

# Improving Hemp Using Heteroduplex Mapping

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[www.cnap.org.uk](http://www.cnap.org.uk)



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# finding solutions in the natural world

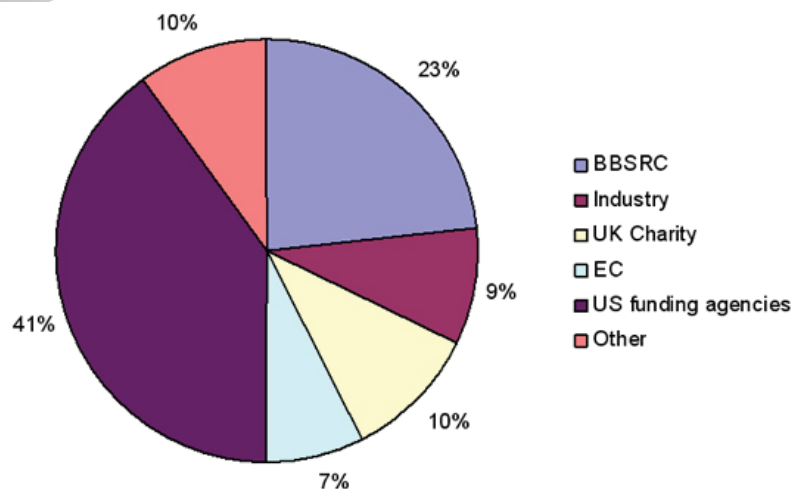
creative  
science

problem  
solving

health

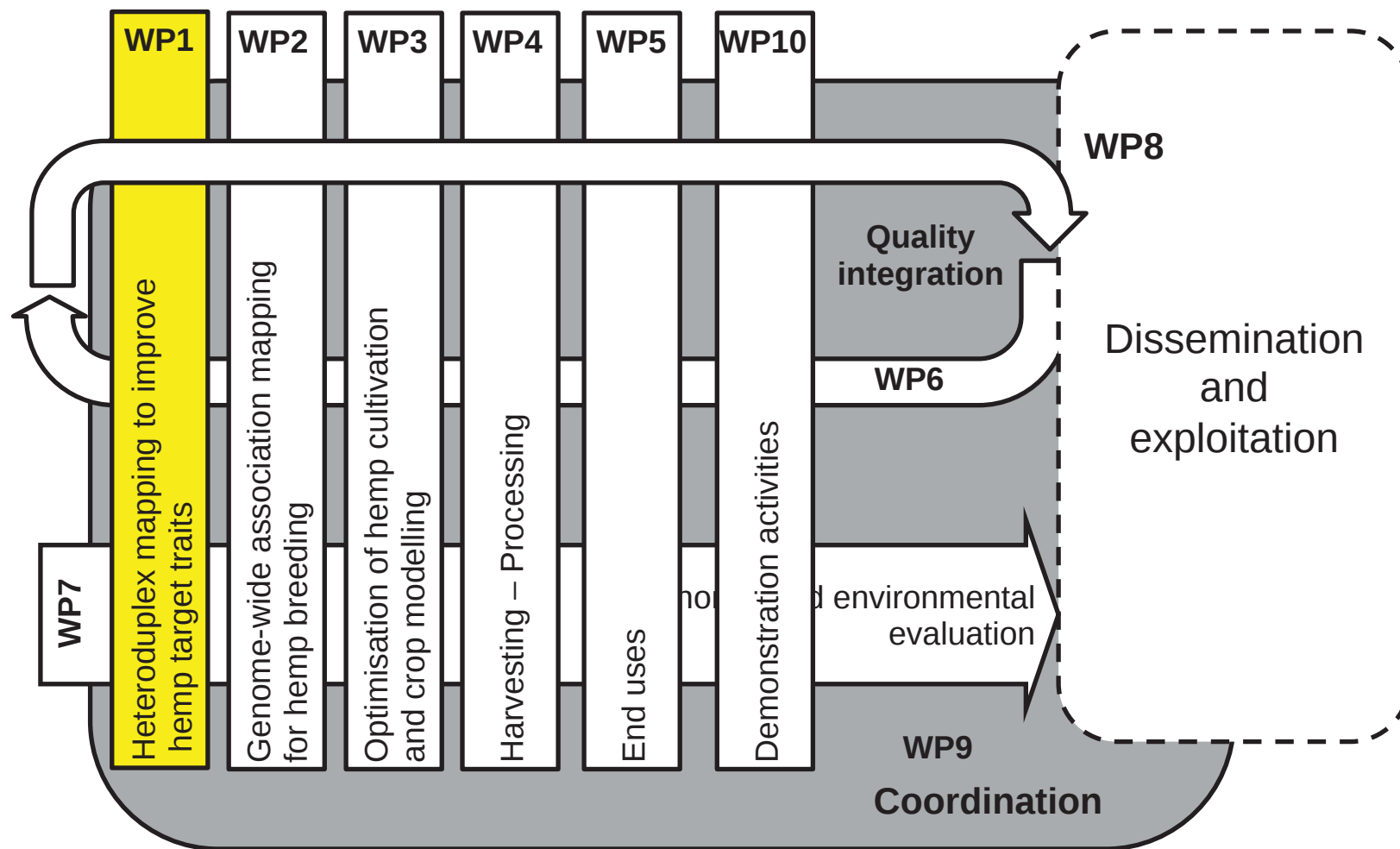
energy/  
biorenewables

remediation and  
clean technology



Current open funding  
is ~ £22 M

# MULTIHEMP



## Crop improvement

- Trait-based selection is forward genetics the classical process for crop improvement- extremely powerful when used in association with genomics- genome wide association mapping- WP2
- Gene-based screening- requires knowledge of the association between a specific gene and a trait- provides a highly targeted way to reach a desired end- sometimes called reverse genetics- WP1
- Both are non-GM approaches to reach desired ends rapidly and efficiently. Non-GM does not risk public acceptance or costly registration issues

# Requirements for heteroduplex mapping

- Good mutant population-High levels of mutations in plants- required to limit the numbers of plants to be screened- we use chemical mutagenesis to increase mutational load in the screening population
- Choice of gene linked to trait of interest -Good knowledge base regarding the genetic components underpinning traits of interest
- Information on chosen hemp gene target- Good gene sequence and expression database from which to identify target genes

## Traits for engineering by reverse genetics- choosing genes for targeting

- Change fatty acid content in seeds for industrial applications
- Lower lignification of fibre- lignin decreases fibre quality and makes decortication harder
- Decrease cell to cell adhesion of fibre to other tissues to improve decortication
- Increase fibre surface charge to improve chemical reactivity with resins and dyes
- In theory- can target essential oil profiles but not in this project at present

## 18 cDNA Libraries

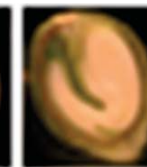
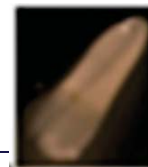
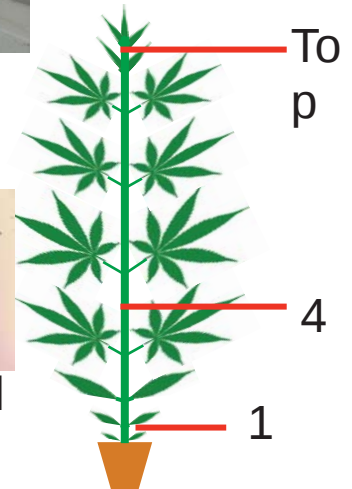
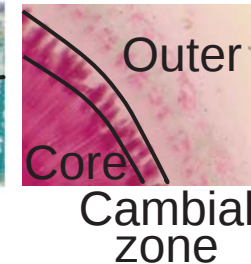
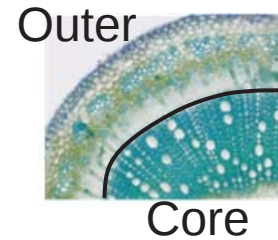
|            |               |
|------------|---------------|
| Root       | (1 week old)  |
| Shoot      | (1 week old)  |
| Whole root | (2 weeks old) |

|                       |               |
