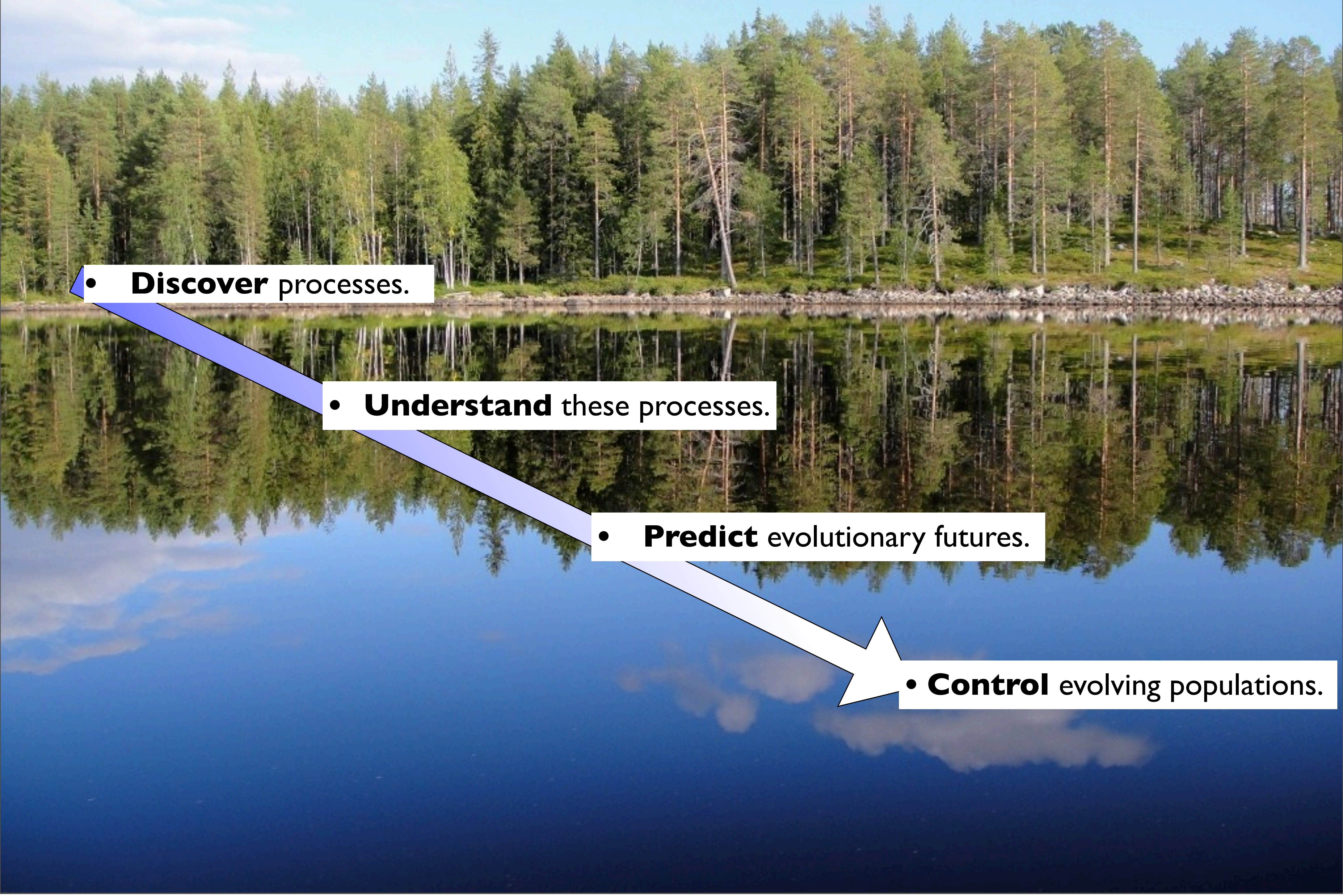
The emergence of resistant clones in heterogeneous populations

Ville Mustonen

Natural progression in the study of evolution



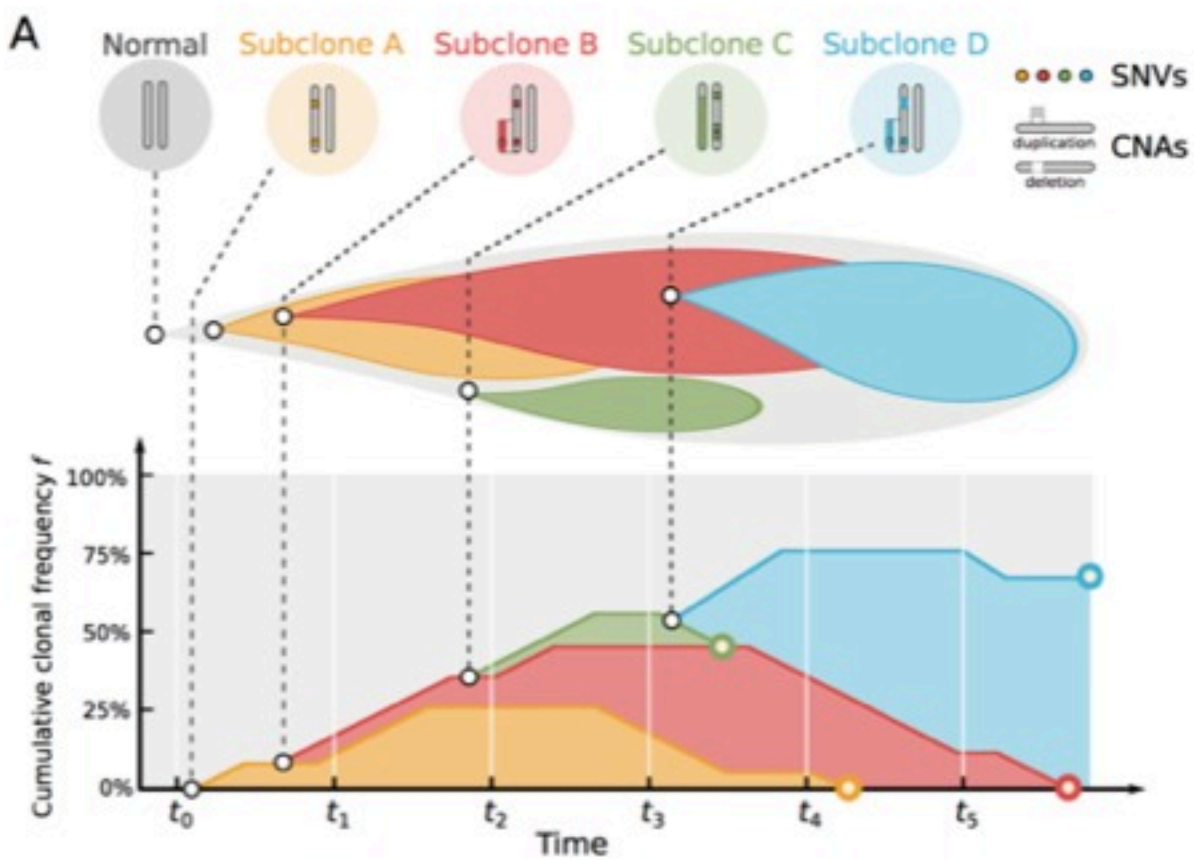
- **Discover** processes.
- **Understand** these processes.
- **Predict** evolutionary futures.
- **Control** evolving populations.

