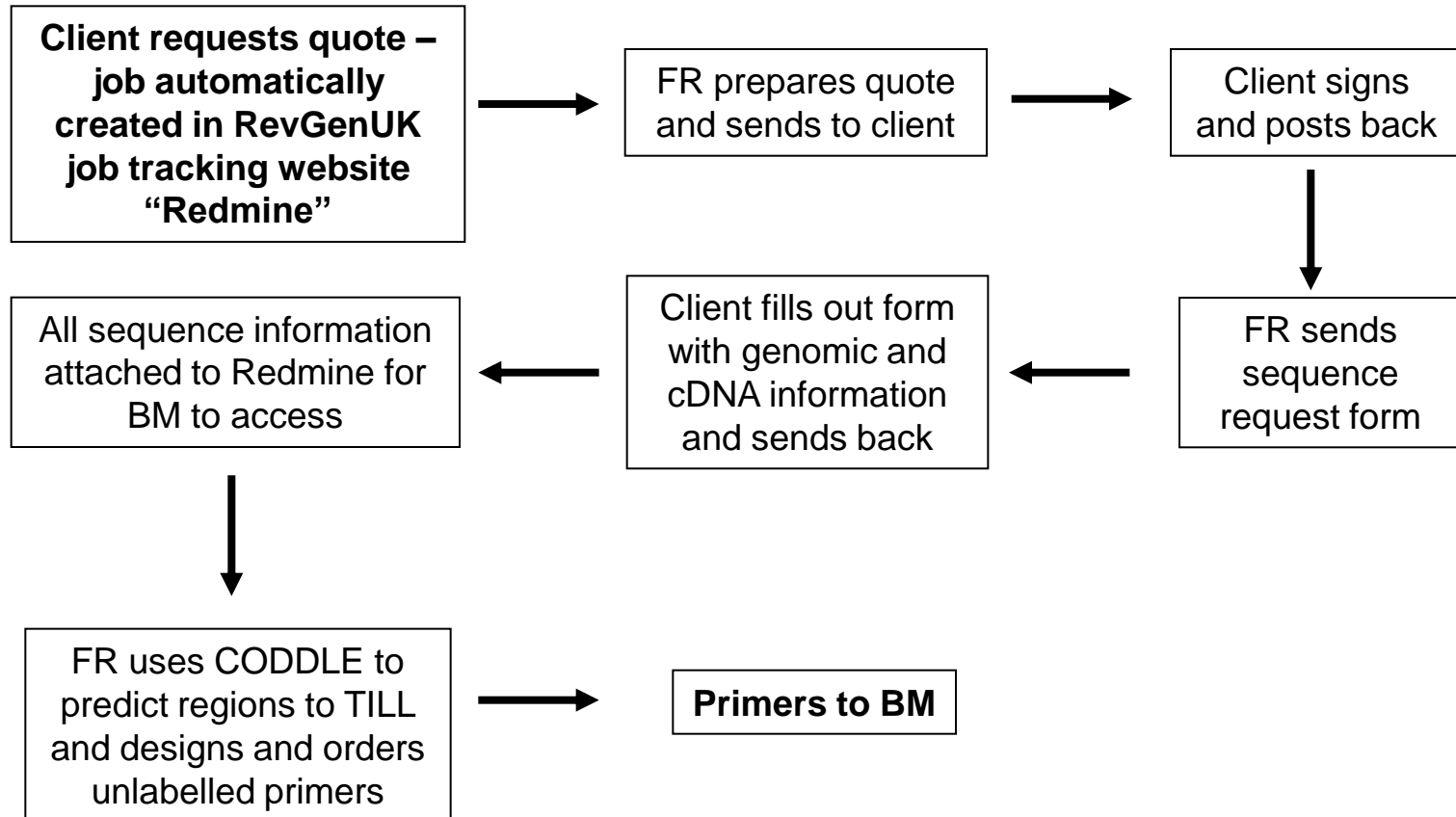
For further information, click '[Contact us](#)'