|-----------------------|---------------|
| Stem Internode 1      | (2 weeks old) |
| Stem Internode 4      | (3 weeks old) |
| Stem core Internode 1 | (4 weeks old) |

|                        |                |
|------------------------|----------------|
| Stem outer Internode 1 | (4 weeks old)  |
| Fibre Stem Internode 1 | (4 weeks old)  |
| Stem top Top Internode | (4 weeks old)  |
| Stem core Internode    | (12 weeks old) |

|                               |                 |
|-------------------------------|-----------------|
| Stem cambial zone Internode 1 | (12 weeks old)  |
| Leaf pair 4 Mid growth        | (2.5 weeks old) |
| Leaf pair 4 Fully expanded    | (4 weeks old)   |
| flower-white Early stage      | (3 weeks old)   |

Embryo Torpedo  
Embryo U-upturned  
Embryo Filled not desiccated



## cDNA Libraries – target gene mining

- RNA extraction, cDNA synthesis, cDNA library generation
- 454 pyrosequencing, ~2,000,000 EST sequences
- Assembled into 26,783 gene sequences
- Identify best hemp orthologues for each target gene
- Analyse relative expression of genes of interest between libraries
- Confirm *in silico* expression data by quantitative PCR
- Confirm activity of target gene product

Gene  
identifier

| Libraries                  | 6406 | 4372 | 23396 | 20996 |
|----------------------------|------|------|-------|-------|
| Root 1week                 | 12   | 0    | 0     | 0     |
| Shoot 1week                | 3    | 9    | 1     | 5     |
| Root 2weeks                | 12   | 0    | 0     | 0     |
| Stem I1 2weeks             | 0    | 10   | 0     | 0     |
| Stem I4 3weeks             | 0    | 23   | 16    | 29    |
| Stem core I4 4weks         | 8    | 9    | 26    | 3     |
| <b>Stem outer I4 4weks</b> | 8    | 12   | 65    | 2     |
| <b>Stem Fibre I4 4weks</b> | 52   | 52   | 0     | 5     |
| Stem top I4 4weks          | 1    | 5    | 0     | 4     |
| Stem core I4 12weks        | 20   | 0    | 9     | 0     |
| Stem camb zone I4 12weks   | 7    | 5    | 9     | 5     |
| Stem outer I4 12weks       | 25   | 3    | 32    | 15    |
| Leaf expanding             | 0    | 6    | 0     | 17    |
| Leaf grown                 | 0    | 5    | 0     | 18    |
| Flower white               | 0    | 3    | 1     | 2     |
| Embryo filled not dessicat | 0    | 0    | 0     | 0     |
| Embryo Torpedo             | 0    | 0    | 0     | 0     |
| Embryo U-upturned          | 0    | 1    | 0     | 0     |