Evolution of drug resistance

- A key problem affecting global health.
- Cancer and infectious disease exhibit the emergence of resistance to drugs.
- Large scale -omics data provide an opportunity to study in detail how resistance evolves.
- New population genetic / evolutionary theory based computational methods and ideas are needed:

- to analyse these data.
- design experiments that ultimately lead to novel approaches in combating resistance.

Tumours are not genetically homogeneous

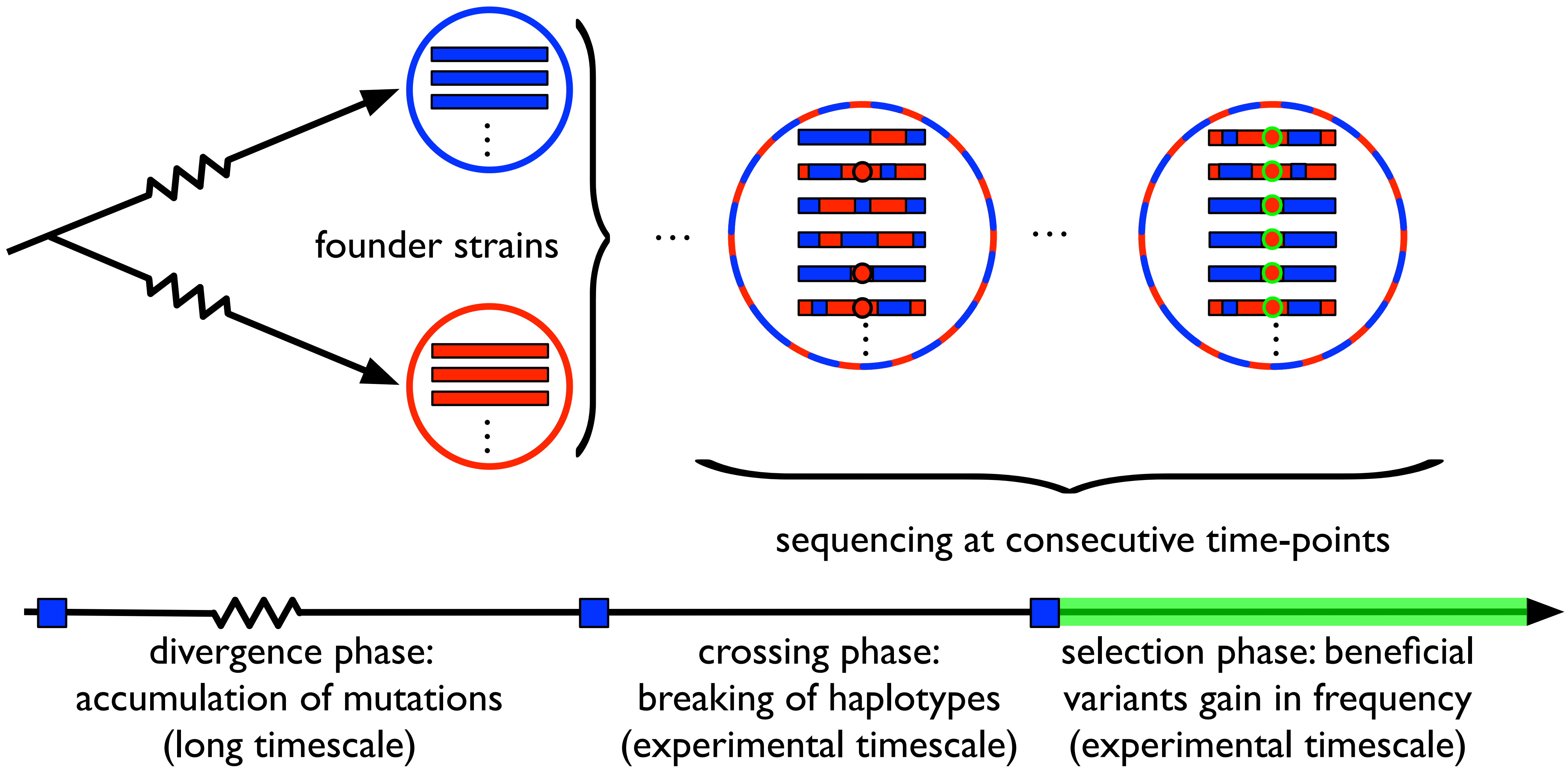


- naive sample extraction strategies will lead to an underestimation of real tumour heterogeneity.
- sub-clones can be resistant to drugs: adaptation via *de novo mutations vs. standing variation.*
- Probability of treatment success is higher in genetically homogenous and/or early stage cancers.

We would like to understand how heterogeneous populations escape from the application of drugs.

Part I: Heterogeneous yeast populations adapting to cancer drugs

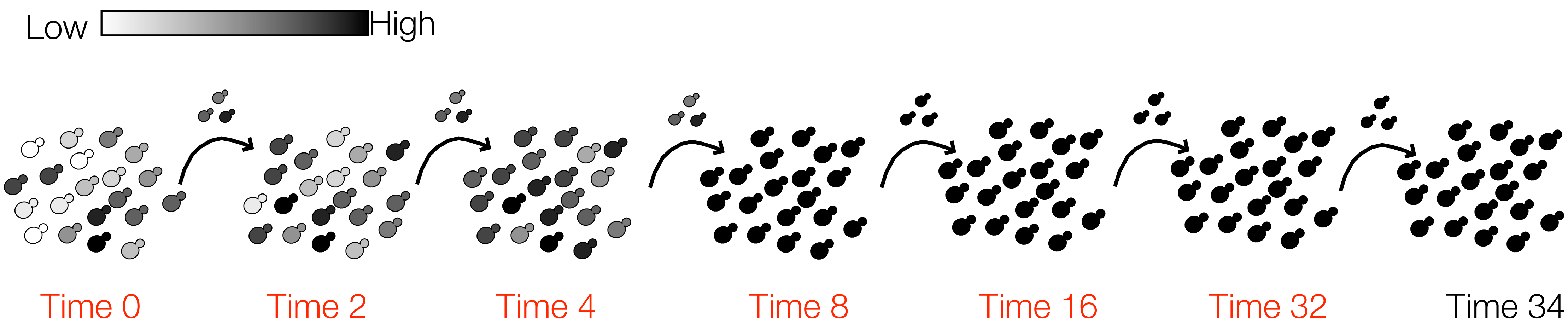
- A collaboration with Gianni Liti Lab, Institute of Research on Cancer and Ageing of Nice (IRCAN).