Reverse Genetics UK

# TILLING job flowchart

## 1) Pre-TILL



# R-0-18 BLAST searches

```
AGCACGCCATAGATCCGATTCTGGCAGCTTCAAACGTTATTGTTAGCTTA
AGCACGCCATAGATCCGATACTGGCAGCTTCAAACATTATCGTTACCTTA
AGCACGCCATAGATCCGATACTGGCAGCTTCAAACATTATCGTTACCTTA
AGCACGCCATAGATCCGATACTGGCAGCTTCAAACATTATCGTTACCTTA
AGCACGCCATAGATCCGATACTGGCAGCTTCAAACATTATCGTTACCTTA
```

```
CAACACCTCGTTTCACGAAGGCAGACCCCTTTAGACTCTCAGGTAGAAAC
CAACACCTTGTTCACGTTGAGGCAGATCCCTTTAGACTCTCAGGTAGAAAC
CAACACCTTGTTCACGTTGAGGCAGATCCCTTTAGACTCTCAGGTAGAAAC
CAACACCTTGTTCACGTTGAGGCAGATCCCTTTAGACTCTCAGGTAGAAAC
CAACACCTTGTTCACGTTGAGGCAGATCCCTTTAGACTCTCAGGTAGAAAC
```

```
ATAACATAATCTTCTTTGGCTCTTTCAAGTGTGATGTATATGCATTGTG
ATAACATAATCTTCTTTGGCTCTTTCAAGTGTGATGTATATGCATTGTG
ATAACATAATCTTCTTTGGCTCTTTCAAGTGTGATGTATATGCATTGTG
ATAACATAATCTTCTTTGGCTCTTTCAAGTGTGATGTATATGCATTGTG
ATAACATAATCTTCTTTGGCTCTTTCAAGTGTGATGTATATGCATTGTG
```

```
TTTG-----GTTACAGTTGCTAAATTTGAAGGCGGTGGTGCTTTTAATG
TTTGCAGGTAGTTACAGTTGCTAAATTTGAAGGAGGGGGTGCTTTTAAT
TTTGCAGGTAGTTACAGTTGCTAAATTTGAAGGAGGGGGTGCTTTTAAT
TTTGCAGGTAGTTACAGTTGCTAAATTTGAAGGAGGGGGTGCTTTTAAT
TTTGCAGGTAGTTACAGTTGCTAAATTTGAAGGAGGGGGTGCTTTTAAT
```

```
TGATTCCAGACTCTGTACTATCGGTGGTACATTACAGAGCCTTTTCAACT
TGATTCCAGACTCTGTACCATCGGTGGTACATTACAGAGCCTTTTCAACT
TGATTCCAGACTCTGTACCATCGGTGGTACATTACAGAGCCTTTTCAACT
TGATTCCAGACTCTGTACCATCGGTGGTACATTACAGAGCCTTTTCAACT
TGATTCCAGACTCTGTACCATCGGTGGTACATTACAGAGCCTTTTCAACT
```