# cDNA library analysis

## Example

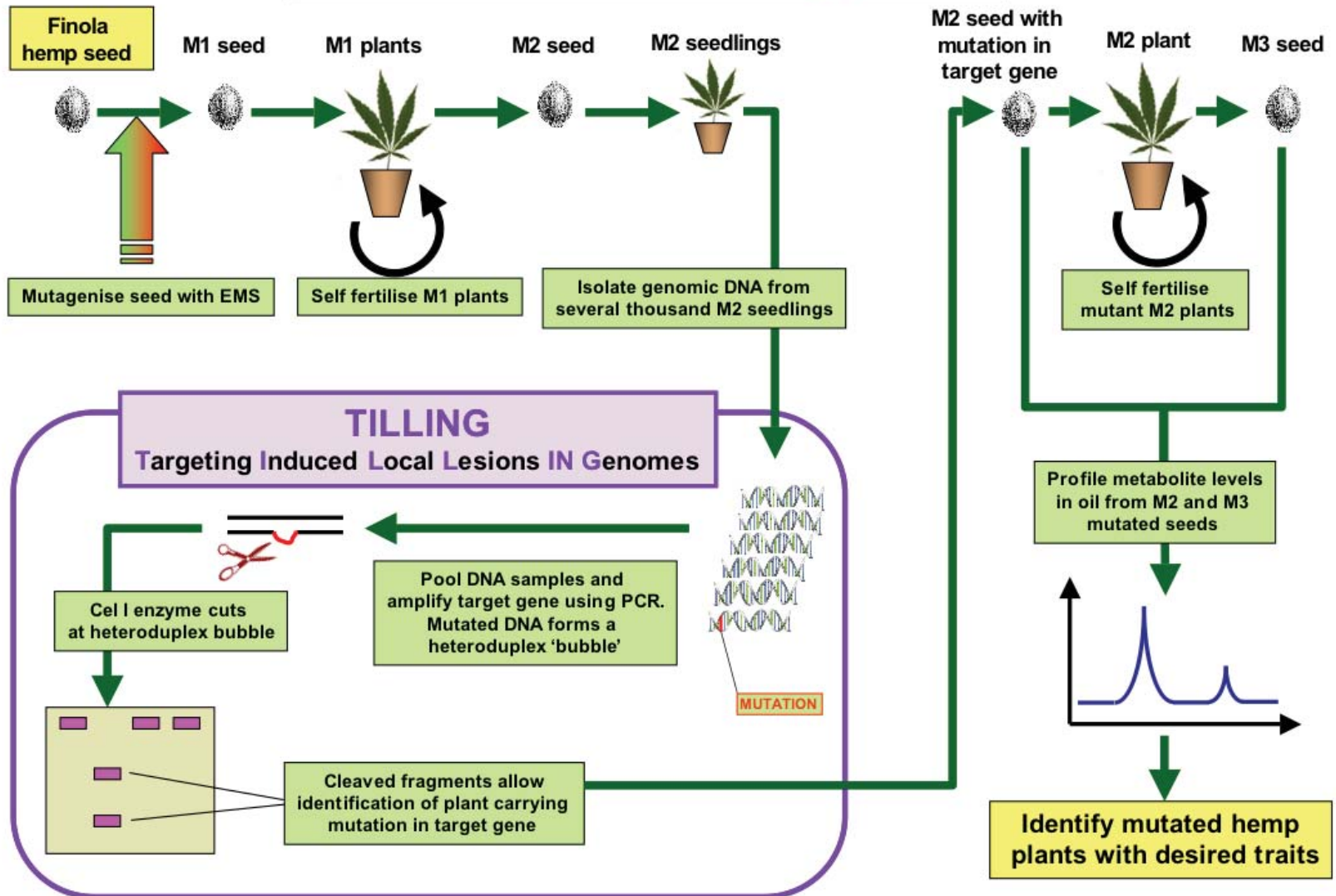
### Relative expression of pectin methylesterase

|       |
|-------|
| 0-9   |
| 10-19 |
| 20-29 |
| 30-39 |
| 40-49 |
| 50-59 |
| 60-69 |