[For details of the cross see Parts et al. Genome Res. 2011]

[See talk by C. Illingworth]

Selection experiment: sequencing the pool under selection



PHL 2 ug/mL

RA 0.025 ug/mL

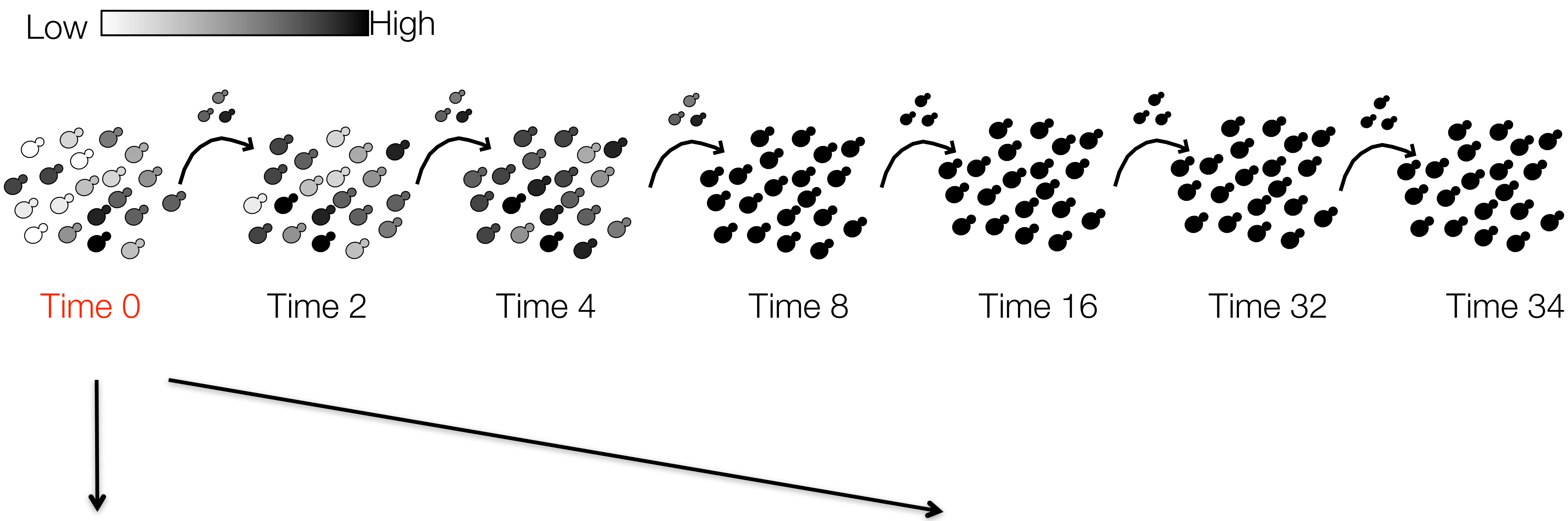
HU 10 mg/mL

PHL 0.5 ug/mL / RA 0.01 ug/mL

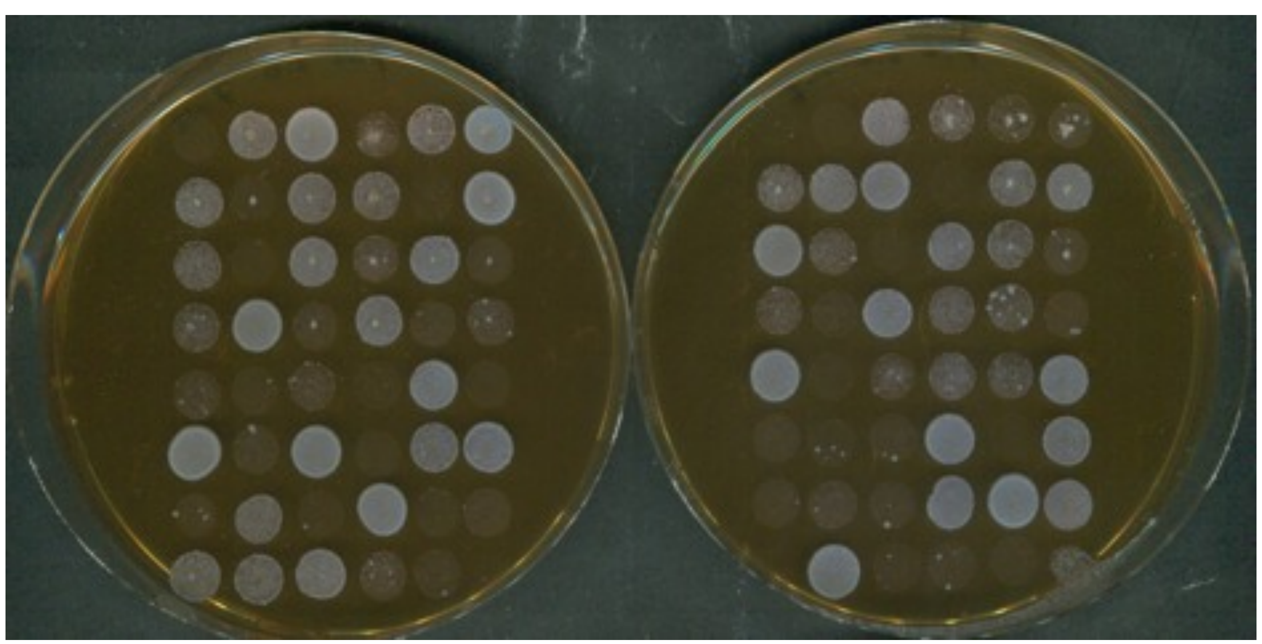
PHL 0.5 ug/mL /HU 1 mg/mL

