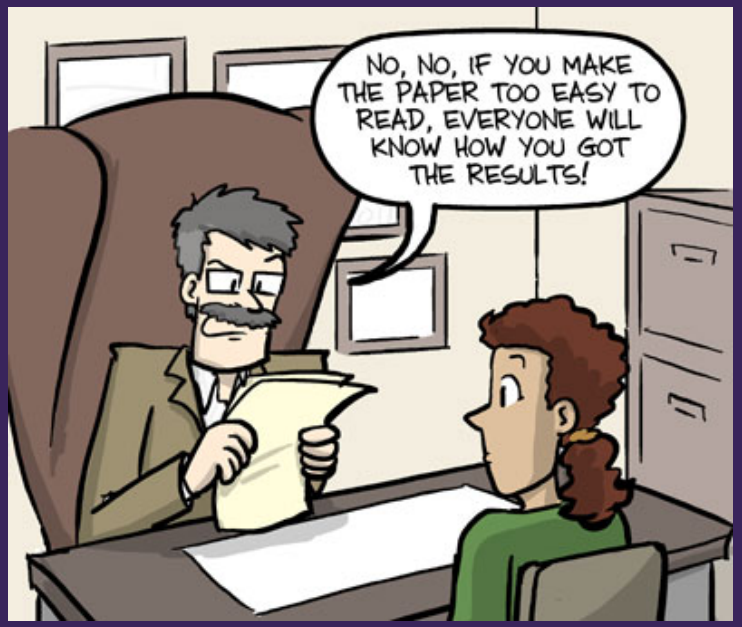
Molekulare Mechanismen der Signaltransduktion



04 - Lesen, Schreiben, Präsentieren
+ Methodisches (EMS Mutagenese + Mapping by sequencing)
Folien:
http://tinyurl.com/Modul-MMS

http://www.nature.com/scitable/content/ne0000/ne0000/ne0000/ne0000/14239512/ECS_scientific-papers_ksm.jpg

Lesen, Schreiben, Präsentieren….

- wissenschaftliche Lehrbücher sind gut zum Erlernen von Grundlagen
  → aber immer veraltet und häufig keine experimentellen Details!
- wissenschaftliche (Original-) Literatur ist die primäre Bezugsquelle relevanter und aktueller Information
- Schult das kritische Lesen/Denken, kann hilfreich für das eigene Verfassen von Artikeln sein
- effizientes Lesen
  = effiziente Forschung!

Artikeltypen

- **Original article**

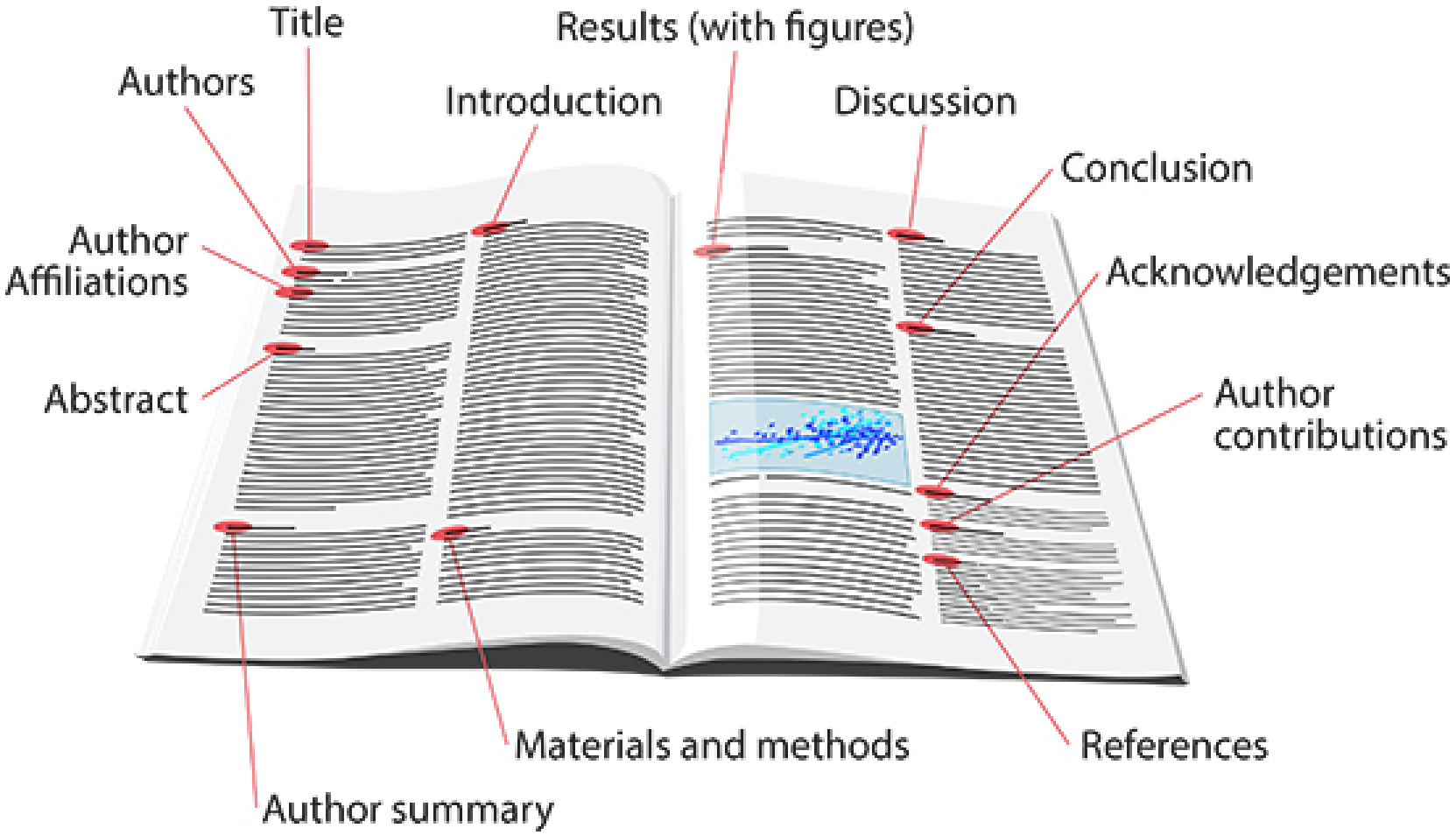
  klassisches paper basierend auf eigenen Forschungsergebnissen
  - full length - “großer artikel” mit mehr Abbildungen
  - short reports - kurze Artikel, meist zu aktuellen Themen
- **Review**

  Übersichtsartikel zu Forschungsergebnissen verschiedenster Autoren (guter Einstieg in ein Thema!)
- **Commentary / opinion paper**

  kurzer Artikel (ähnlich review) mit starker persönlicher Einschätzung
- **Letter to the Editor**

  kurz und knapp, Ergebnisse oder opinion zu (aktuellem) wichtigem Thema

Aufbau - original article



Reihenfolge/Abschnitte teilweise unterschiedlich (je nach Zeitschrift)

https://askabiologist.asu.edu/sites/default/files/resources/articles/anatomy_of_an_article/anatomy_paper_diagram3.jpg

einzelne Abschnitte - original article

- **Title:**
  ideal: trifft den Inhalt, ist “catchy”, enthält wichtige Stichwörter (→ databases/search engines) und **kurz**

- **Authors:**

Vol 452|27 March 2008|doi:10.1038/nature06758 nature

ARTICLES

Erstautor:
Höchster Anteil an praktischer Arbeit
(+ Schreiben)

Genetics of gene expression and its effect on disease

Valur Emilsson[1,2], Gudmar Thorleifsson[1], Bin Zhang[2], Amy S. Leonardson[2], Florian Zink[1], Jun Zhu[2], Sonia Carlson[2], Agnar Helgason[1], G. Bragi Walters[1], Steinunn Gunnarsdottir[1], Magali Mouy[1], Valgerdur Steinthorsdottir[1], Gudrun H. Eiriksdottir[1], Gyda Bjornsdottir[1], Inga Reynisdottir[1], Daniel Gudbjartsson[1], Anna Helgadottir[1], Aslaug Jonasdottir[1], Adalbjorg Jonasdottir[1], Unnur Styrkarsdottir[1], Solveig Gretarsdottir[1], Kristinn P. Magnusson[1], Hreinn Stefansson[1], Ragnheidur Fossdal[1], Kristleifur Kristjansson[1], Hjortur G. Gislason[3], Tryggvi Stefansson[3], Bjorn G. Leifsson[3], Unnur Thorsteinsdottir[1], John R. Lamb[2], Jeffrey R. Gulcher[1], Marc L. Reitman[4], Augustine Kong[1], Eric E. Schadt[2]* & Kari Stefansson[1]*

Senior /corresponding author:
konzeptioneller Anteil / Geldbeschaffer / Author

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Vol 452|27 March 2008|doi:10.1038/nature06758 nature

alle Autoren sind für den Inhalt des papers verantwortlich!

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einzelne Abschnitte - original article

- **Abstract** - Zusammenfassung (~ 150-250 Wörter)
  - Muss Hintergrund, (Methode), Ergebnis und Fazit enthalten
  - Wichtigster Teil des Papers!!!

    hier entscheidet sich ob man den Artikel liest oder nicht

einzelne Abschnitte - original article

- **Abstract** - Zusammenfassung (~ 150-250 Wörter)
  - Muss Hintergrund, (Methode), Ergebnis und Conclusion enthalten
  - Wichtigster Teil des Papers!!!

    hier entscheidet sich ob man den Artikel liest oder nicht
- **Introduction** - ausführliche Beschreibung der Hintergründe, der Fragestellung und Herangehensweise (teilweise auch: Ergebnisse)

einzelne Abschnitte - original article

- **Methods**
  - alle notwendigen Infos um Experimente reproduzieren zu können
  - Info über Statistik, Stichproben, biol. vs. tech. repl., etc....
- **Results**
  - Präsentation der Ergebnisse (in logischer Reihenfolge)
- **Discussion/Conclusions** -
  - Einordnung der Ergebnisse in den bisherigen Wissensstand + eventl. hypothet. Model

Lesen, Schreiben, Präsentieren….

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effizientes Lesen

=„aktives Lesen“ - nicht einfach konsumieren

→ Hinterfragen der Relevanz, Herangehensweise, Methoden, Ergebnisse und Interpretation

→ paper werden i.d.R. nicht direkt „am Stück“ gelesen

1) Entscheiden ob überhaupt relevant
2) Überfliegen → Glaubwürdigkeit und Relevanz
3) detailiertes Durcharbeiten mit Notizen

Preparation
- Quiet place.
- Pencil, paper, photocopy of article.

Deciding what to read
- Read title, abstract.
- Read it, file it or skip it?

Read for breadth
- What did they do?
- Skim introduction, headings, graphics, definitions, conclusions and bibliography.
- Consider the credibility.
- How useful is it?
- Decide whether to go on.

Read in depth
- How did they do it?
- Challenge their arguments.
- Examine assumptions.
- Examine methods.
- Examine statistics.
- Examine reasoning and conclusions.
- How can I apply their approach to my work?

Take notes
- Make notes as you read.
- Highlight major points.
- Note new terms and definitions.
- Summarize tables and graphs.
- Write a summary.

http://www.cse.ogi.edu/~dylan/efficientReading.html

effizientes Lesen

Glaubwürdigkeit:
Figures, Methods (Statistik!), Conclusions
(+ Modell)
nicht unbedingt relevant: Wer + Wo

Preparation
- Quiet place.
- Pencil, paper, photocopy of article.

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- Read title, abstract.
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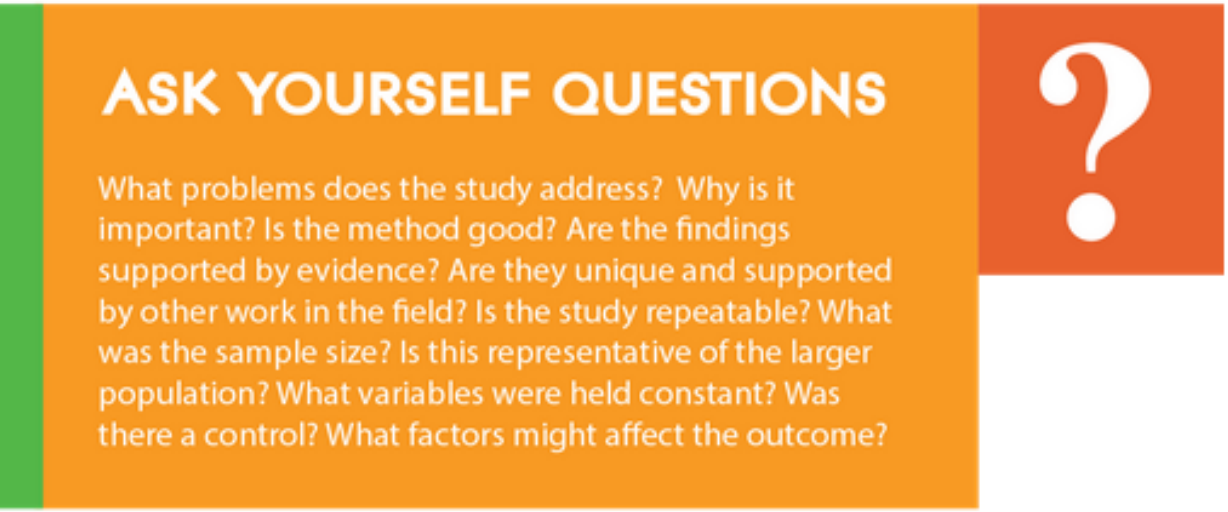
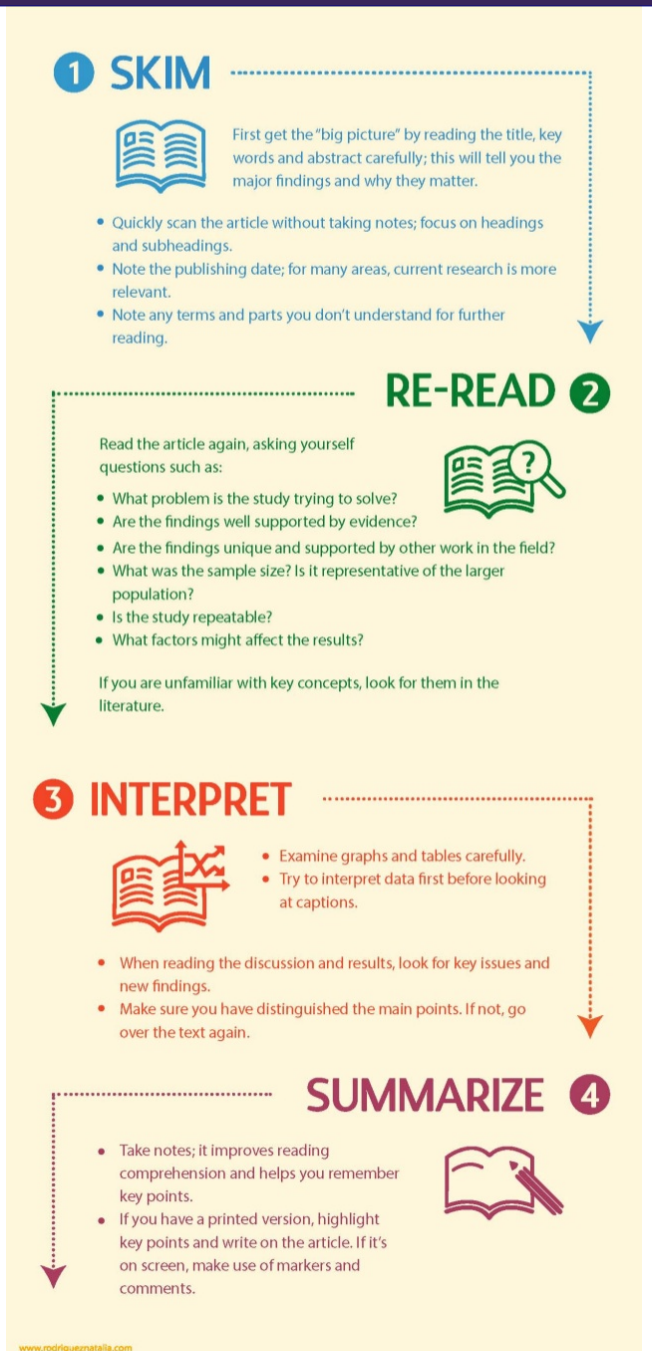
effizientes Lesen