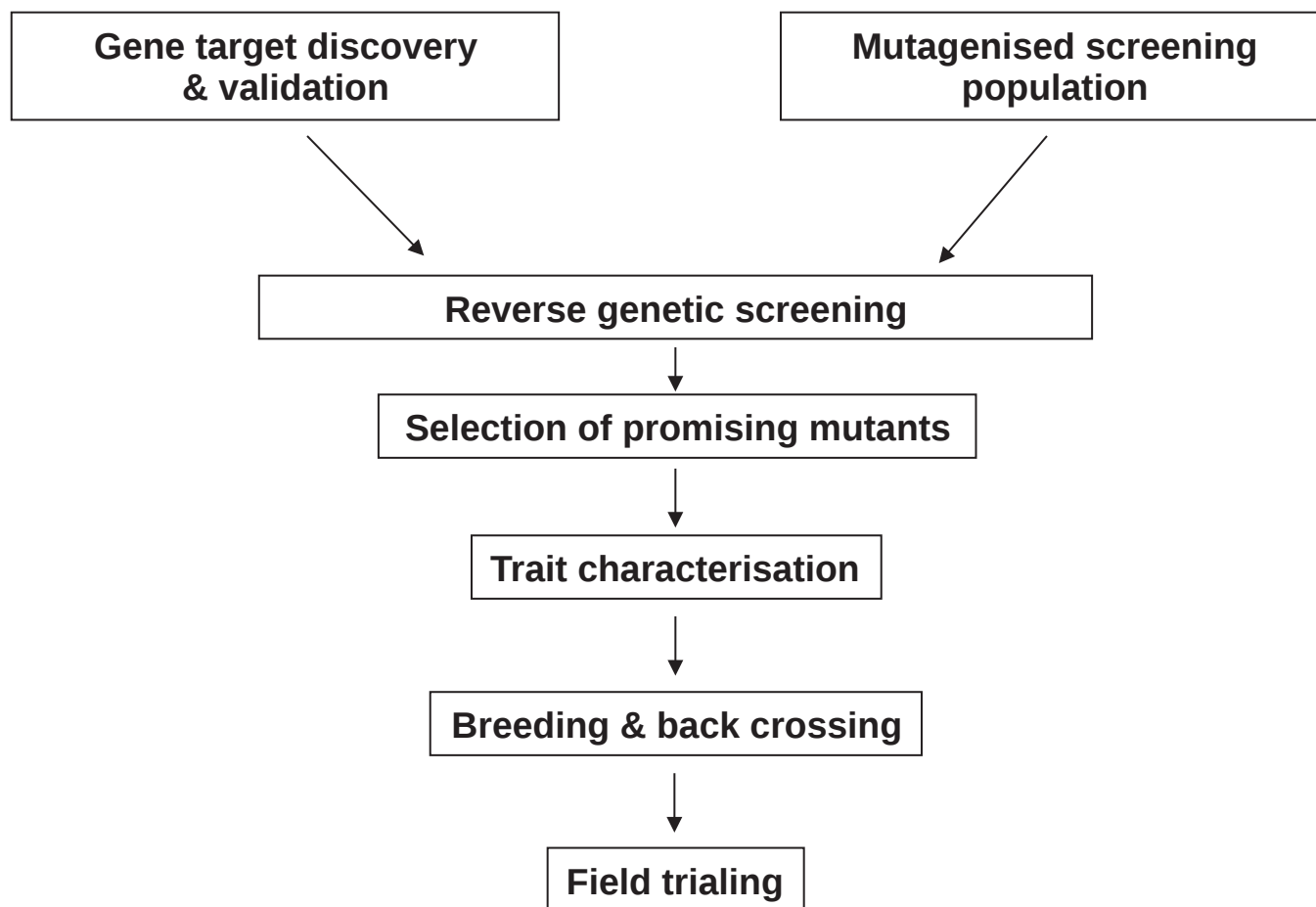
Normalised EST  
counts

# Heteroduplex mapping

## The CNAP fast-track breeding platform

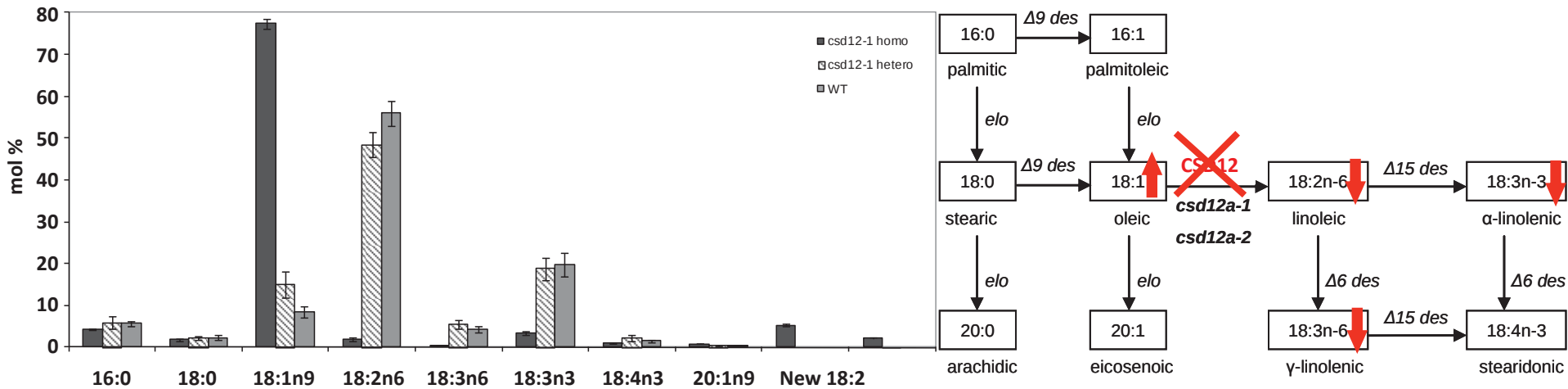
THE  
ARCHIVER

## Experimental work flow for development of new hemp varieties



*cds12a-1* and *cds12a-2* are functional knockout alleles of  $\Delta 12$  desaturase resulting in high-oleic acid phenotype