Control no anticancer drugs

High phenotypic diversity of the pool at T0

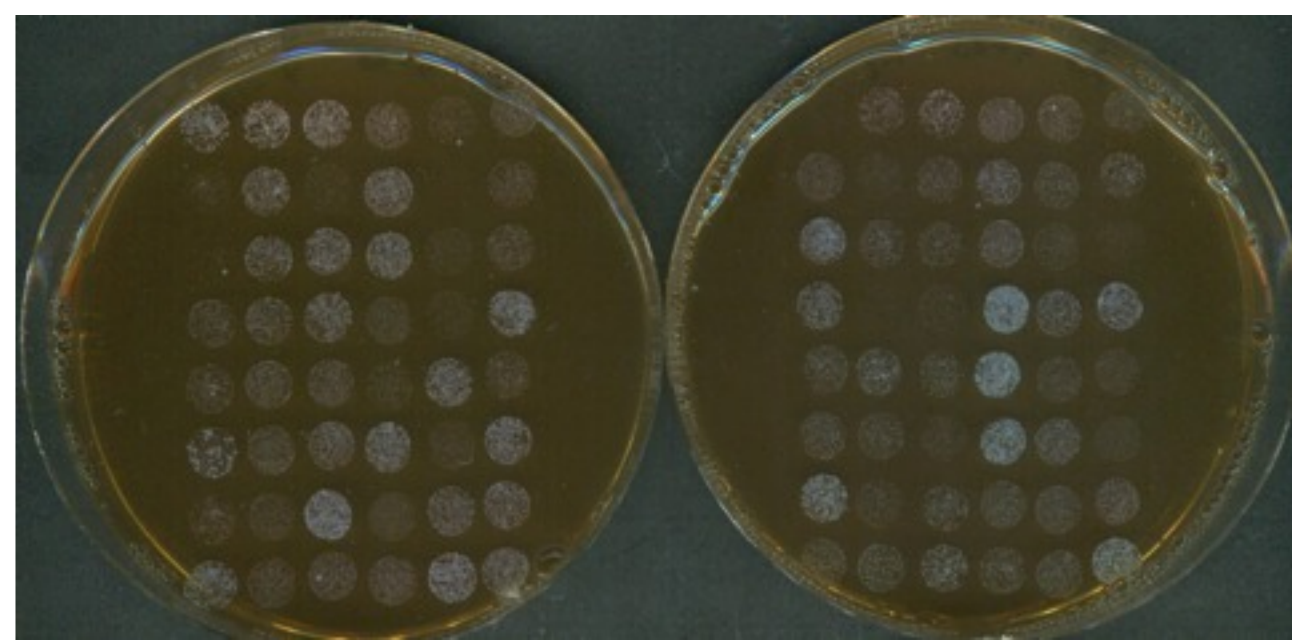


PHL 2 ug/mL



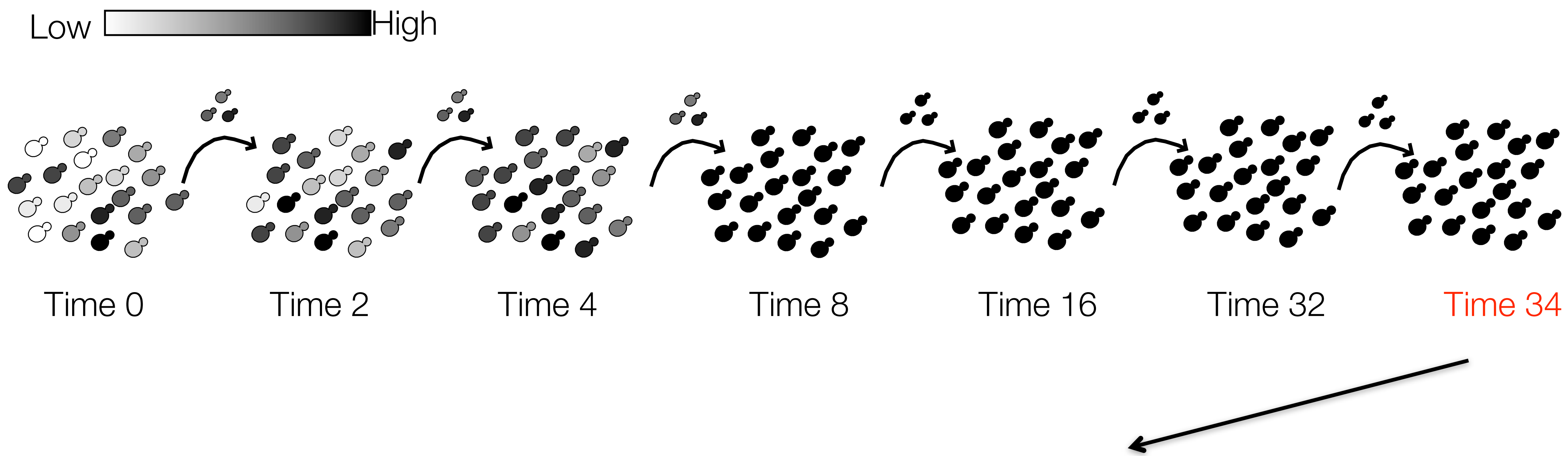
R1 R2

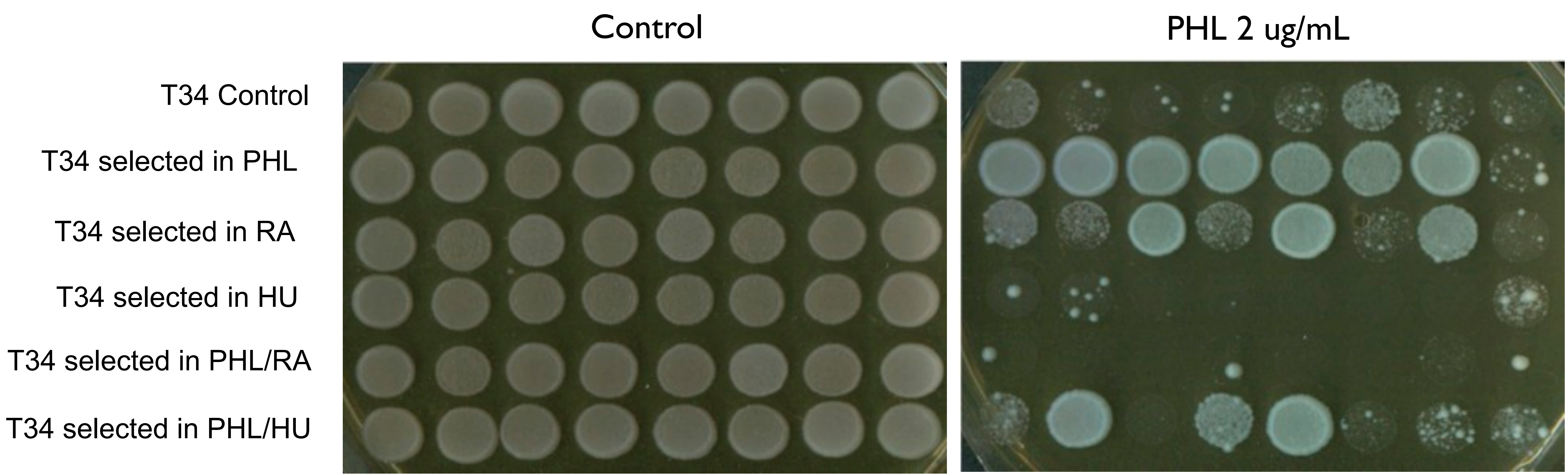
RA 0.025 ug/mL



R1 R2

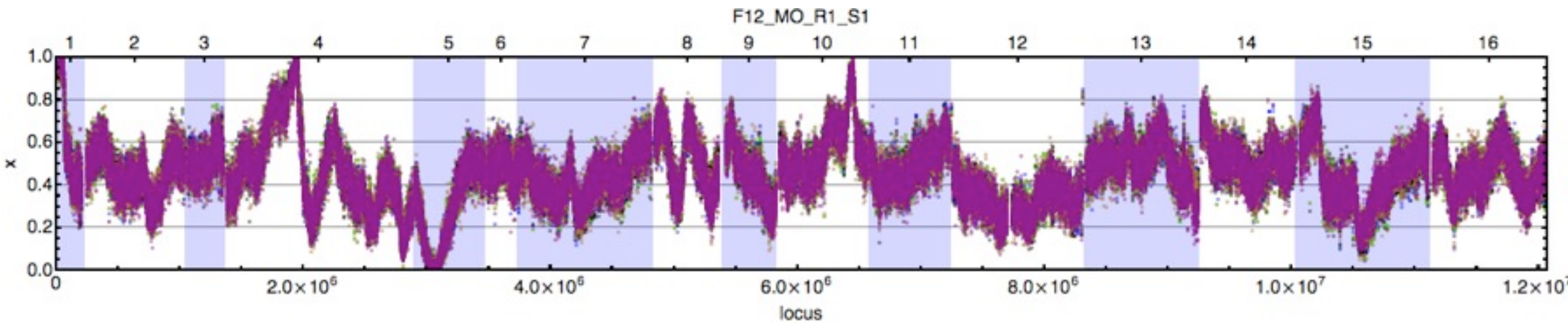
Reduction in phenotypic diversity of the pool at T34