Zahlreiche Varianten:

z.B. THE THREE-PASS APPROACH
http://blizzard.cs.uwaterloo.ca/keshav/home/Papers/data/07/paper-reading.pdf

Wichtig (egal welche Methode):

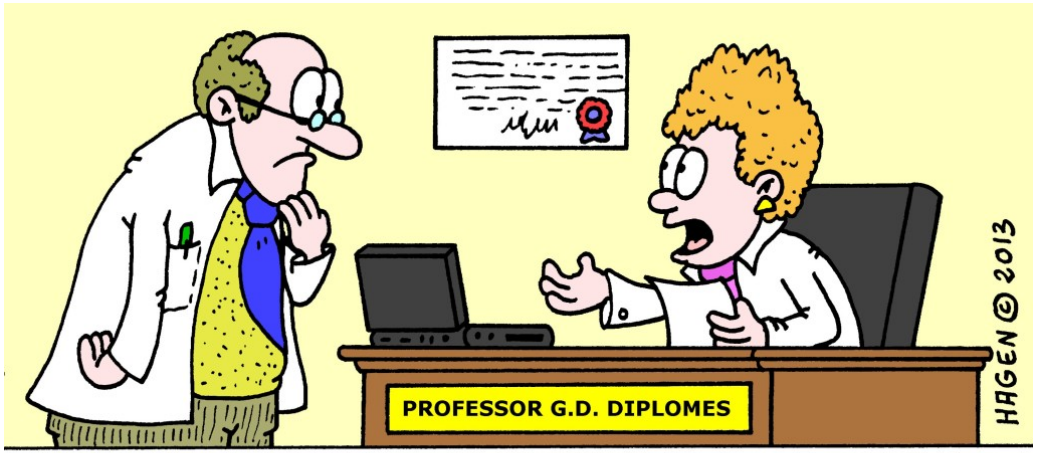




https://www.elsevier.com/connect/infographic-how-to-read-a-scientific-paper

Lesen, Schreiben, Präsentieren….

- Verbreitung von neuen wissenschaftlichen Erkenntnissen
- Publikationen in (guten) wissenschaftlichen Zeitschriften ist die „Währung“ eines Wissenschaftlers
  → peer reviewed journals
  → journals mit hohem Impact Factor
- auch: Abschlussarbeiten, Anträge (Forschungsgelder/Stipendien), Tagungsteilnahmen (Anmeldungen für Vorträge)



It's kind of depressing: We've spent three years on this research paper, and probably only ten people in the World will ever read it...

Zeitschriften ranking - Impact Factor

→ Impact factor (ISI /Thomson Reuters)

$$IF = \frac{\text{Zahl der Zitate im Bezugsjahr auf die Artikel der vergangenen zwei Jahre}}{\text{Zahl der Artikel in den vergangenen zwei Jahren}}$$

Aspekte die beeinflussen (sollten) ob man in journal mit hohem IF publiziert:

- gute Forschung!
- zeitgemäße Themen und Methoden
- gut erzählt + präsentiert

Grundregeln fürs Schreiben

Die zwei Regeln der drei „Cs“:

1. clear, concise, correct

   clear: einfache Wortwahl, kurze Sätze

   concise: „we performed a detailed analysis of“ = „we analyzed“

   correct: genau Beschreibung der Analyse/Experimente/Ergebnisse

2. context, content, conclusion

   gilt für paper gesamt, aber auch pro Abschnitt (insbesondere Ergebnisteil!)

   jeder Abschnitt:

   - beginnt mit Grund/Fragestellung für das jeweilige Experiment
   - zeigt das Ergebnis
   - schließt mit Fazit ab (das auch als Überleitung für den nächsten Abschnitt dient)

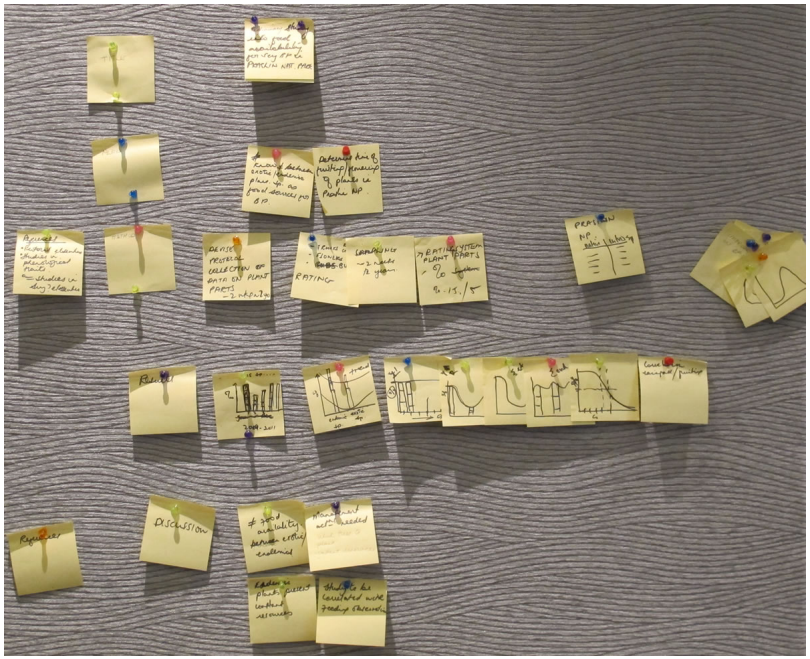
ein gutes paper

- erzählt eine Geschichte (aber keine Fiktion!!)

  Hintergrund - Fragestellung - Herangehensweise - Ergebnisse - Fazit ergeben sich „flüssig“
- ist klar strukturiert, verzichtet auf „Schnörkel“ und Überreibungen
- hat gute (selbsterklärende) Abbildungen + Bildunterschriften
- kritische Berwertung eigener Daten (+Literatur)
- beschreibt Daten und Methoden nachvollziehbar

wie schreibt man ein gutes paper

1. Überlegen welche Daten vorhanden sind → liste mit major result
2. Struktur der „Geschichte“ überlegen (logisch nicht unbedingt chronologisch!)
3. Journal auswählen (scope beachten)
4. author instructions lesen und Manuskriptformat festlegen
5. Figures fertig stellen
6. Abschnitte (außer Abstract) schreiben
7. Abstract + Titel
8. Letzte Formatierungen
9. (Editor) und Gutachtervorschläge überdenken
10. Einreichen!



Korrektur lesen lassen nicht vergessen!
+ alle Co-Authoren müssen zustimmen

der Abstract

*One or two sentences providing a **basic introduction** to the field, comprehensible to a scientist in any discipline.*

*Two to three sentences of **more detailed background**, comprehensible to scientists in related disciplines.*

*One sentence clearly stating the **general problem** being addressed by this particular study.*

*One sentence summarising the main result (with the words "**here we show**" or their equivalent).*

*Two or three sentences explaining what the **main result** reveals in direct comparison to what was thought to be the case previously, or how the main result adds to previous knowledge.*

*One or two sentences to put the results into a more **general context**.*

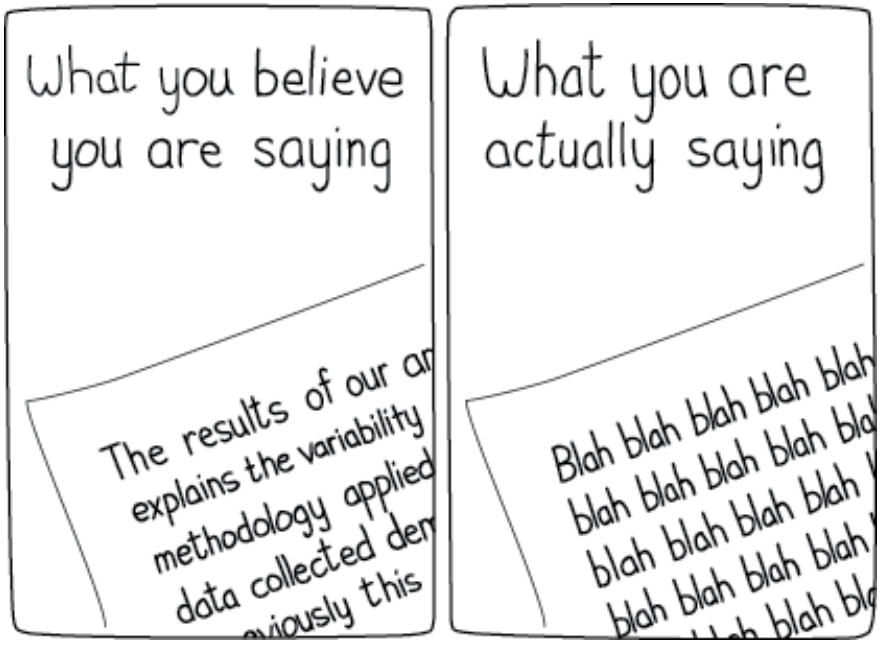
*Two or three sentences to provide a **broader perspective**, readily comprehensible to a scientist in any discipline, may be included in the first paragraph if the editor considers that the accessibility of the paper is significantly enhanced by their inclusion. Under these circumstances, the length of the paragraph can be up to 300 words. (The above example is 190 words without the final section, and 250 words with it).*

During cell division, mitotic spindles are assembled by microtubule-based motor proteins[1,2]. The bipolar organization of spindles is essential for proper segregation of chromosomes, and requires plus-end-directed homotetrameric motor proteins of the widely conserved kinesin-5 (BimC) family[3]. Hypotheses for bipolar spindle formation include the 'push–pull mitotic muscle' model, in which kinesin-5 and opposing motor proteins act between overlapping microtubules[2,4,5]. However, the precise roles of kinesin-5 during this process are unknown. Here we show that the vertebrate kinesin-5 Eg5 drives the sliding of microtubules depending on their relative orientation. We found in controlled *in vitro* assays that Eg5 has the remarkable capability of simultaneously moving at ~20 nm s^{-1} towards the plus-ends of each of the two microtubules it crosslinks. For anti-parallel microtubules, this results in relative sliding at ~40 nm s^{-1}, comparable to spindle pole separation rates *in vivo*[6]. Furthermore, we found that Eg5 can tether microtubule plus-ends, suggesting an additional microtubule-binding mode for Eg5. Our results demonstrate how members of the kinesin-5 family are likely to function in mitosis, pushing apart interpolar microtubules as well as recruiting microtubules into bundles that are subsequently polarized by relative sliding. We anticipate our assay to be a starting point for more sophisticated *in vitro* models of mitotic spindles. For example, the individual and combined action of multiple mitotic motors could be tested, including minus-end-directed motors opposing Eg5 motility. Furthermore, Eg5 inhibition is a major target of anti-cancer drug development, and a well-defined and quantitative assay for motor function will be relevant for such developments.

http://www.nature.com/nature/authors/gta/2c_Summary_para.pdf

allgemeine Tipps zum Schreiben

- LESEN!LESEN!LESEN!
- Reference Manager verwenden
  (Zotero, EndNote, etc...)
- Vektorgrafikprogramme verwenden
  (Inkscape, Illustrator, etc…)
- Zeit in gute Abbildungen investieren
- Modell in Diskussion
- feedback einfordern!
- Korrekturlesen von
  native speakern



manuscript → paper

1. Hürde: Editor
   → Elsevier journals 30 - 50 % Ablehnungen
   → Nature 80 %

Chancen erhöhen:

1. Passendes Journal wählen
   → aim and scope of journal prüfen
2. Form beachten
   → Formatierungen des journals beachten = Guter Eindruck
3. „Sauberes“ Manuskript einreichen
   → Fehler vermeiden, Figures und Text publication ready
4. Guten cover letter schreiben
   → erste Chance den Editor von der Relevanz der Arbeit zu überzeugen
   + Wahl des Journals begründen

paper review

Preprint - Manuscript

Postprint – Accepted Manuscript

Published – Version of record

Submit to publisher

Peer Review

Edit

Accepted by publisher

Copyediting

Typesetting

neuer Versuch anderes Journal

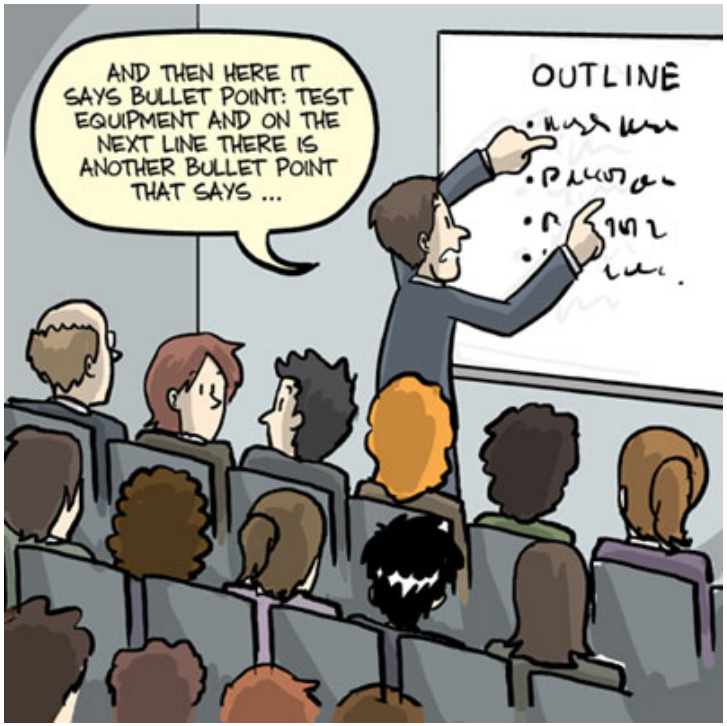
no

http://s3.amazonaws.com/libapps/accounts/29502/images/versions-eng.PNG

Lesen, Schreiben, Präsentieren….

- Vorträge:
  - Vorstellung der Ergebnisse (und sich selbst) vor einem breiteren Publikum
  - Zielgruppenorientiert zusammenstellen
  - visuell attraktiv
  - Informationsgehalt balancieren!

- Qualität von Vorträgen
  - Forschungsergebnisse
  - Präsentationsfähigkeiten
    - → Üben!
    - → Kurse!
    - → feedback einfordern / Film



http://www.nature.com/scitable/content/ne0000/ne0000/ne0000/ne0000/14239658/ECS_presentations_ksm.jpg

ein guter Vortrag:

Inhaltlich ähnlich wie beim paper:

- erzählt eine Geschichte (aber keine Fiktion!!)

  Hintergrund - Fragestellung - Herangehensweise - Ergebnisse - Fazit ergeben sich „flüssig“
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- hat gute (selbsterklärende) **Abbildungen** + Bildunterschriften
- kritische Berwertung eigener Daten (+Literatur)
- beschreibt Daten und Methoden nachvollziehbar

→ **aber (fast) alles mit weniger Details!**

Vortragsgliederung

- Einleitung / Hintergrund
- **Fragestellung / Ziel der Arbeit!**
- Herangehensweise / Fragestellung des Experimentes (konkret)
- Ergebnis1 + Diskussion
- Herangehensweise / Fragestellung des Experimentes (konkret)
- Ergebnis 2 + Diskussion
- …..
- Zusammenfassung u/o. Modell
- Danksagung

Auch hier gelten die 2 Regeln der drei „Cs“

1. clear, concise, correct
2. context, content, conclusion

Presentation skills

→ Die Art der Präsentation hat großen Einfluss auf den Erfolg!