Fatty acid composition of the seed lipids from seeds segregating allele *cds12a-1*



• *cds12a-1* (Q167\*)



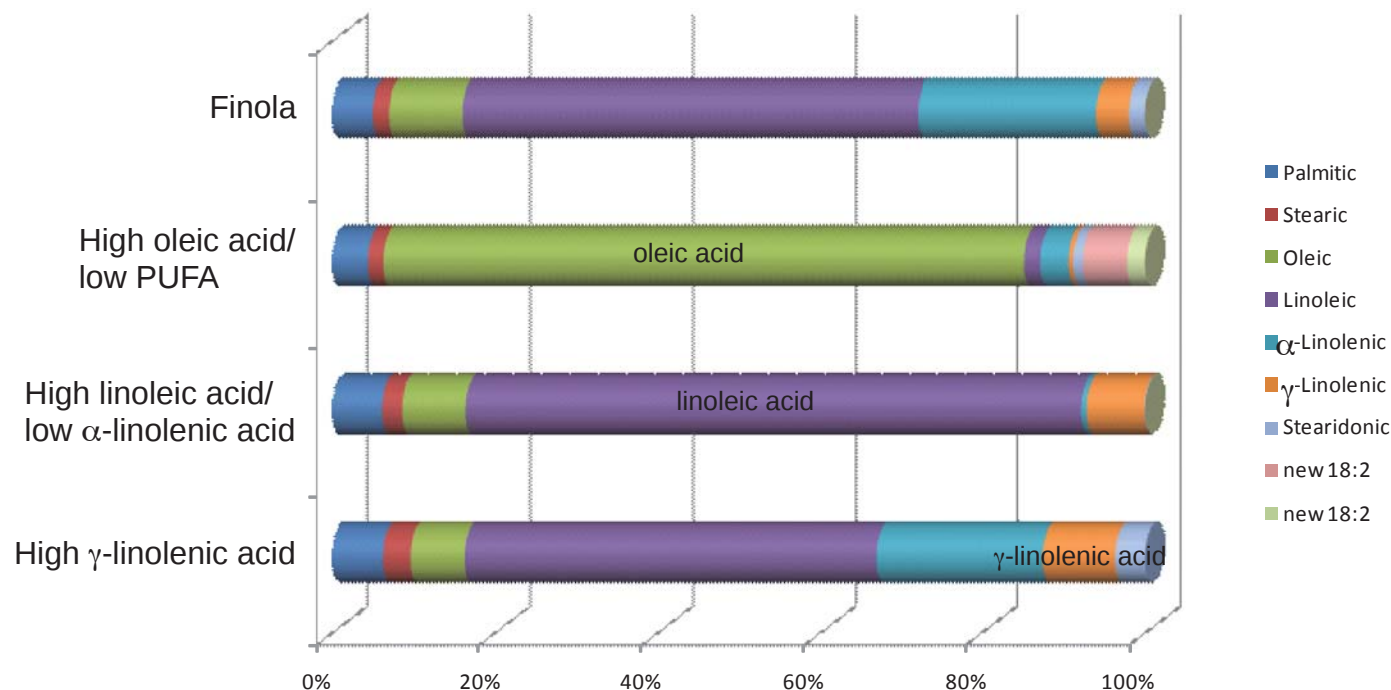
• *cds12a-2* (P218L/P375L)



• high levels of oleic acid (9%  $\longrightarrow$  77%) at expense of PUFAs (>80%  $\longrightarrow$  14%)

• *cds12a-1* and *cds12a-2* starting point for development of high-oleic acid hemp cultivars