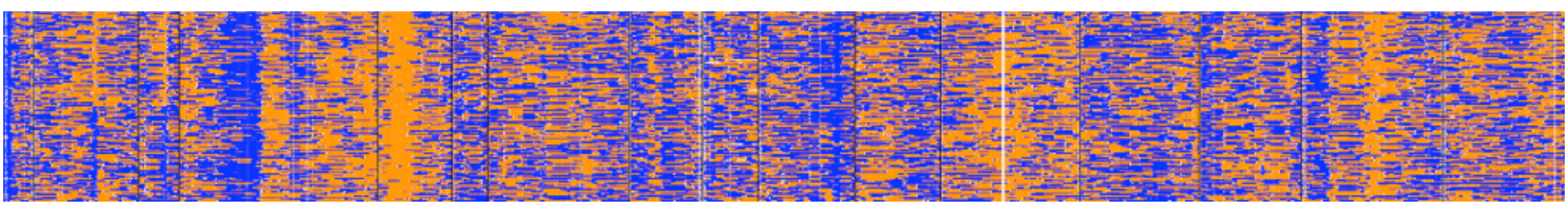


Control condition

Time T0 Time T2 Time T4 Time T8 Time T16 Time T32



Full haplotypes from the initial pools



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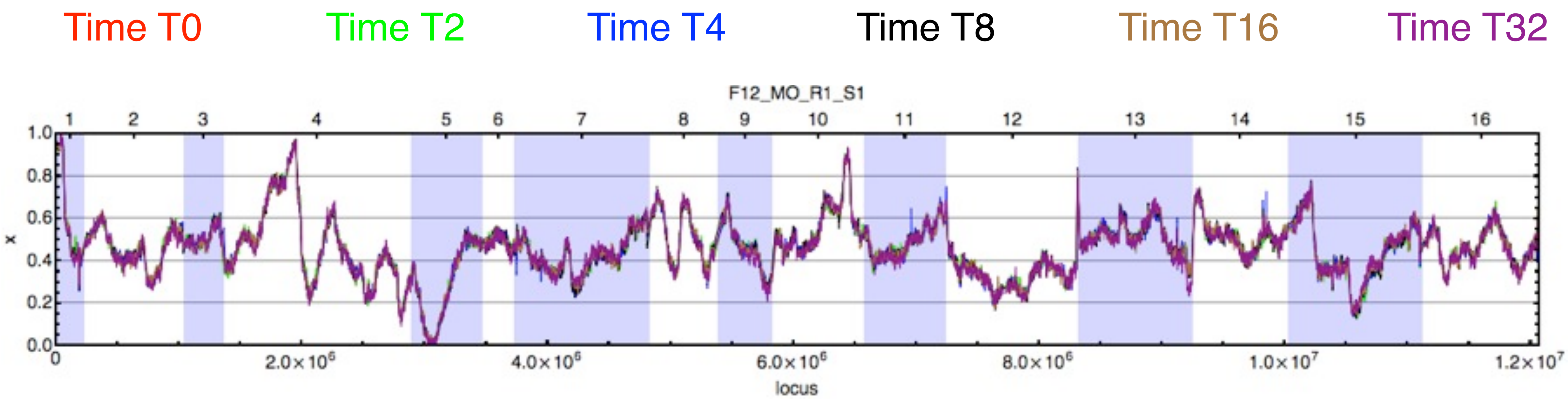

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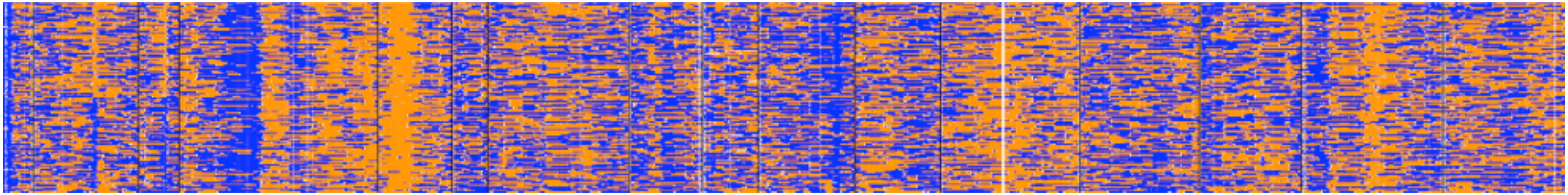
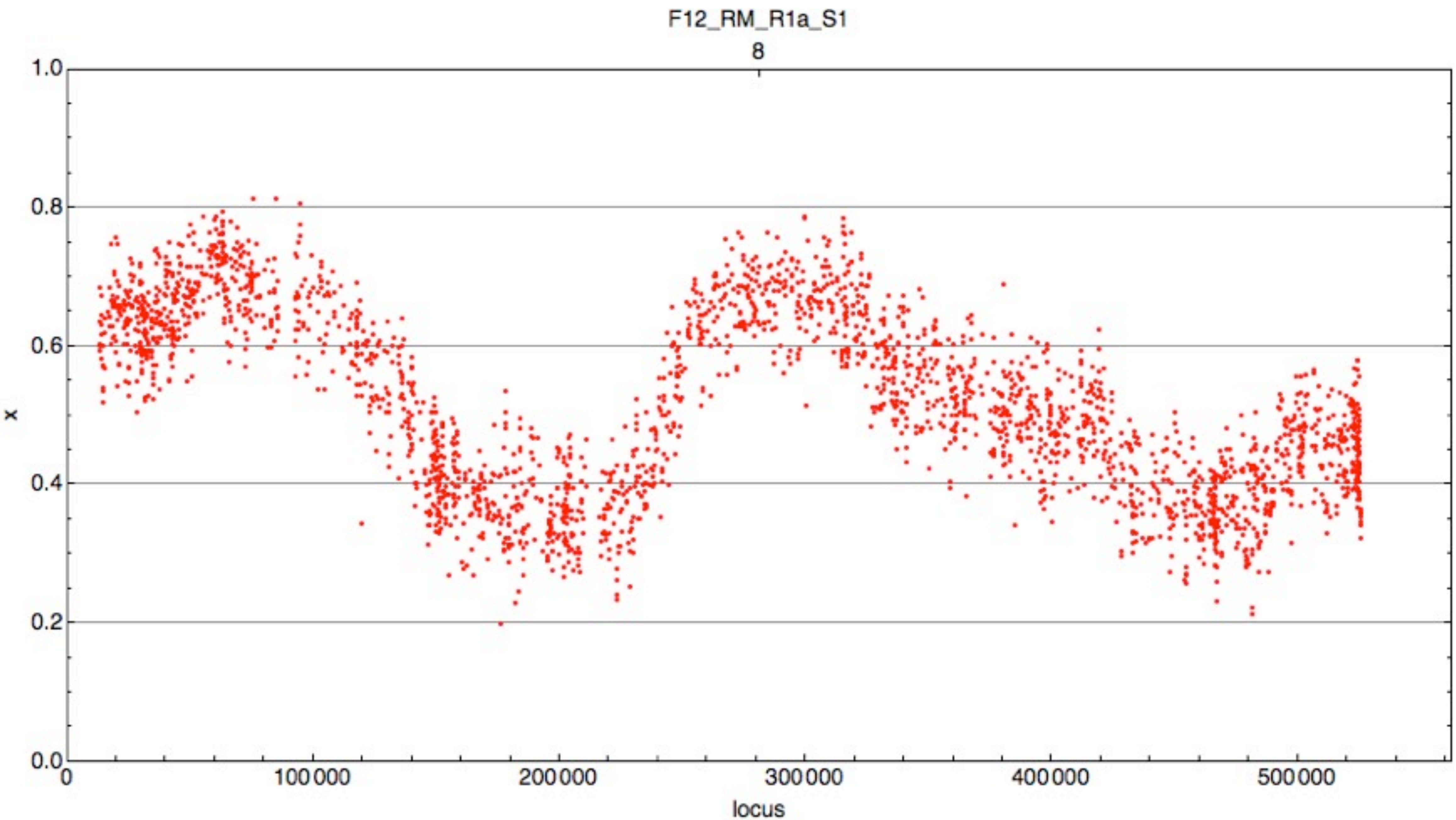