Presentation skills

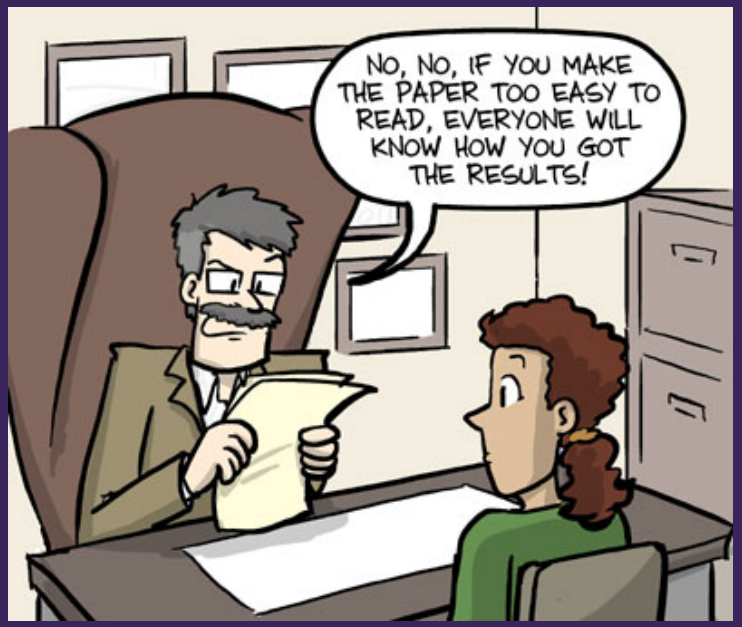
- Sprachkenntnisse (Englisch)
  → Kurse, Sprach-Stammtisch, int. Kollegen/Studenten
  → Üben (Filme, Bücher, Hörbücher, Selbstgespräche)

- Enthusiasmus
  → nur wenn Sie begeistert vom Thema sind, können Sie auch andere dafür begeistern

- authentisch
  → es gibt viele nützliche Tipps - nicht alle werden für jeden funktionieren

- feedback
  → holen Sie sich Rat und feedback von (kritischen) Leuten

Molekulare Mechanismen der Signaltransduktion



04 - Lesen, Schreiben, Präsentieren

+ Methodisches (EMS Mutagenese + Mapping by sequencing)

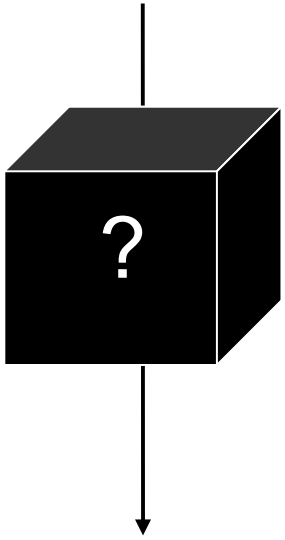
Folien:

http://tinyurl.com/Modul-MMS

http://www.nature.com/scitable/content/ne0000/ne0000/ne0000/ne0000/14239512/ECS_scientific-papers_ksm.jpg

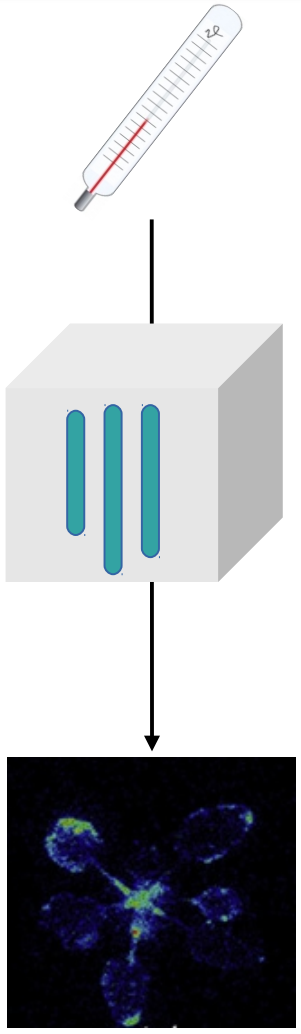
forward genetic screens

stimulus



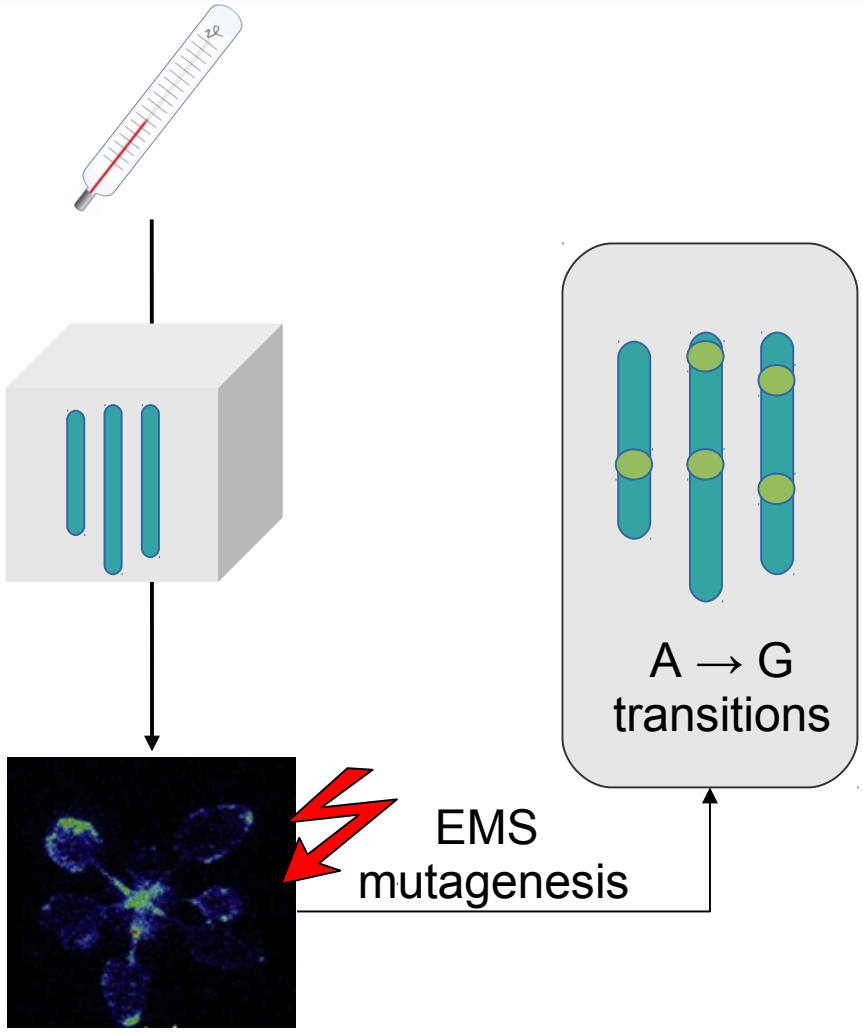
response

forward genetic screens



[*HSP70:LUC*]
(Kumar & Wigge 2010)

forward genetic screens



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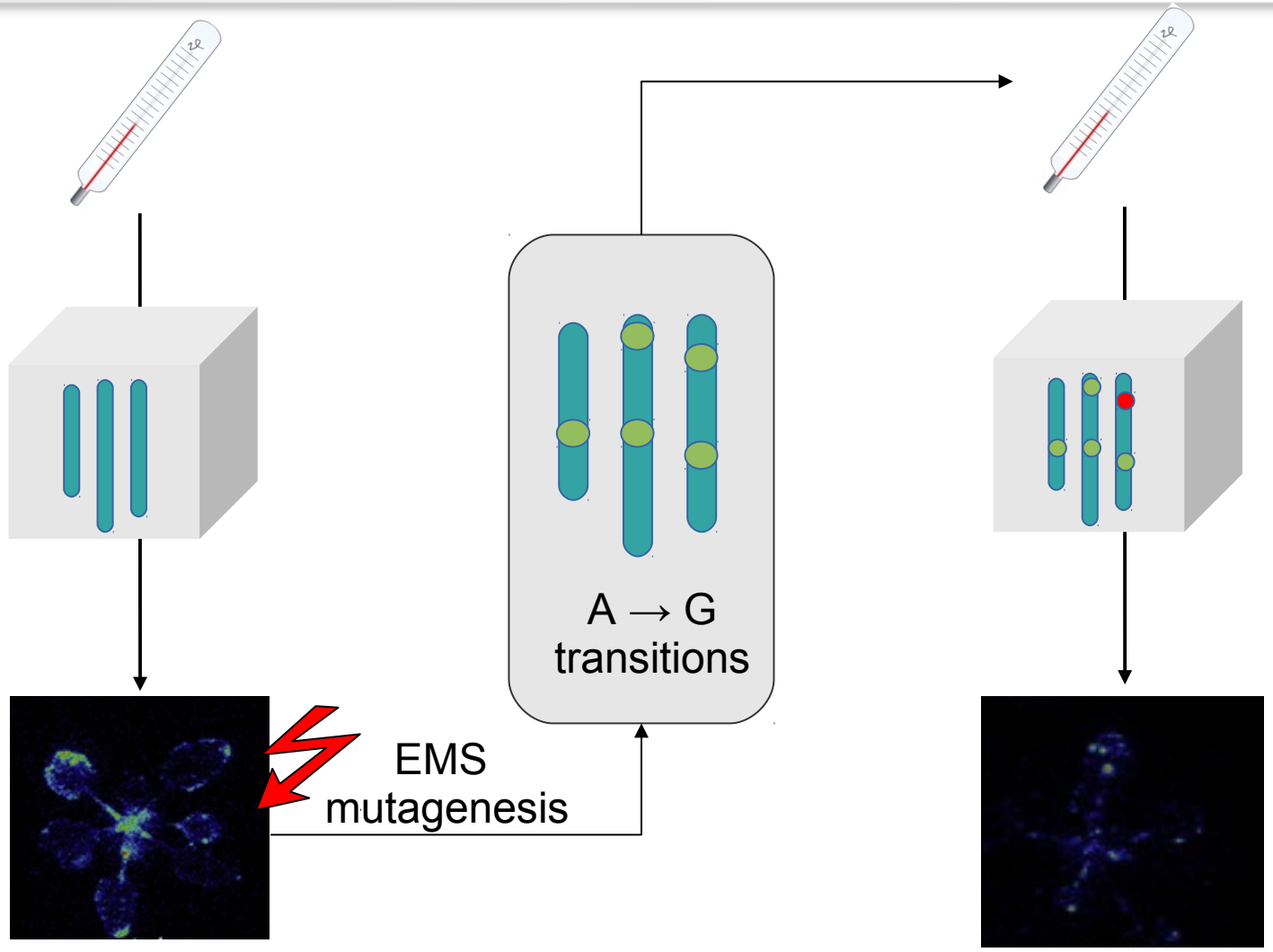

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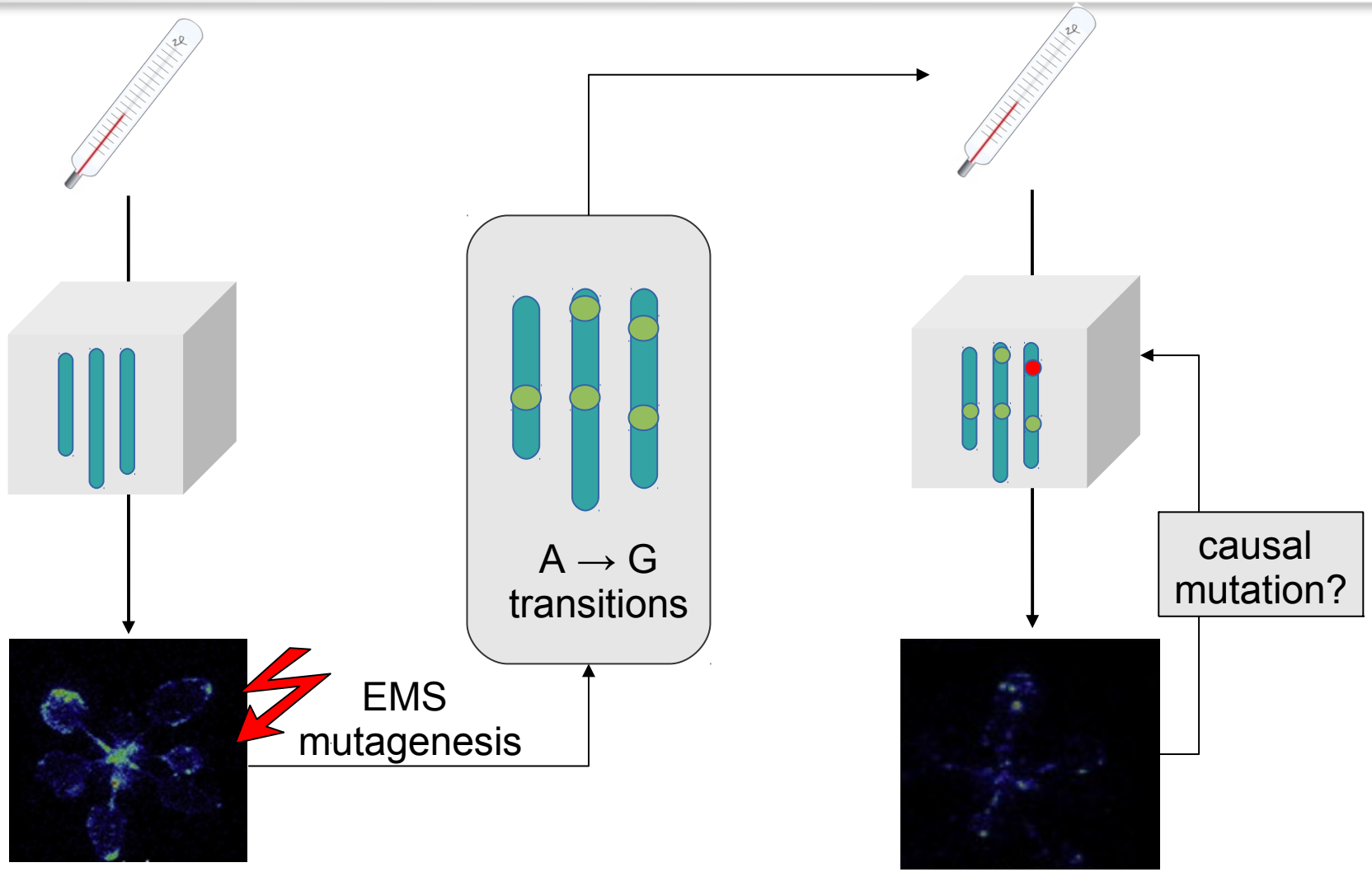