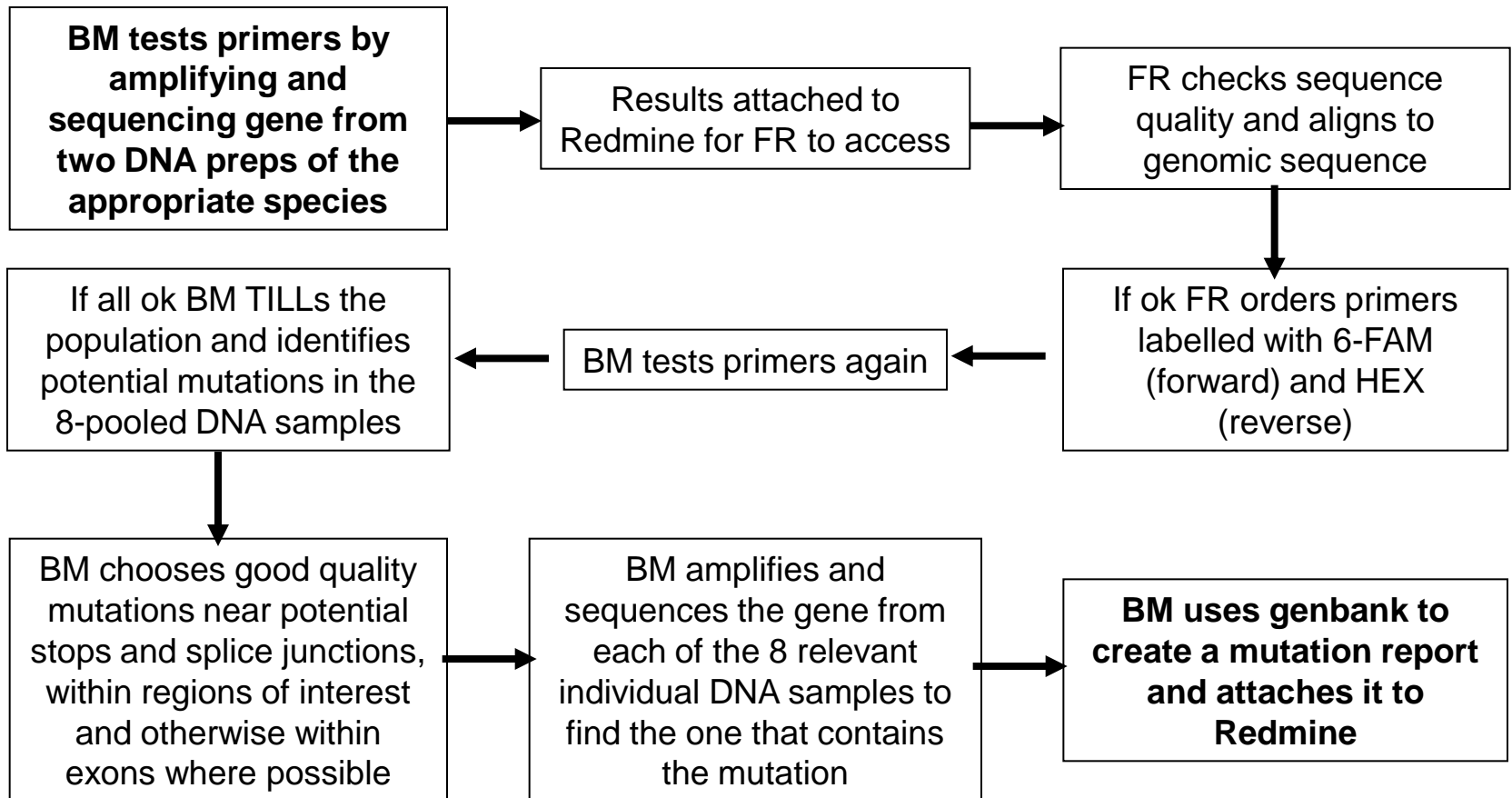
```
AAAGCTTTACACAACCTCAAGAAAAGAATTGACAGGTTTACACAACCTCA
AAAAGCTTTACACAACCTCAAGAAAAGAATTGACAGG-----TCA
AAAAGCTTTACACAACCTCAAGAAAAGAATTGACAGG-----TCA
AAAAGCTTTACACAACCTCAAGAAAAGAATTGACAGG-----TCA
AAAAGCTTTACACAACCTCAAGAAAAGAATTGACAGG-----TCA
```

- 1) Verified polymorphisms identified between client's Chiifu sequence and our R-o-18 sequence
- 2) Identified polymorphisms between R-o-18 and the client's Canadian Cultivar in potential site of primer design
- 3) Assisted in extending sequenced region in a client's gene when they were unable to amplify R-0-18 using primers designed to Chiifu