Yeast adapting to cancer drugs T=0,2,4



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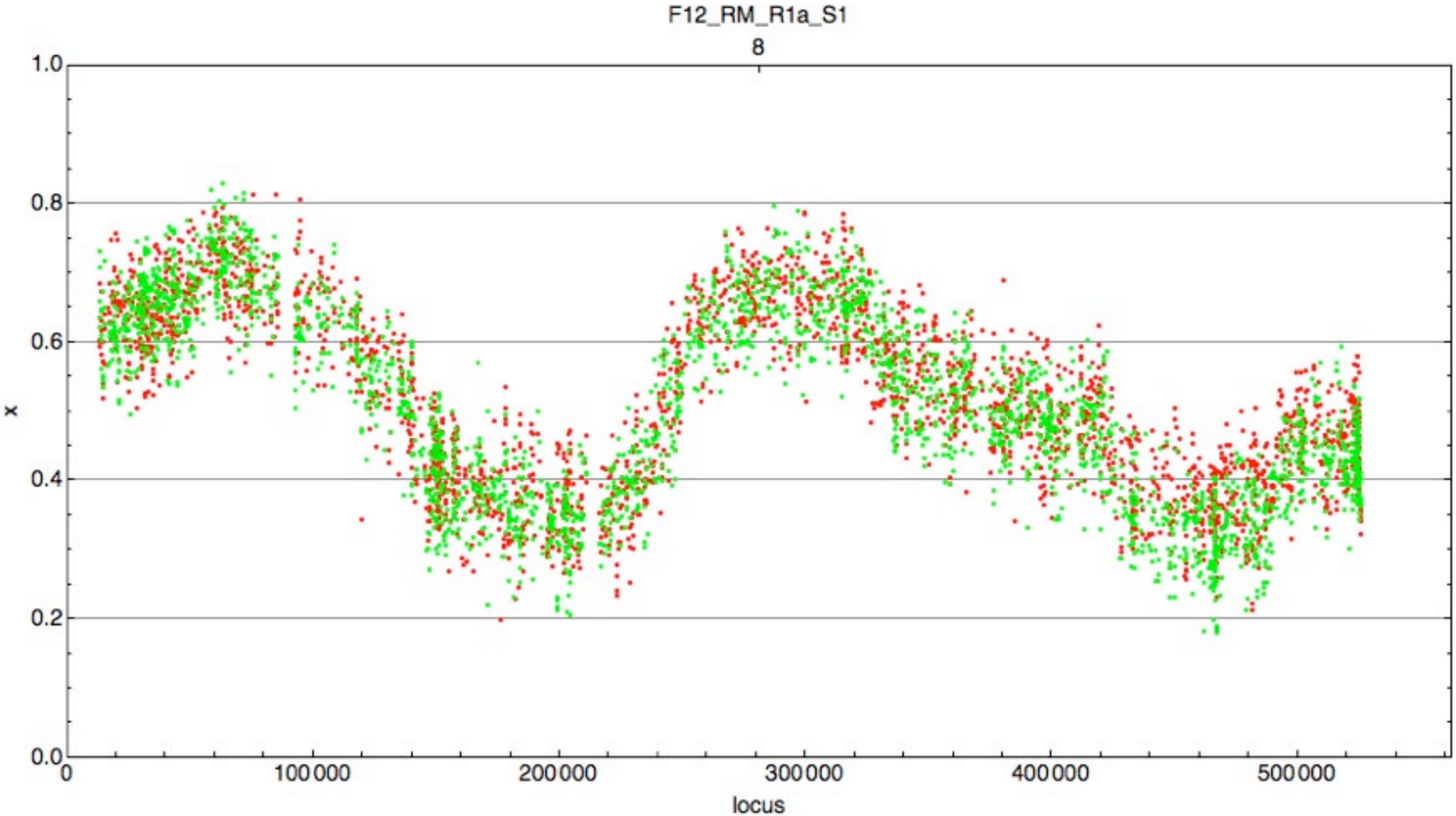