[*HSP70:LUC*]
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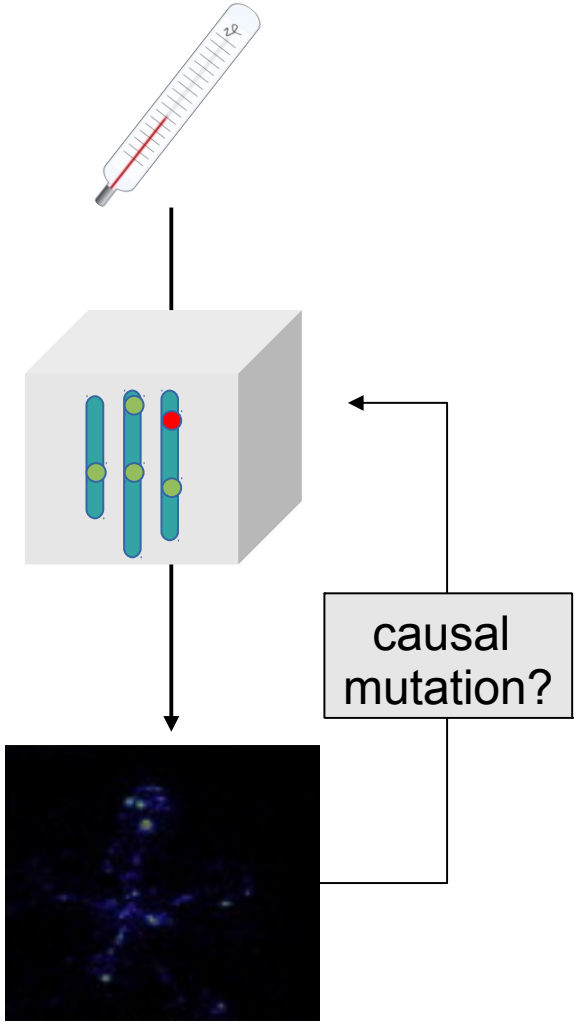
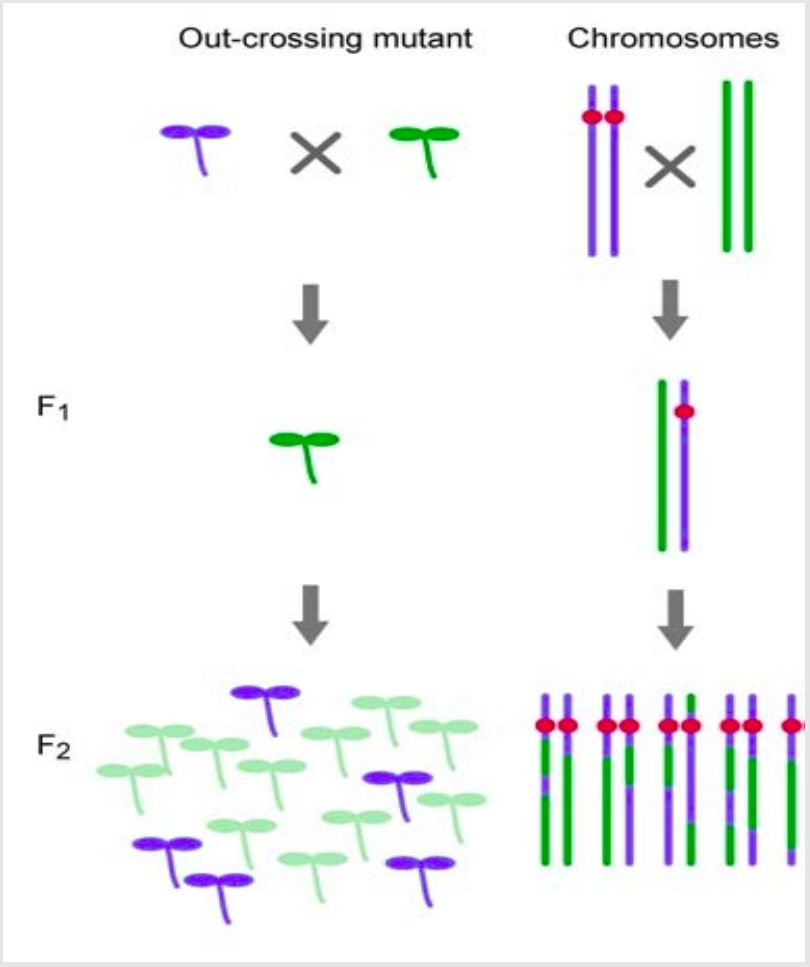
response to moderate temperature shifts

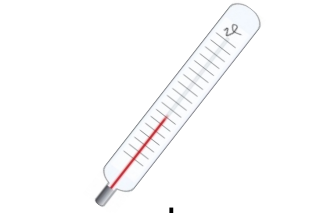


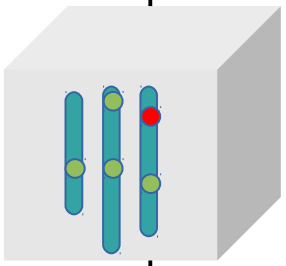
map-based cloning



Schneeberger & Weigel (2010, TiPS)

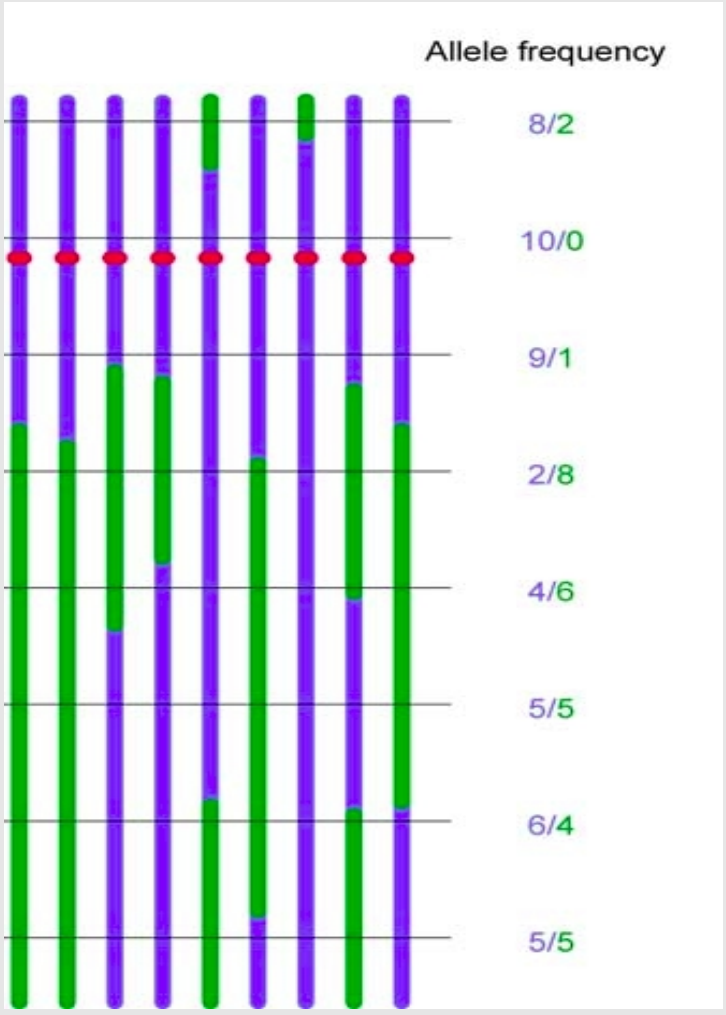
response to moderate temperature shifts





causal mutation?

map-based cloning



Schneeberger & Weigel (2010, TiPS)

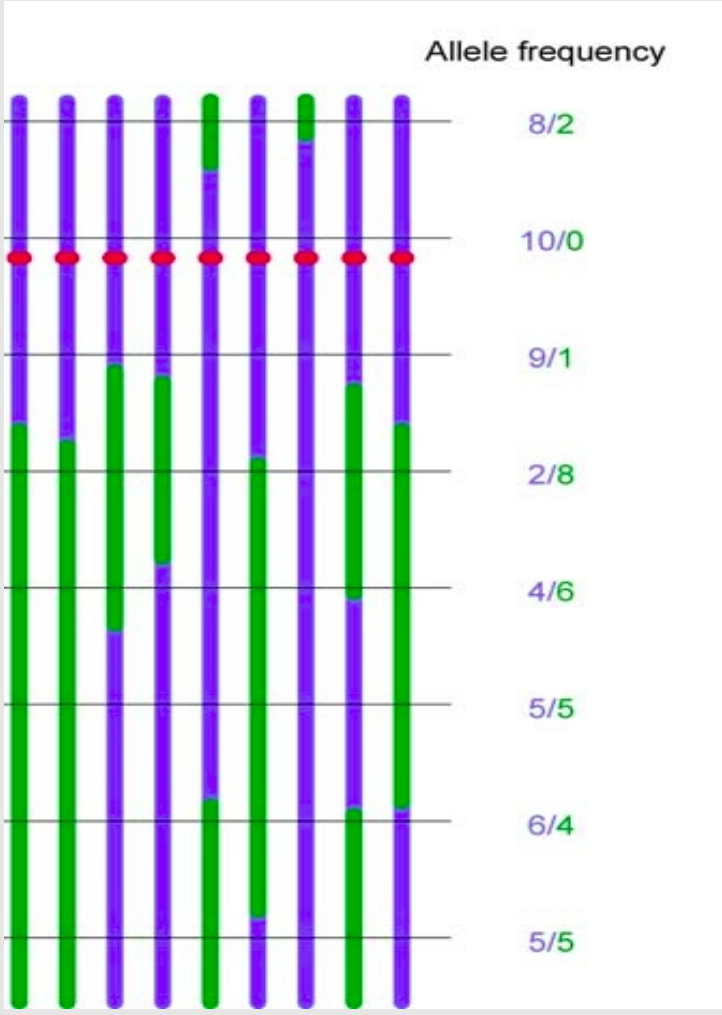
response to moderate temperature shifts

classic map-based cloning requires:

- marker (sequence) information → genotyping
- high number of F2 individuals (subsequent crosses → F3)

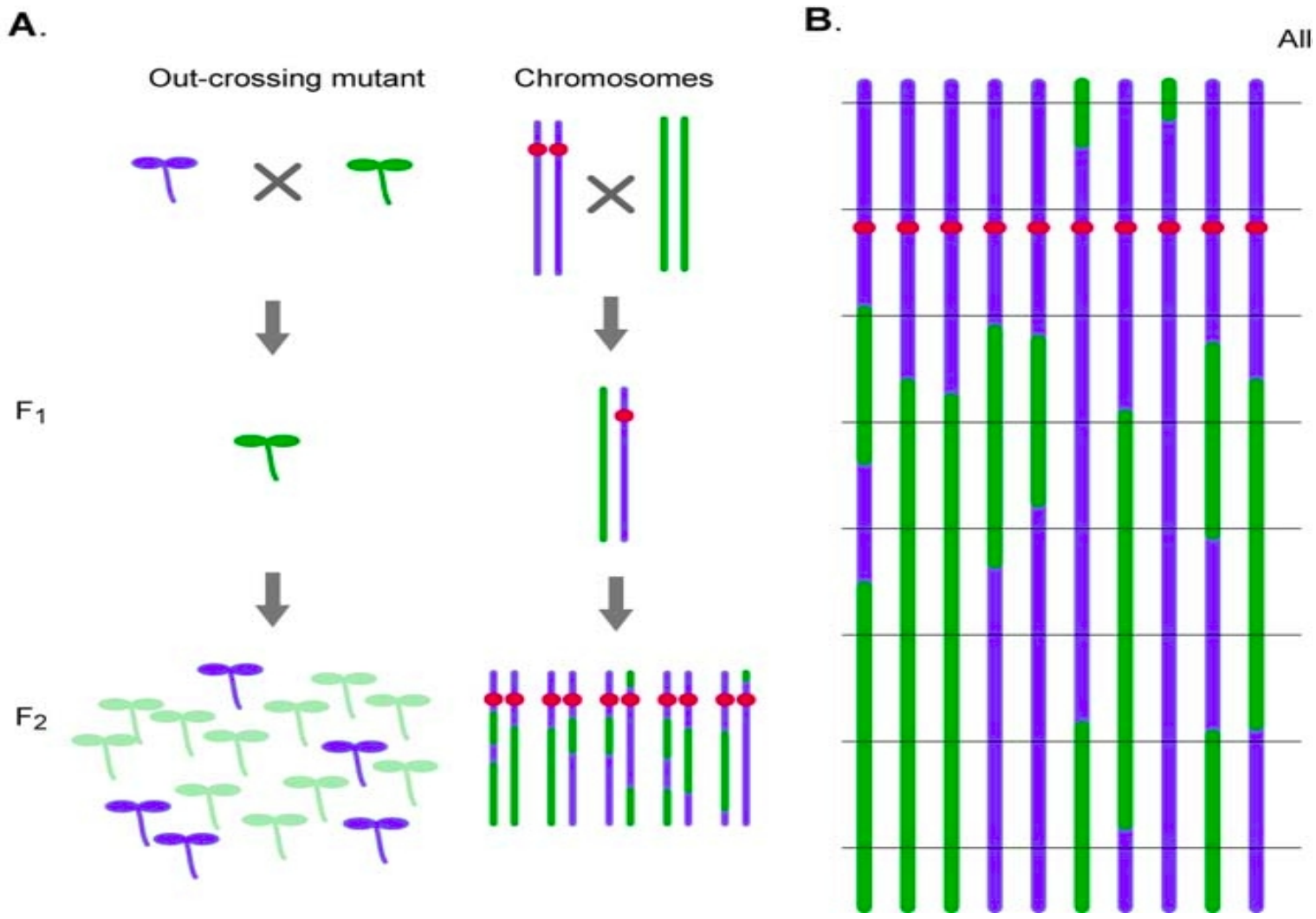
→ time-intensive

map-based cloning



Schneeberger & Weigel (2010, TiPS)

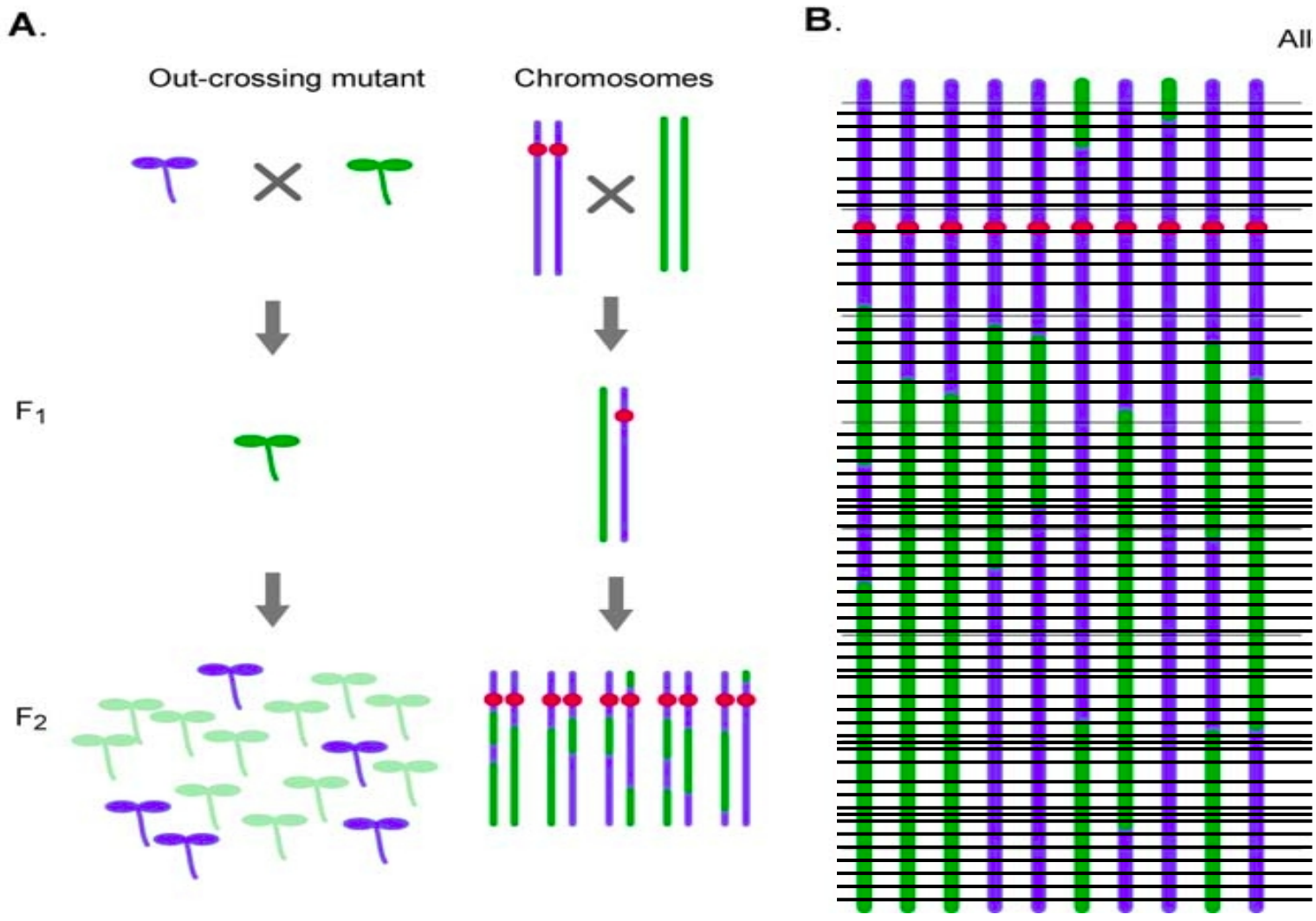
next generation mapping



sequencing of an (out-cross) mapping population → high resolution map

Schneeberger & Weigel (2010, TiPS)

next generation mapping

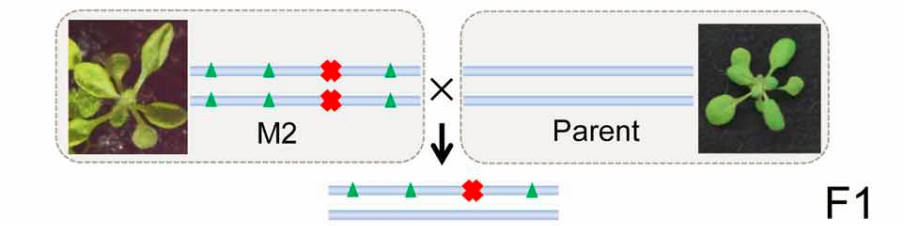


sequencing of an (out-cross) mapping population → high resolution map

Schneeberger & Weigel (2010, TiPS)

next generation mapping (BC1)

back-cross (BC) population also suitable for mapping by sequencing!