## Summary & Outlook

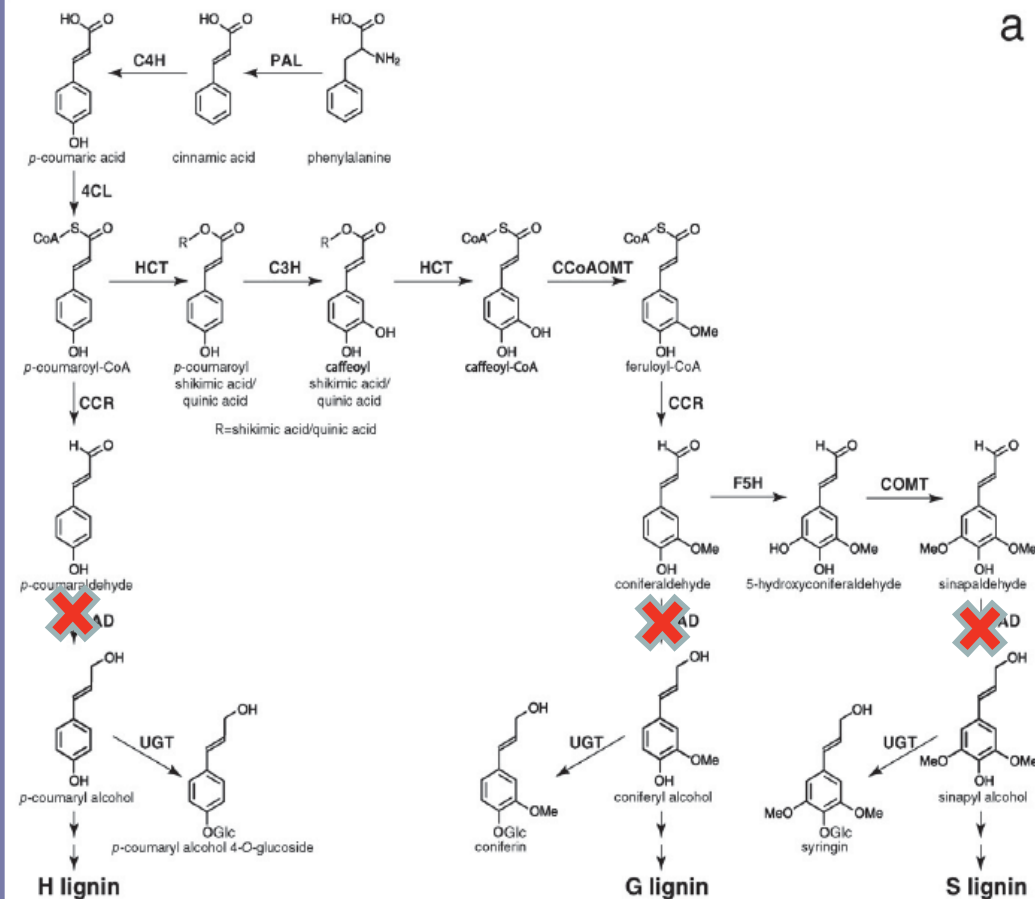


- advanced development of three novel types of hemp seed oil cultivars
- further characterisation of mutants/oils
- field trials with '1. phase cultivars'
- use double mutants to generate further optimised '2. phase cultivars'

# Reducing lignification of fibres

a

- Fibre lignification is temporarily separated from other stem tissues- may be specific genes involved
- Work in other species shows CAD5 is best target for making more extractable lignin
- Identified hemp CAD5 orthologues expressed in older tissues in fibres



# Reducing fibre adhesion

- Fibres joined to one another and surrounding tissues through middle lamella
- Middle lamella glue is essentially pectin-polygalacturonic acid that forms charge-based crosslinks that make up glue
- Polygalacturonic acid is secreted from cells with most charges masked by methyl esters on the carboxyl groups
- Charges are revealed by removal of methyl esters by pectin methyl esterase enzymes leading to increased adhesion
- Target knockout in fibre-specific pectin methyl esterase genes



# BDC Biorenewables Development Centre

**Plants • Processes • Products**

**Home**  
**Business**  
**Research**  
**About us**  
**Facilities**  
**Contact us**

**What does the Biorenewables Development Centre do?** The BDC is a not-for-profit company which provides industry with new processes to convert plants and biowastes into high value products. It addresses the plant feedstock, the conversion processes and the products in the following units

- Plants: [Feedstock development unit](#)
- Processes: [Process development unit](#)
- Products: [Business development unit](#)

**What is unique about the BDC?** The BDC offers a unique combination of internationally recognized analytical science, fast track plant breeding and novel extraction and processing technologies. Focused on business needs, we bridge the gap between laboratory development and commercial manufacture by providing open access scale-up processing facilities