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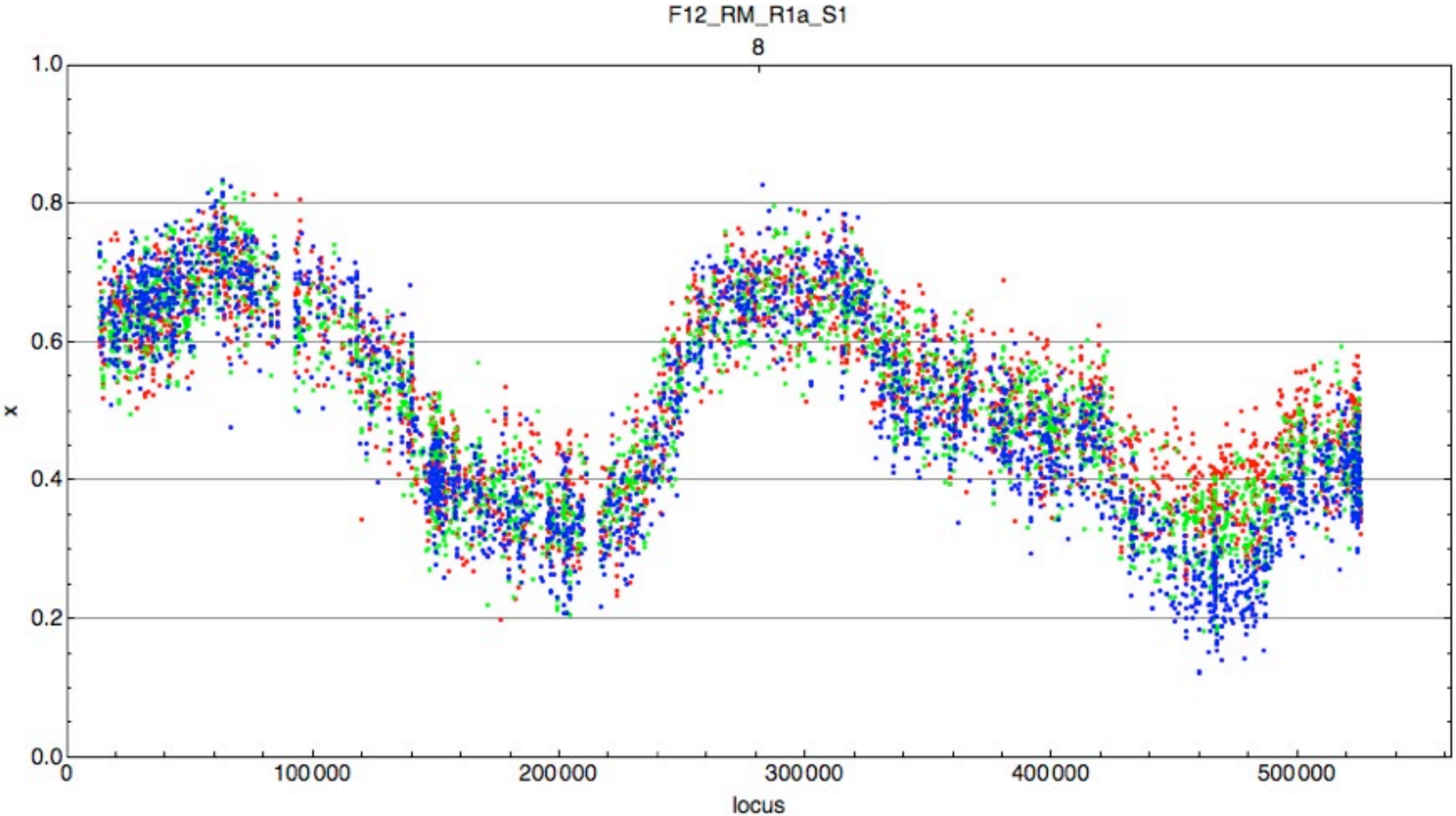

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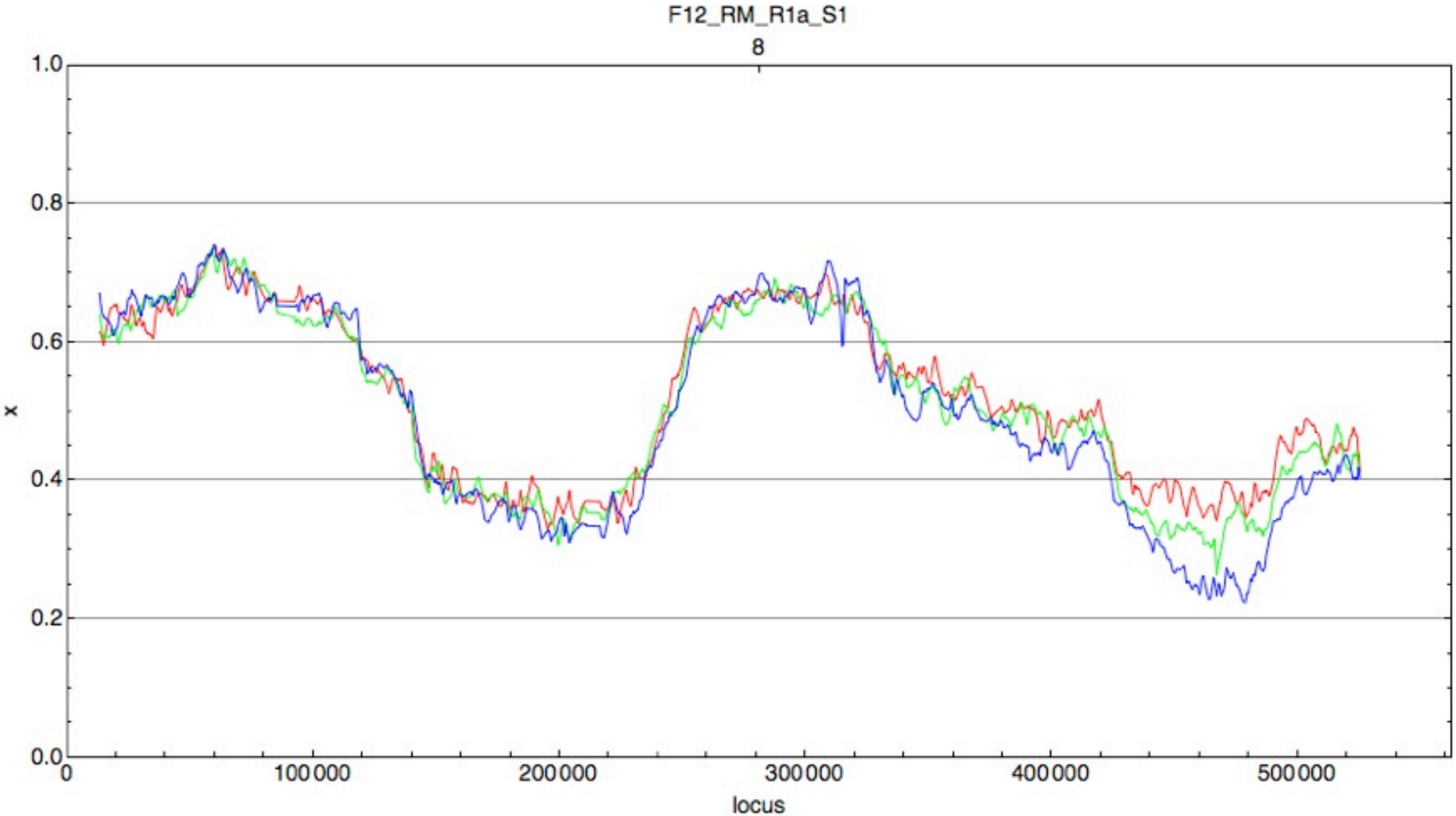