# TILLING job flowchart

## 2) Primer tests, TILL and sequence confirmation



# Customer reports

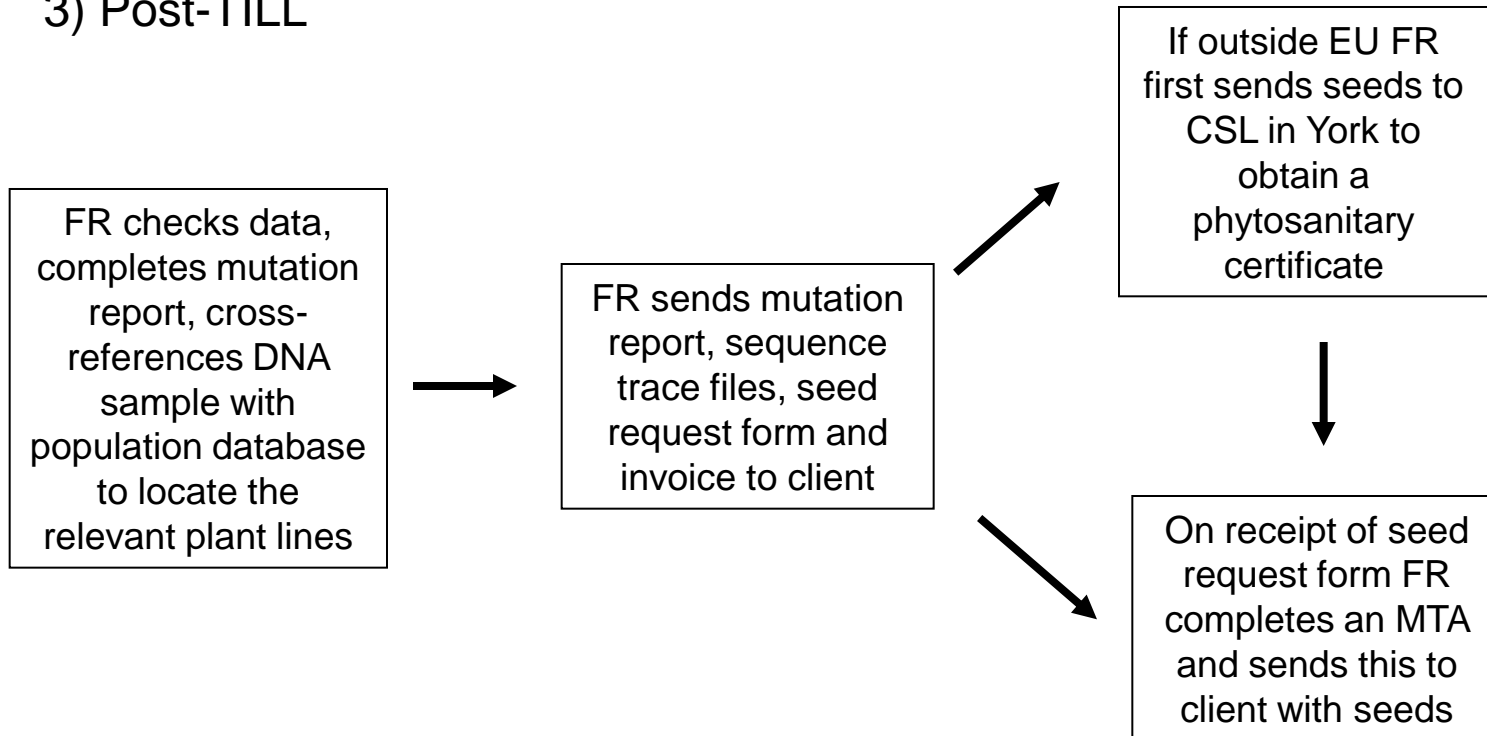
Mutation Surveyor table used to generate Customer Report and all information linked to trace files and stored in database