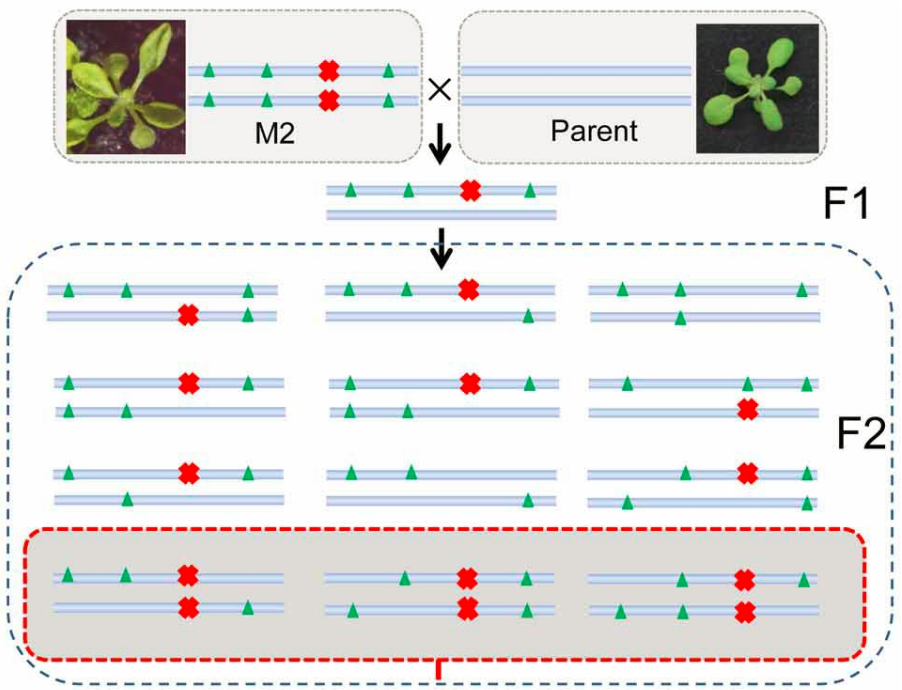


Allen et al. (2013, Front. Plant Sci)

next generation mapping (BC1)

back-cross (BC) population also suitable for mapping by sequencing!

BC1 F2 individuals are selected by mutant phenotype → DNA pooled



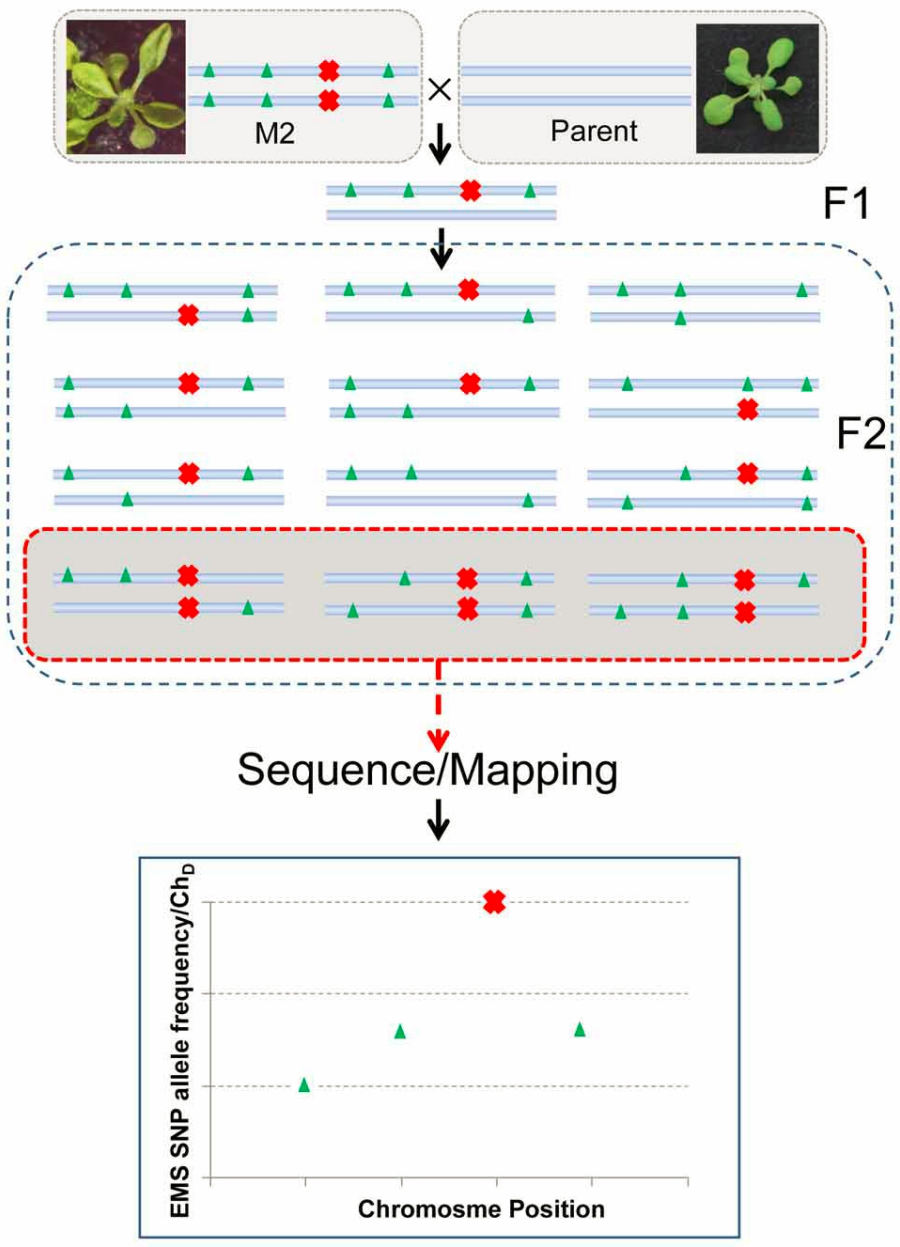
Allen et al. (2013, Front. Plant Sci)

next generation mapping (BC1)

back-cross (BC) population also suitable for mapping by sequencing!

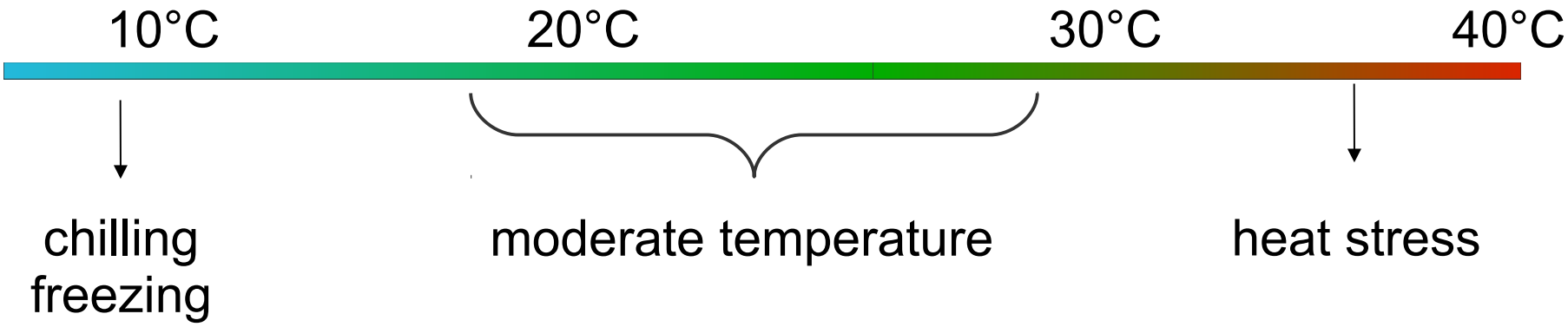
BC1 F2 individuals are selected by mutant phenotype → DNA pooled

EMS-induced SNPs used for mapping

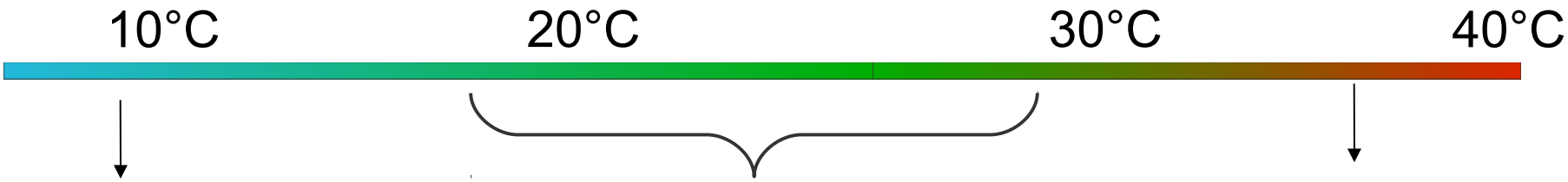


Allen et al. (2013, Front. Plant Sci)

from theory to real life.....



from theory to real life.....



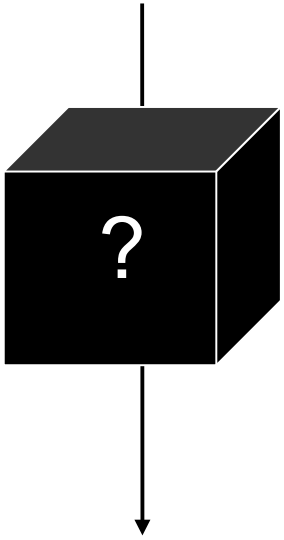
chilling
freezing

moderate temperature

heat stress

ΔT (20 → 28°C)

stimulus



response

from theory to real life.....

10°C 20°C 30°C 40°C

chilling
freezing

moderate temperature

heat stress

ΔT (20 → 28°C)

sensor?

PIF4

auxin biosynthesis

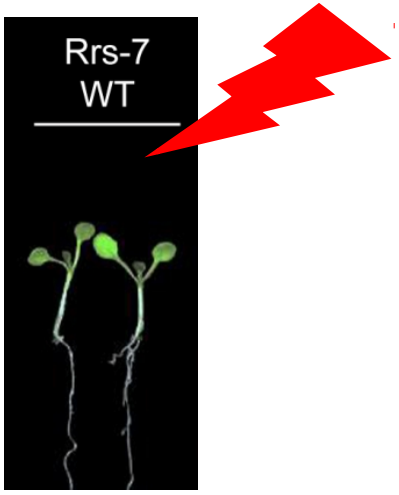
auxin response

physiological response

flowering time

elongation growth

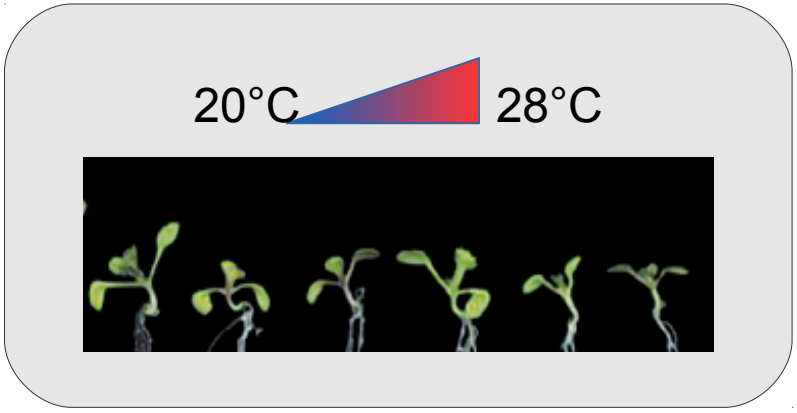
the *opi* screen



EMS

screen of
45 000 M2
seedlings

reduced
temp.-induced hypocotyl elongation
(TIHE)

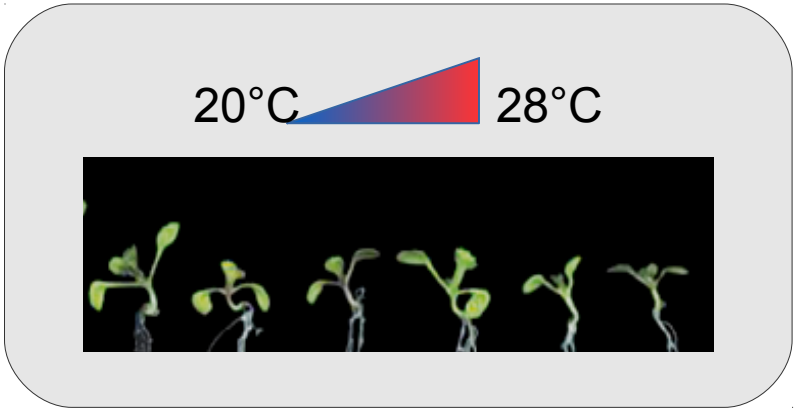


the *opi* screen

EMS

screen of
45 000 M2
seedlings

reduced
temp.-induced hypocotyl elongation
(TIHE)