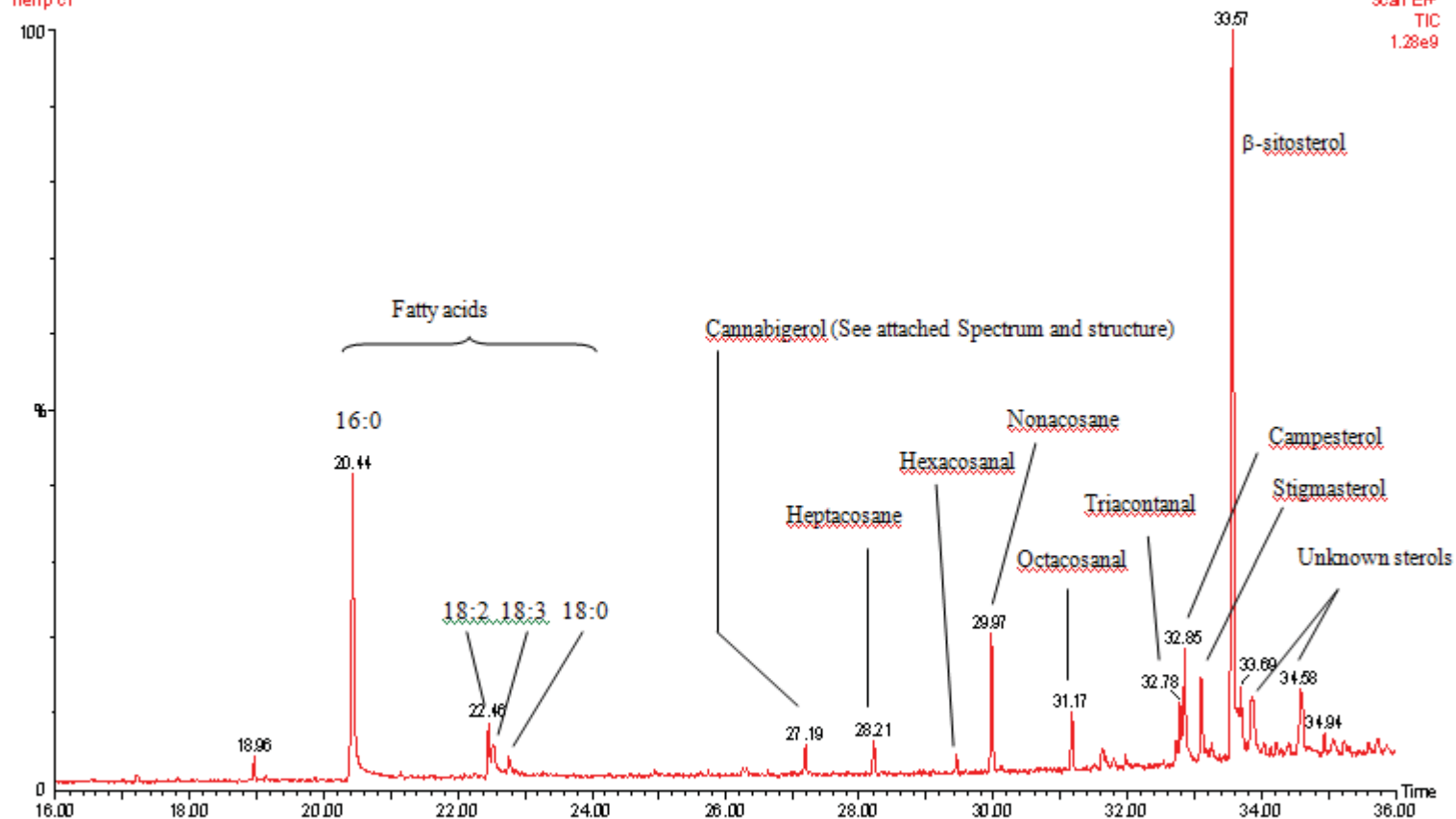
# Supercritical CO<sub>2</sub> extraction of whole hemp stem

Hemp straw SCO<sub>2</sub> extract - expanded Chromatogram

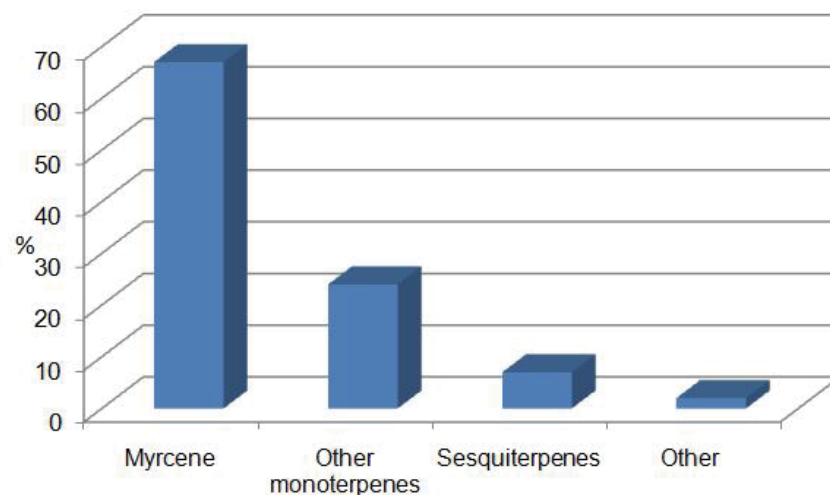
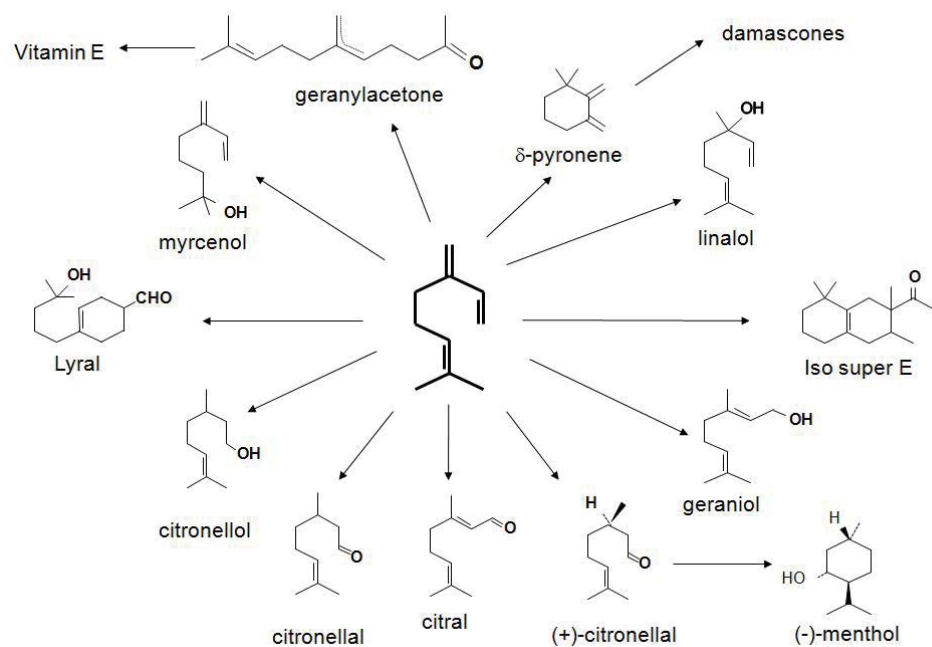
hemp c1