Date	Primer Set	Plate Position	Plant Name	Sequence													G/A or C/T	Het/Hom	Coding Region	Missense	Truncations		Silent			
				-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5				6	7	Missense	Stop	Splice Site	Non-Coding Region	No Change in AA
29/09/2008	LJCK3	5:H11	533-1	C	A	C	T	A	C	A	G	G	A	C	C	T	G	C	G/GA	Het	2854G>GA:422G>G/R	x				
29/09/2008	LJCK3	8:H12	869-1	T	T	T	G	T	T	A	C	A	A	A	A	T	T	G	C/CT	Het	2568C>CT				x	
29/09/2008	LJCK3	3:G2	247-1	T	C	A	G	A	A	G	T	G	G	A	G	A	A	C	T/TTC	Het	2715T>TC:375S>S/S					x
29/09/2008	LJCK3	2:G1	137-1	G	A	C	T	A	T	G	G	G	A	T	G	T	T	C	G/GA	Het	2748G>GA:386W>W/X		x			
29/09/2008	LJCK3	4:E12	428-1	C	C	C	T	T	T	A	G	C	C	G	A	C	C	A	G/GA	Het	2301G>GA:309A>A/T	x				
29/09/2008	LJCK3	11:B4	1108-1	A	C	T	C	A	C	A	G	A	A	G	C	T	A	C	G/GA	Het	2507G>GA				x	
29/09/2008	LJCK3	34:G5	4521-1	A	A	G	T	T	T	T	C	C	A	C	A	A	A	A	C/CT	HET	3010C>CT				x	
29/09/2008	LJCK3	32:D11	4374-1	T	A	T	C	A	T	T	C	A	T	A	A	A	A	G	C/CT	HET	2833C>CT:415H>H/Y	x				
29/09/2008	LJCK3	31:A2	4201-1	T	C	A	C	A	A	G	C	A	C	T	A	T	G	G	G/GA	HET	2741G>GA:384G>G/E	x				
29/09/2008	LJCK3	32:A2	4297-1	A	G	A	A	G	T	G	G	A	G	A	A	C	T	G	G/GA	HET	2717G>GA:376E>E/K	x				
29/09/2008	LJCK3	47:E1	5719-1	T	T	T	T	C	C	A	C	A	A	A	A	T	T	A	C/CT	HET	3013C>CT				x	
29/09/2008	LJCK3	54:A10	6543-1	A	A	T	C	T	T	G	G	A	T	T	T	T	G	A	G/A	HOM	2800G>A:404D>N	x				
29/09/2008	LJCK3	33:E3	4407-1	C	A	T	T	T	T	T	G	T	G	C	A	G	G	A	G/GA	HET	2614G>GA				x	
29/09/2008	LJCK3	49:H11	6038-1	T	T	T	A	G	C	C	G	A	C	C	A	T	C	C	G/GA	HET	2304G>GA:310D>D/N	x				

All information linked to trace files and stored in database for eventual web access

# TILLING job flowchart

## 3) Post-TILL



# RevGenUK database

- plant information – pedigrees
- seed information – unique identifiers
- gene information – interrogate all the species
- future: web user interface, ‘track and trace’

Actions  
[Search](#)  
[Back to Hub](#)

Message :

### Search Population Database

#### Quick Search

Go to  Number

#### Full Search

Search for

#### Query Details

Species

Ecotype

Population

Generation

Location

Line

Mutagen Options -

Mutant Allele Options -

Transgene Options -

Phenotype

Created By

Is Mutant

Is Transgenic

Is Cross

Is Clone

Planted By

You are required to select search on phenotype.

# RevGenUK TILLING team

Trevor Wang (PI)

Jillian Perry

Fran Robson

Beth McCullagh

Rob Cook

Thanks also to

Catherine Sumner and JIC Horticulture Staff

Dave Baker (TGAC)

Graham King (RRes)

RevGenUK *TILLING*  
*collaborators*

JIC

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Lars Østergaard (Co-I)

Pauline Stephenson

Jon Clarke (Co-I)

Martin Trick (Co-I)

INRA

Richard Thompson

Christine Le Signor

# TILLING service

## CONTACT

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<http://revgenuk.jic.ac.uk/>

for

*Lotus japonicus* 'Gifu' and 'MG20'

*Medicago truncatula* 'jemalong'

*Brassica rapa* 'R-o-18'