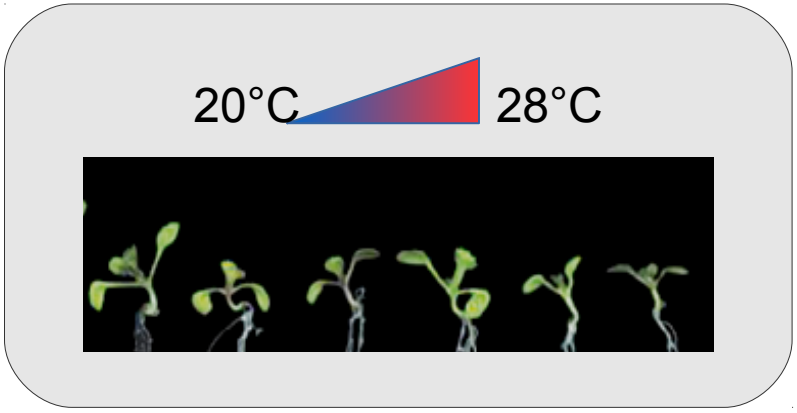
“giraffe”
phenotype

the *opi* screen

EMS

screen of
45 000 M2
seedlings

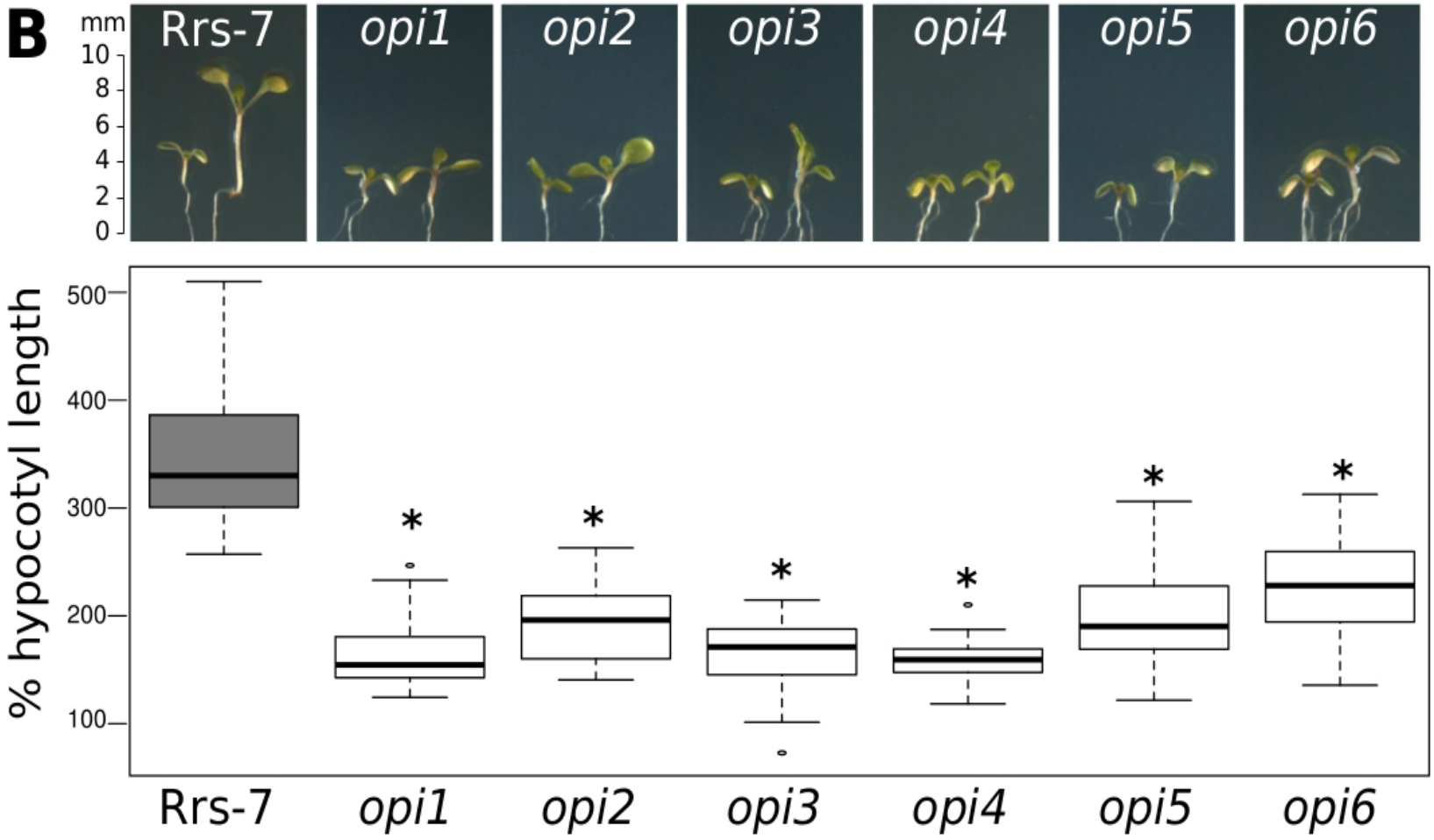
reduced
temp.-induced hypocotyl elongation
(TIHE)



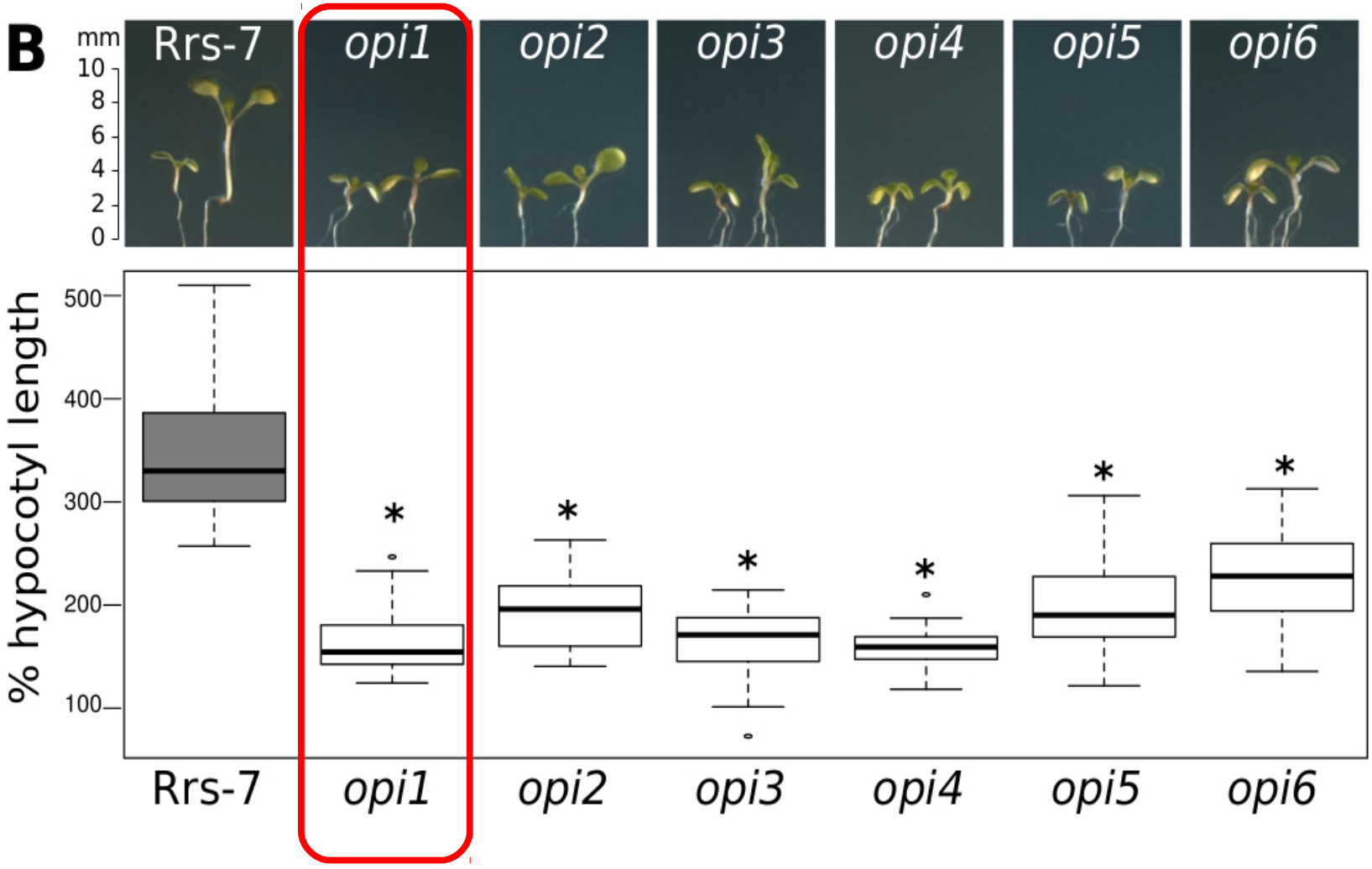
"giraffe"
phenotype

"*okapi*"
(*opi*)
mutants

the *opi* screen

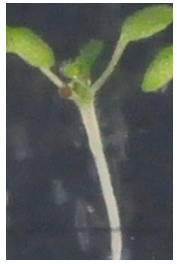


the *opi* screen



mapping by sequencing (coll. K. Schneeberger, MPI Köln)

mapping by sequencing of *opi1*

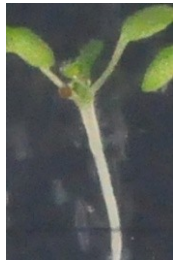
P:  X  → F1: 

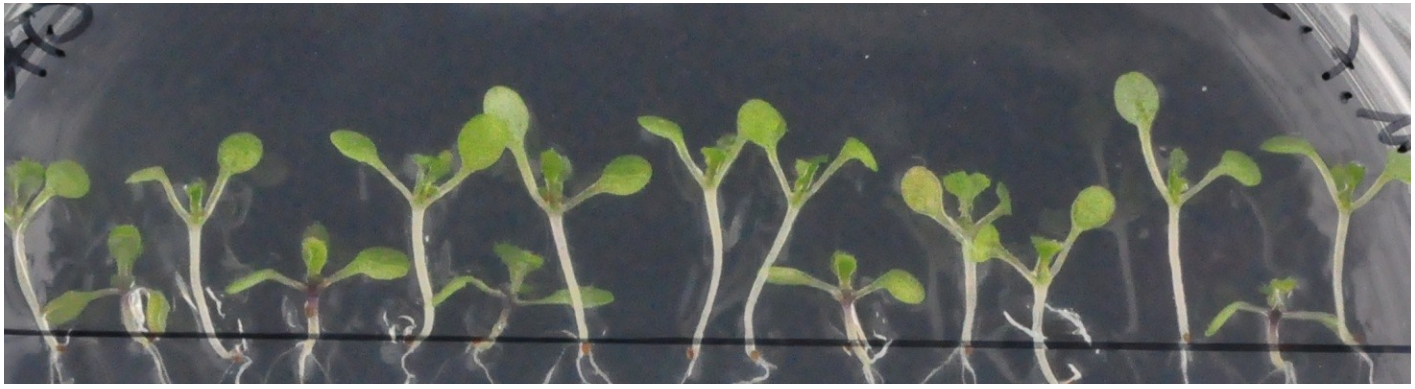
WT *opi1*

mapping by sequencing of *opi1*

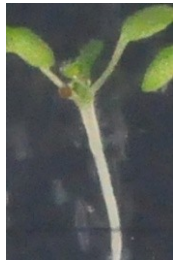
P:  X 

WT *opi1*

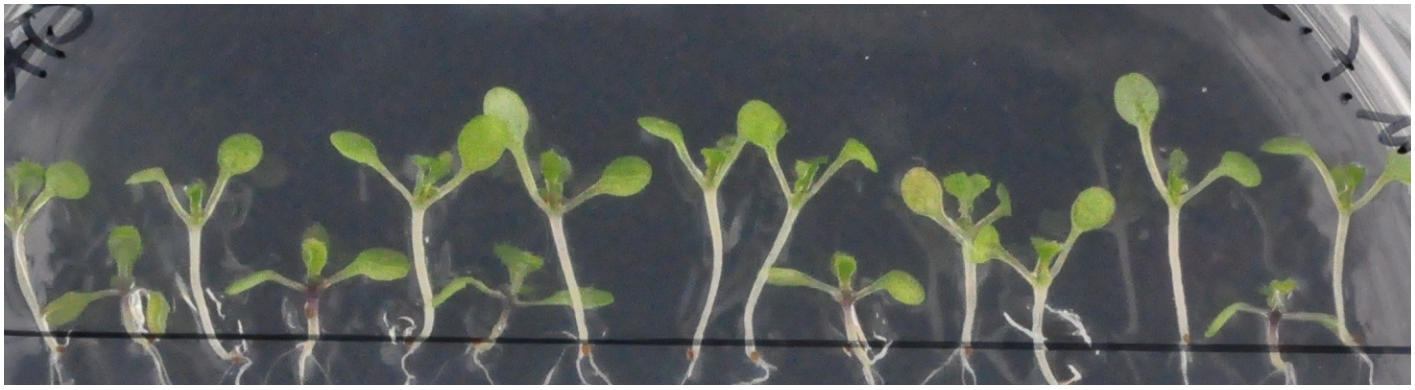
F1: 

F2: 

mapping by sequencing of *opi1*

P:  X  → F1: 

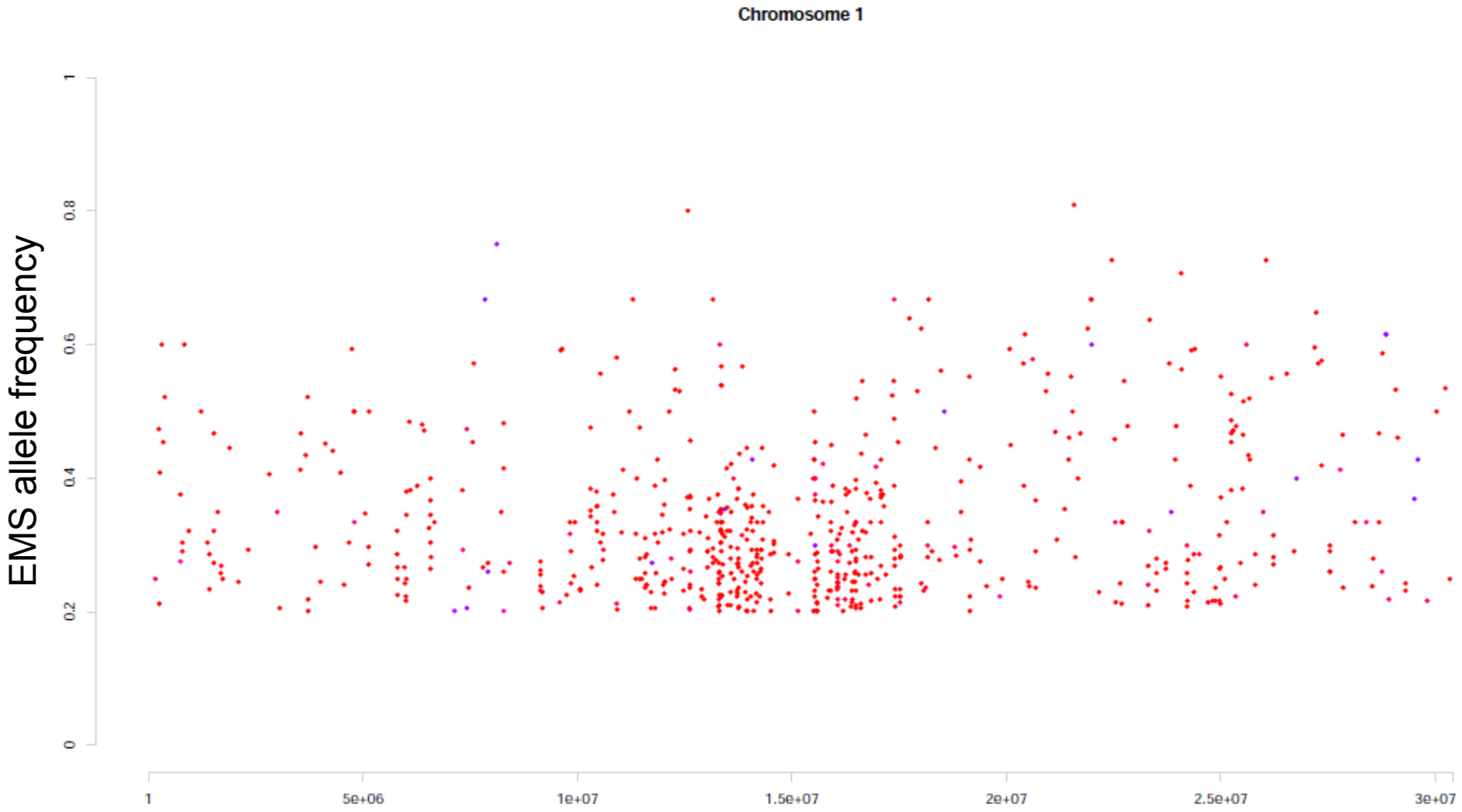
WT *opi1*

F2: 