, 21-Dec-2009 + 13:09:13

Scan EH+  
TIC  
1.28e9

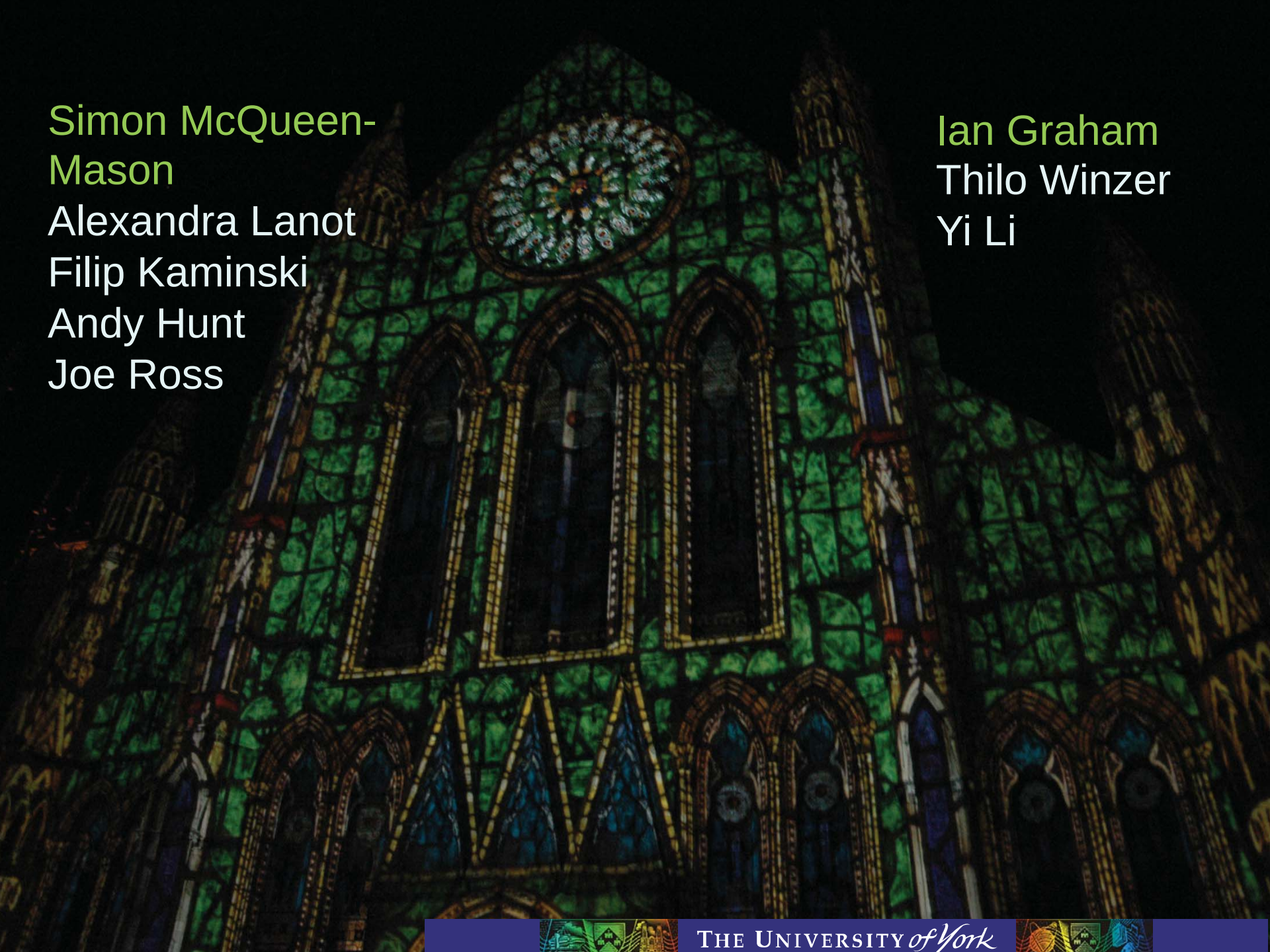


# Essential oils from hemp



Distribution of myrcene in hemp oil and flavour and fragrance applications

- Shortly begin scCO<sub>2</sub> extraction of flower and seed heads from the 2012 field trial of Finola and new high oleic acid variety
- Aim to scale up to allow industrial evaluation



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Andy Hunt  
Joe Ross

Ian Graham  
Thilo Winzer  
Yi Li



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