136 confirmed F2 BC1 individuals
→ pooled DNA for sequencing

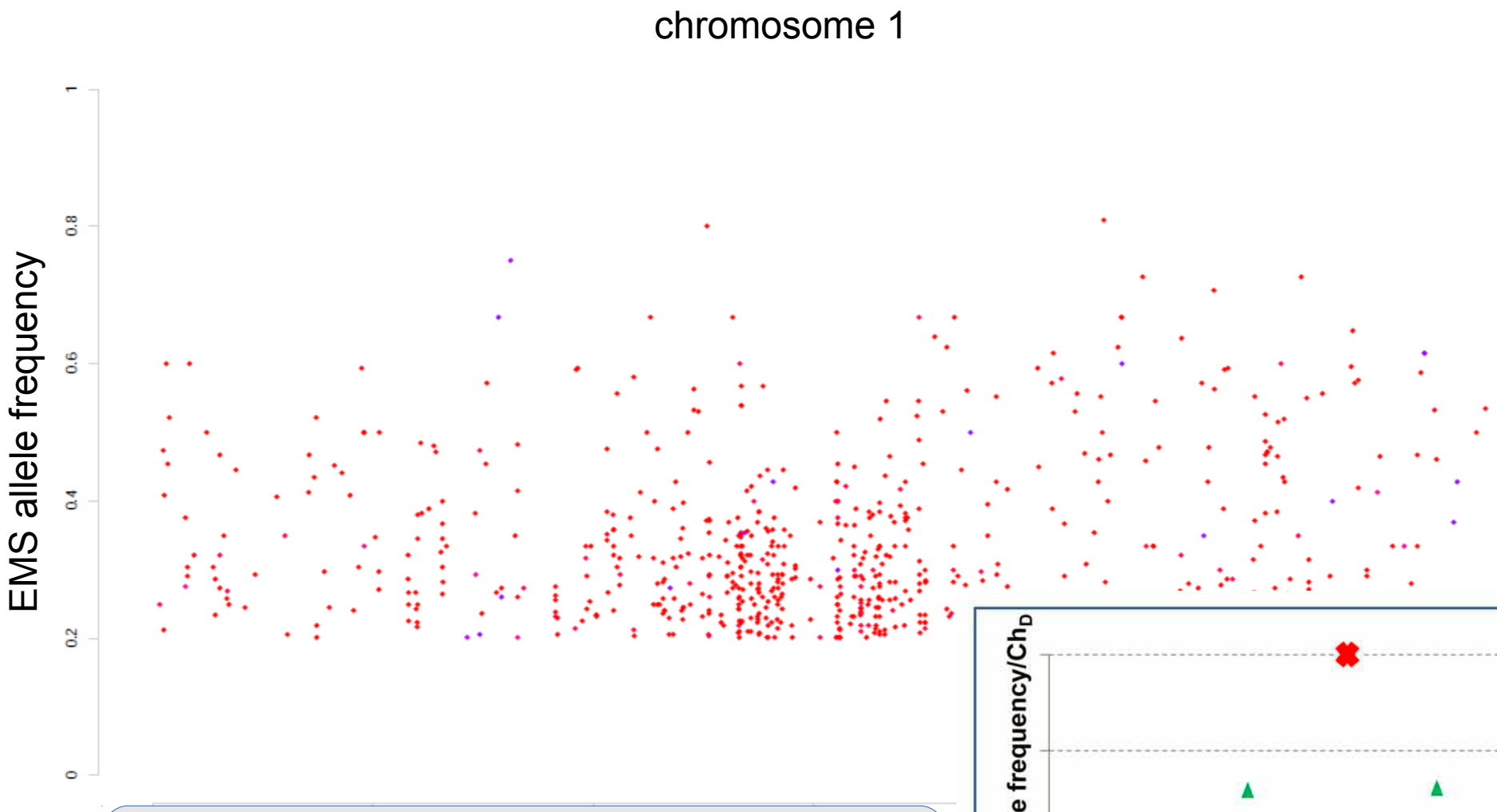
40x coverage (1/5 lane, Illumina HighSeq 2000)

mapping by sequencing of *opi1*

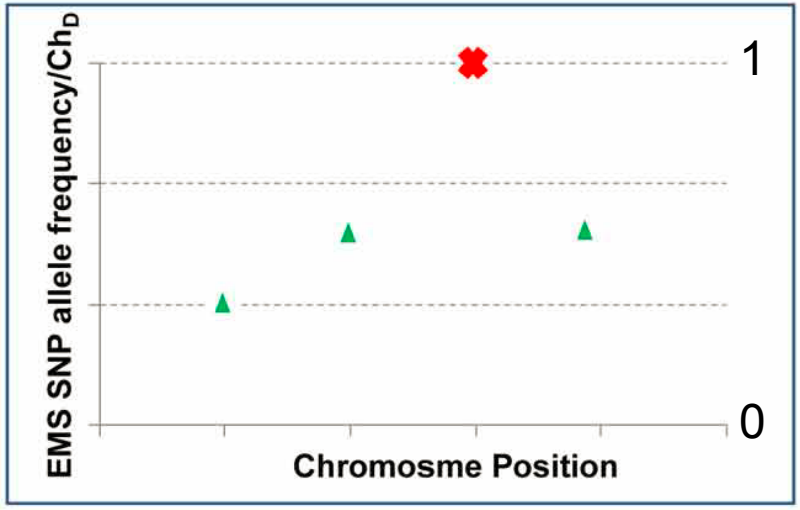


SHOREmap (http://1001genomes.org/downloads/shore.html); Schneeberger et al. (2009, Nature Methods)

mapping by sequencing of *opi1*



no interval of SNP allele frequency = 1 on chr. 1,2,3 or 5



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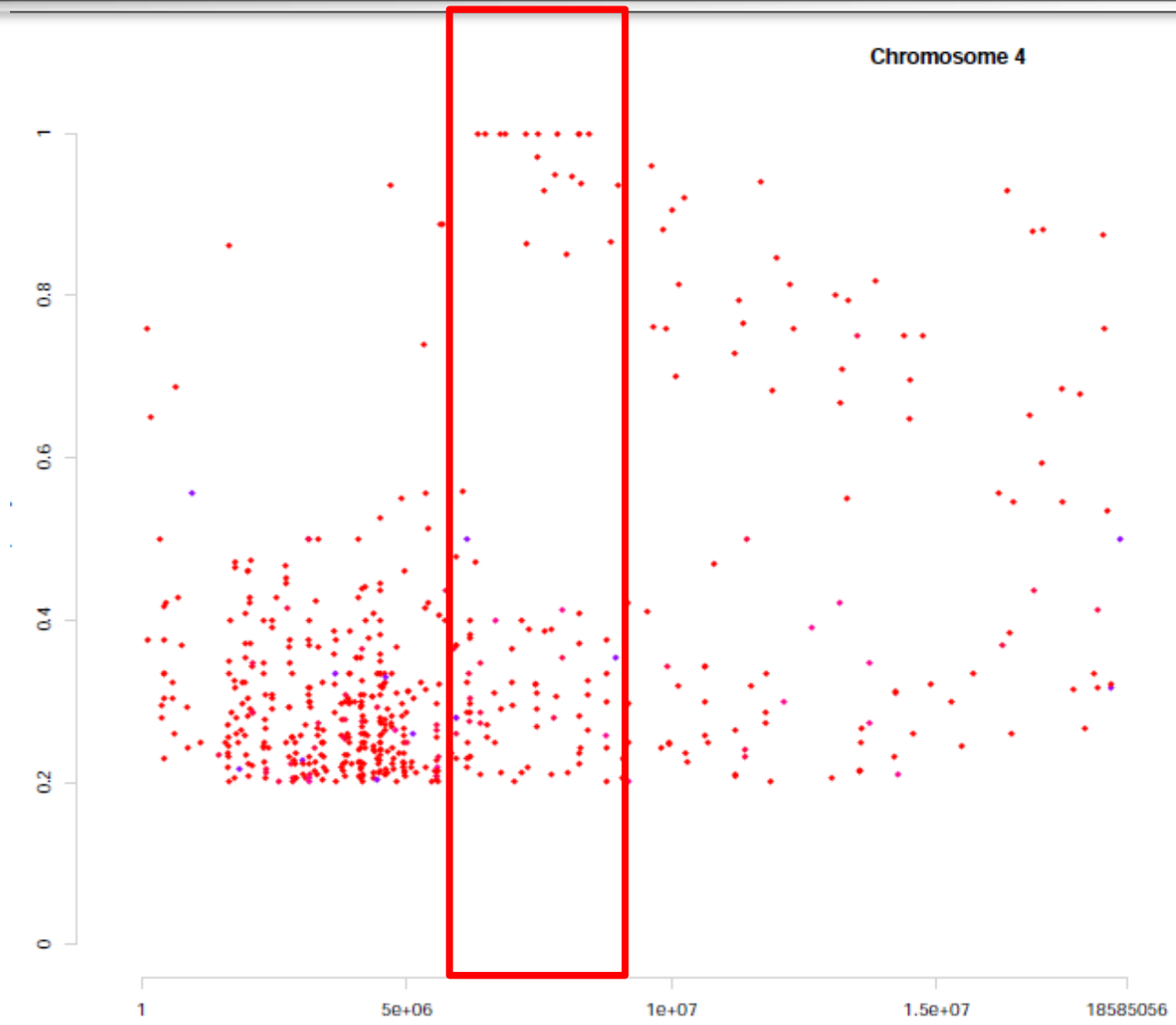
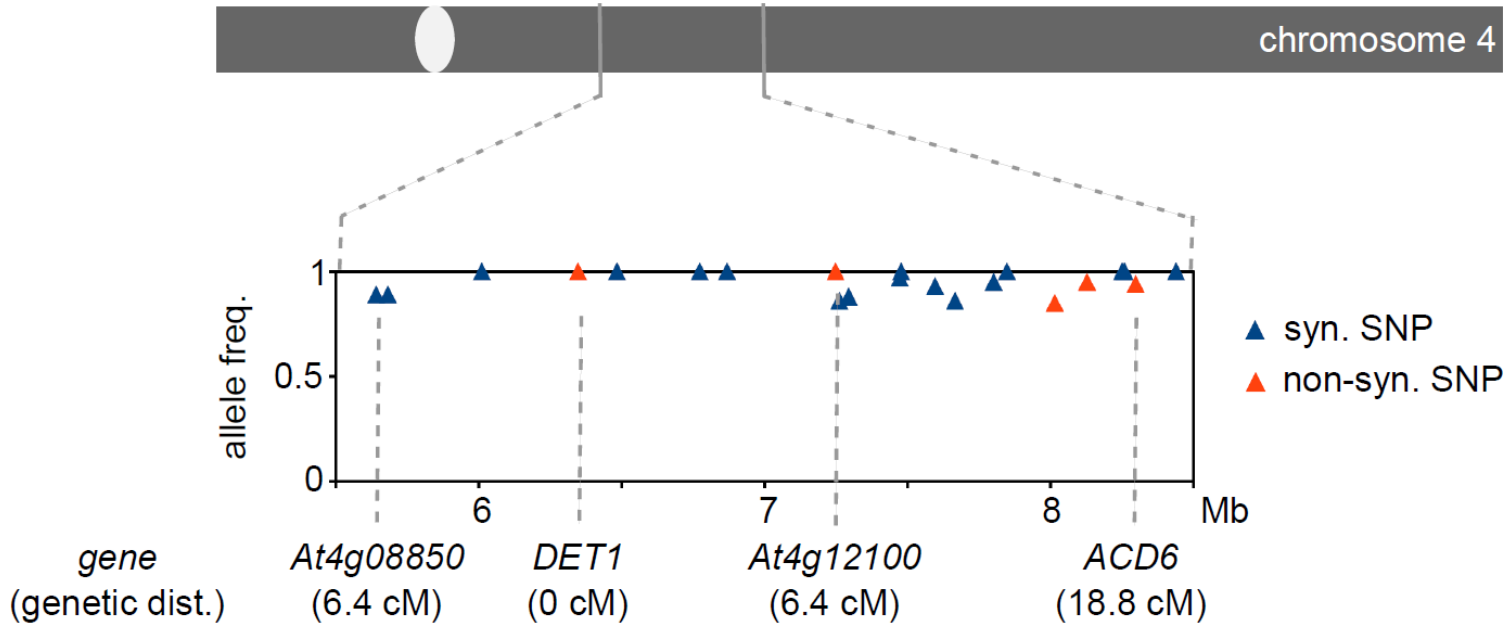
mapping by sequencing of *opi1*



interval of SNP allele frequency = 1
→ candidate interval

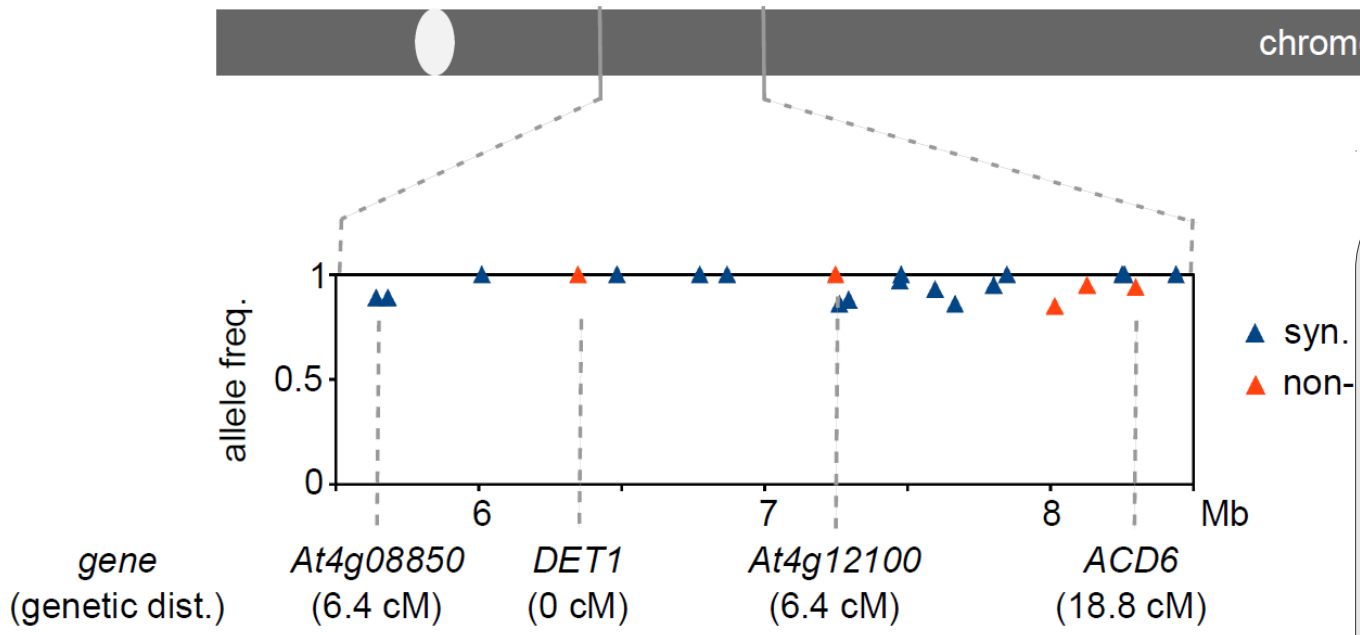
opi1 is a novel *det1* allele



G-58-E

```
Zea            YSFRKFTDDGHYFVAFSRNHQDLIVYRPIWLTYSCN-EECDPHE-LPPKAKK-------- 101
Brachypodium   YSFRKFTDDGHYLVAFSRNHQDLIVYRPIWLTFSCH-EECDSHD-LPDQARR-------- 101
At_Col         HCFRKFTEDGLFLISFSRNHQELIVYRPSWLTYSTTDDSTTTLPPLPRRASK-------- 100
A.lyr          HCFRKFTEDGLFLISFSRNHQELIVYRPSWLTYSTTSDDDDSTP-LPRRASK-------- 99
Medicago       HSFRKFTEDGQYLISFSRNHQELIVYRPRWLSFSCNDEDCDQHD-LPSKARR-------- 102
Glycine        HSFRKFTDDGQYLISFSRNHQELIVYRPRWLSFSCKDEDCDKHD-LPSRAKR-------- 99
Vitis          HSFRKFTDDGQYLISFSRNNQDLIVYRPTWLSFSCKEEDCDSYD-LPLKAKR-------- 99
Ricinus        HSLRKFTDDGQYLISFSRSHQELIVYRPKWLSYSCKGEHCD----LPSRANR-------- 136
S.tub          HSFRKFTDDGLYLVSFSRNHQDLVVYRPTWLTFSCKEEDCDTHD-LPLKARK-------- 99
S.lyc          HSFRKFTDDGLYFVSFSRNHQDLVVYRPTWLTFSCKEEDCDTHD-LPLKARK-------- 99
Physcomitrella QWWRRFTHDGQYLICFSKAQQDLLIYRPLWPTYCSTSDTIND---LPSKSKE-------- 98
Bos            CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILANGNDQRSVNIRGR 119
Homo           CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILSNGNDQRSVNIRGR 130
Heterocephalus CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILSNGNDQRSVNIRGR 160
Gallus         CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILANGNDQRSVNIRGR 119
Xenopus        CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLHGYEGEILANSNDQRSVNIRGR 119
Danio          CFLRKFSPDGRCFIAFSSDQTSLEIYEYQGCQAAEDLLQGQEGETLANGNDQRSFNIRGH 131
                  *:*:  :*  ::.**  : .* :*.      .          *.    .
```

opi1 is a novel *det1* allele



G-58-E

```
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Brachypodium  YSFRKFTDGHYLVAFSRNHQDLIVYRPIWLTFSCH-EECDSHD-LPDQARR-------- 101
At_Col        HCFRKFTEGLFLISFSRNHQELIVYRPSWLTYSTTDDSTTTLPPLPRRASK-------- 100
A.lyr         HCFRKFTEGLFLISFSRNHQELIVYRPSWLTYSTTSDDDDSTP-LPRRASK-------- 99
Medicago      HSFRKFTEGGYLISFSRNHQELIVYRPRWLSFSCNDEDCDQHD-LPSKARR-------- 102
Glycine       HSFRKFTDGGYLISFSRNHQELIVYRPRWLSFSCKDEDCDKHD-LPSRAKR-------- 99
Vitis         HSFRKFTDGGYLISFSRNNQDLIVYRPTWLSFSCKEEDCDSYD-LPLKAKR-------- 99
Ricinus       HSLRKFTDGGYLISFSRSHQELIVYRPKWLSYSCKGEHCD----LPSRANR-------- 136
S.tub         HSFRKFTDGLYLVSFSRNHQDLVVYRPTWLTFSCKEEDCDTHD-LPLKARK-------- 99
S.lyc         HSFRKFTDGLYFVSFSRNHQDLVVYRPTWLTFSCKEEDCDTHD-LPLKARK-------- 99
Physcomitrella QWWRRFTHGGYLICFSKAQQDLLIYRPLWPTYCSTSDTIND---LPSKSKE-------- 98
Bos           CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILANGNDQRSVNIRGR 119
Homo          CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILSNGNDQRSVNIRGR 130
Heterocephalus CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILSNGNDQRSVNIRGR 160
Gallus        CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLQGYEGEILANGNDQRSVNIRGR 119
Xenopus       CFLRKFSPDGRYFIAFSSDQTSLEIYEYQGCQAAEDLLHGYEGEILANSNDQRSVNIRGR 119
Danio         CFLRKFSPDGRCFIAFSSDQTSLEIYEYQGCQAAEDLLQGQEGETLANGNDQRSFNIRGH 131
                 *:*:  :*  ::.**  : .* :*.      .          *.    .
```
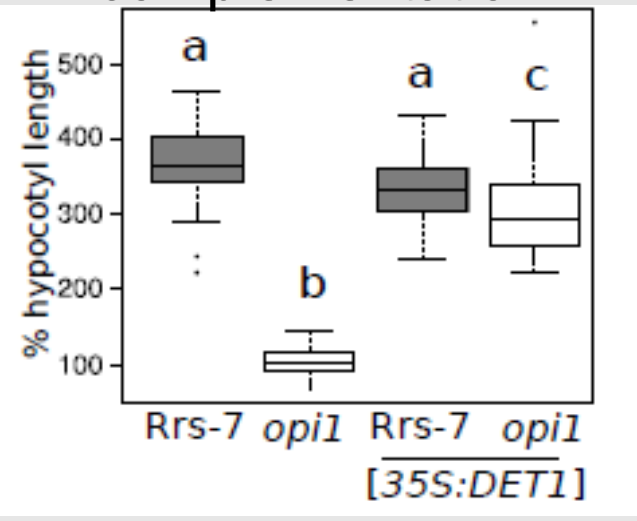
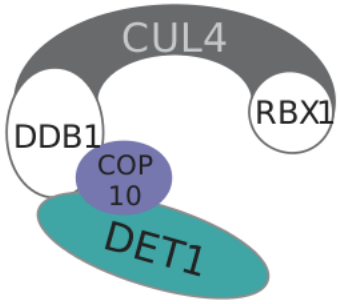

transgenic complementation



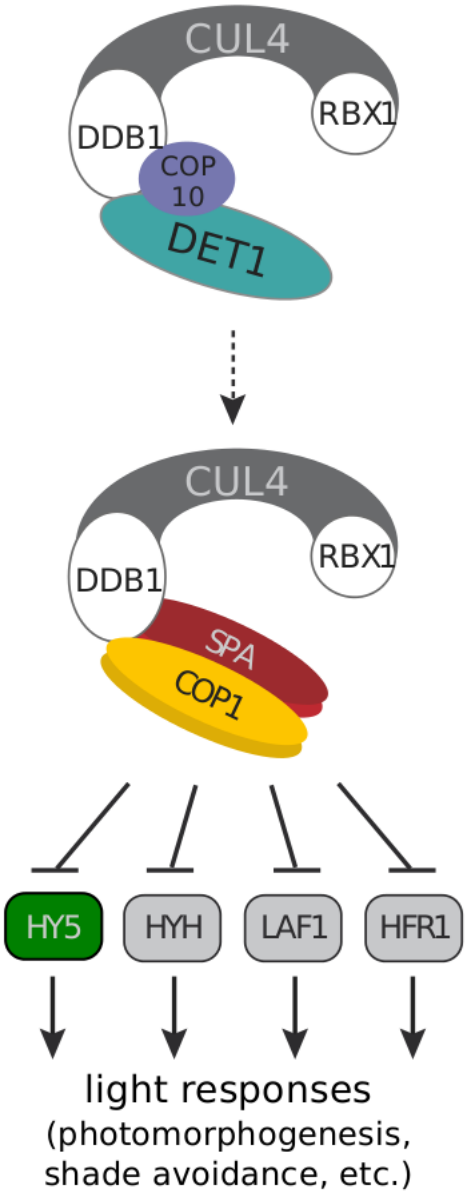
WT *DET1* expression in *opi1* → increase in TIHE response

DET1 is a known regulator in light signaling



DET1 integrates in an E3-ligase-type multi-protein complex (CDD complex)

DET1 is a known regulator in light signaling

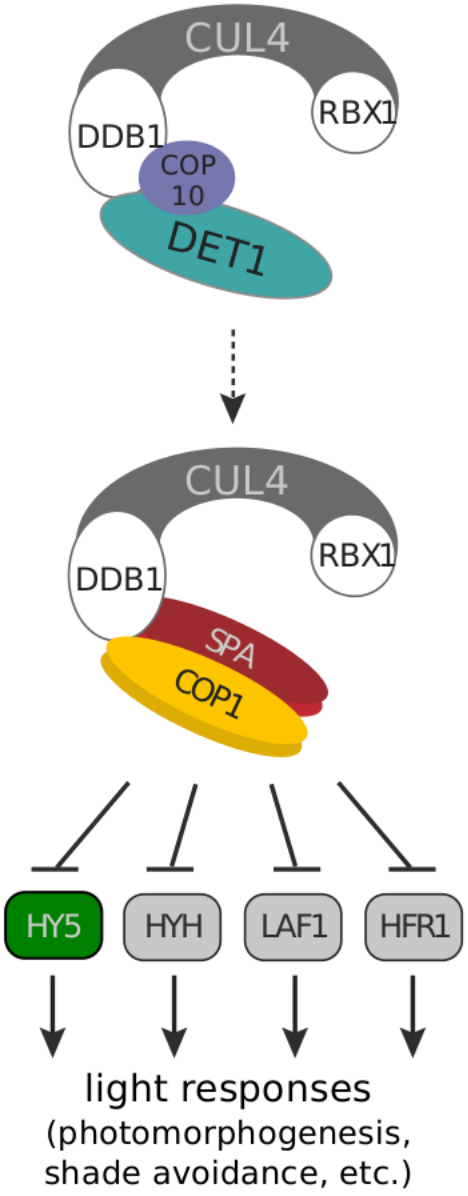


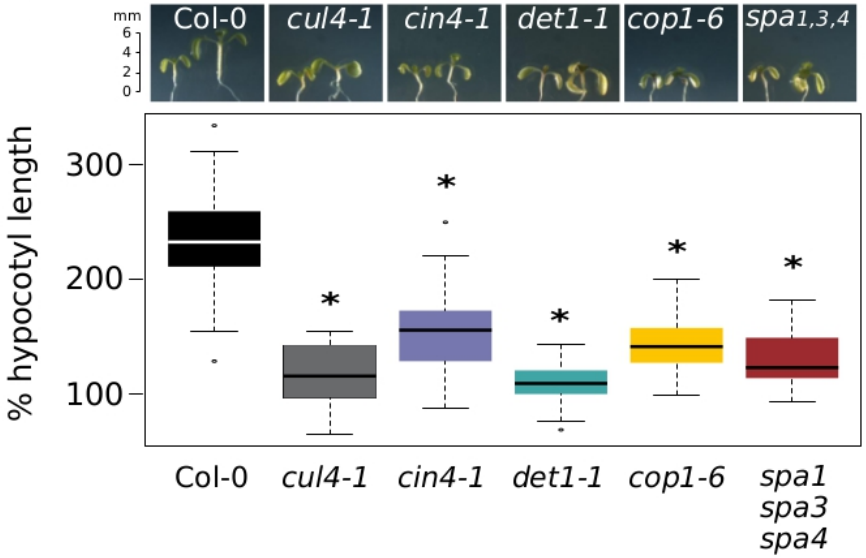
DET1 integrates in an E3-ligase-type multi-protein complex (CDD complex)

DET1 activates the COP1/SPA complex to target transcription factors for proteasomal degradation

is the CDD-COP1/SPA cascade involved in ambient temperature signaling

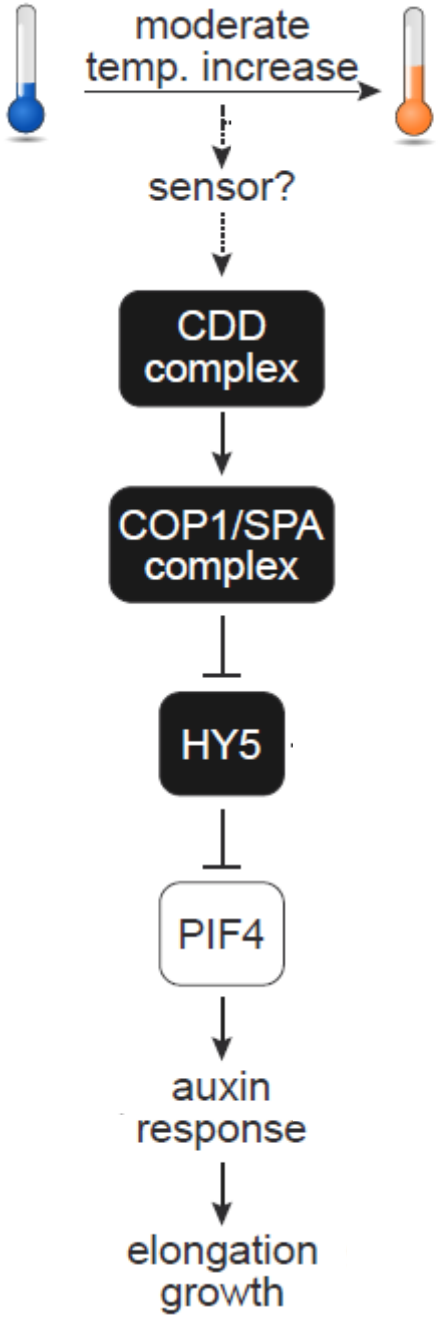
CDD/COP1-SPA/HY5 mediate temp. response



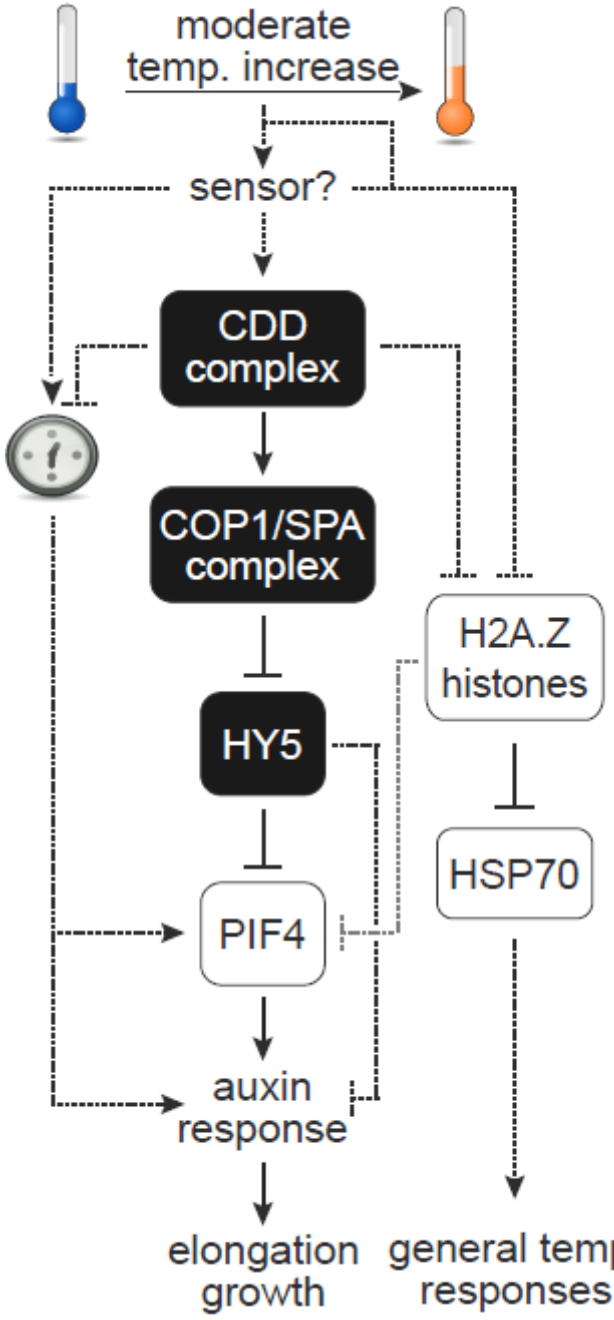


mutants of CDD-complex components
→ reduced TIHE

CDD/COP1-SPA/HY5 connects to PIF4



CDD/COP1-SPA/HY5 connects to PIF4



✔ forward genetic screen → isolation of 6 *opi* mutants

✔ *opi1* identification of causal mutation → mapping by sequencing of BC1 F2 DNA pool

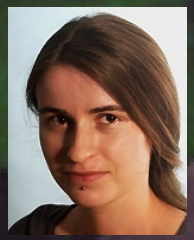
✔ SNP in DET1 → loss of TIHE

✔ ancient light signaling pathway (CDD/COP1-SPA/HY5) regulates PIF4 expression in ambient temperature signaling

The Quint Lab temperature team

Marcel Quint (PI)

Carla Ibanez



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Ute Hoecker (Universität Köln)
Hanjo Hellmann (WSU, USA)
Christian Fankhauser (Lausanne)

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IPB

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