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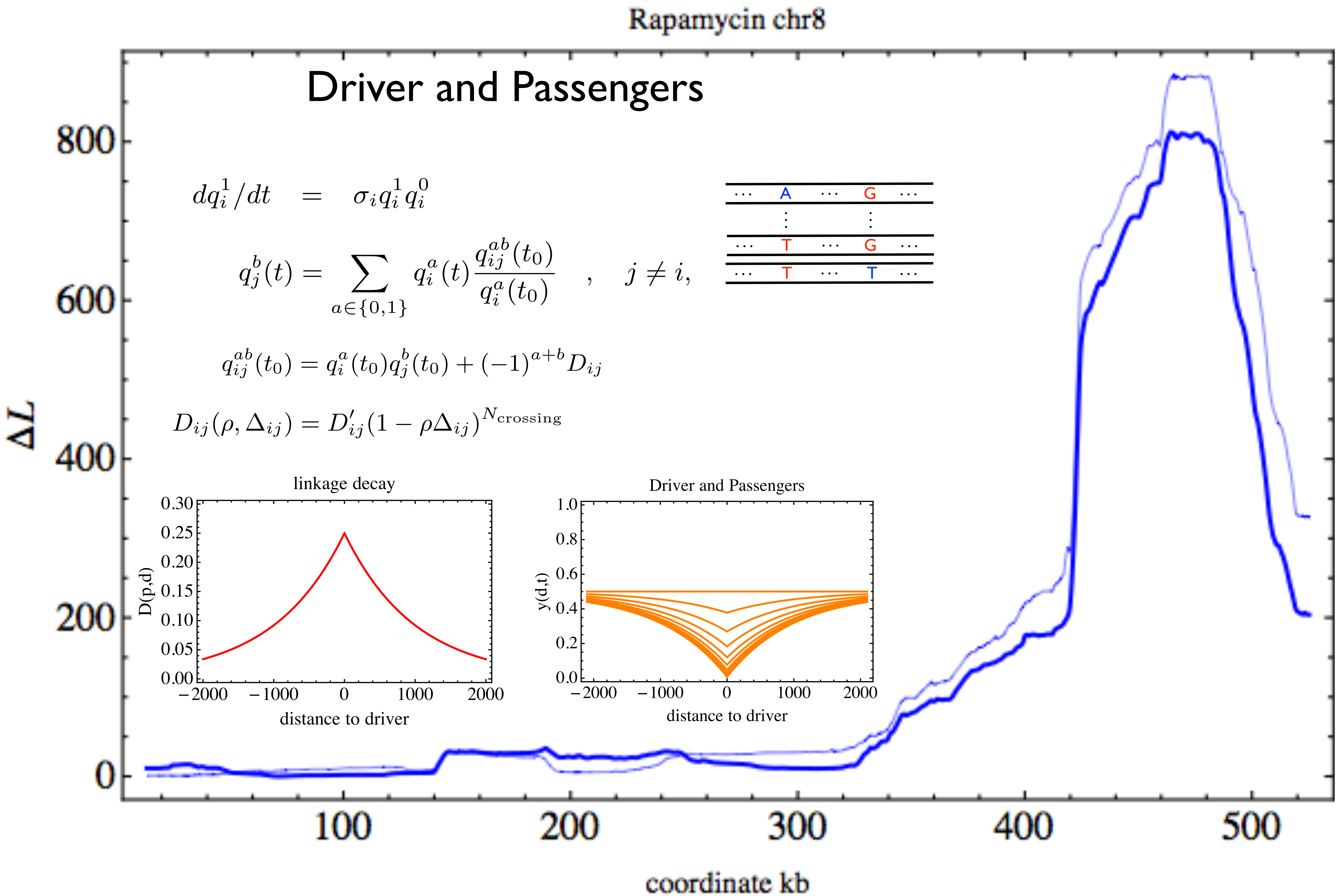

$$dq_i^1/dt = \sigma_i q_i^1 q_i^0$$

$$q_j^b(t) = \sum_{a\in\{0,1\}} q_i^a(t)\frac{q_{ij}^{ab}(t_0)}{q_i^a(t_0)} \quad , \quad j \neq i,$$

$$q_{ij}^{ab}(t_0) = q_i^a(t_0)q_j^b(t_0) + (-1)^{a+b}D_{ij}$$

$$D_{ij}(\rho,\Delta_{ij}) = D'_{ij}(1-\rho\Delta_{ij})^{N_{\text{crossing}}}$$

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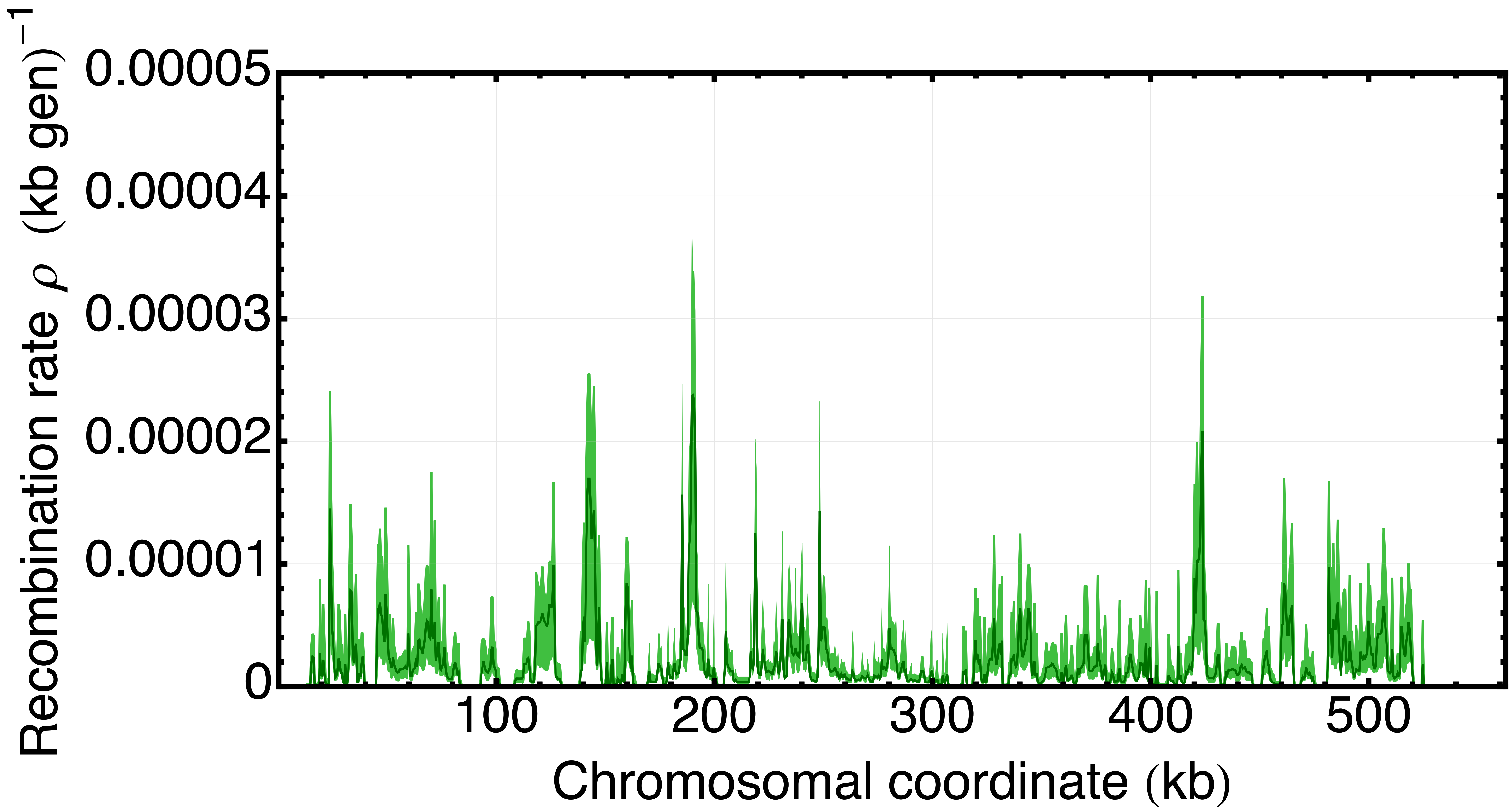

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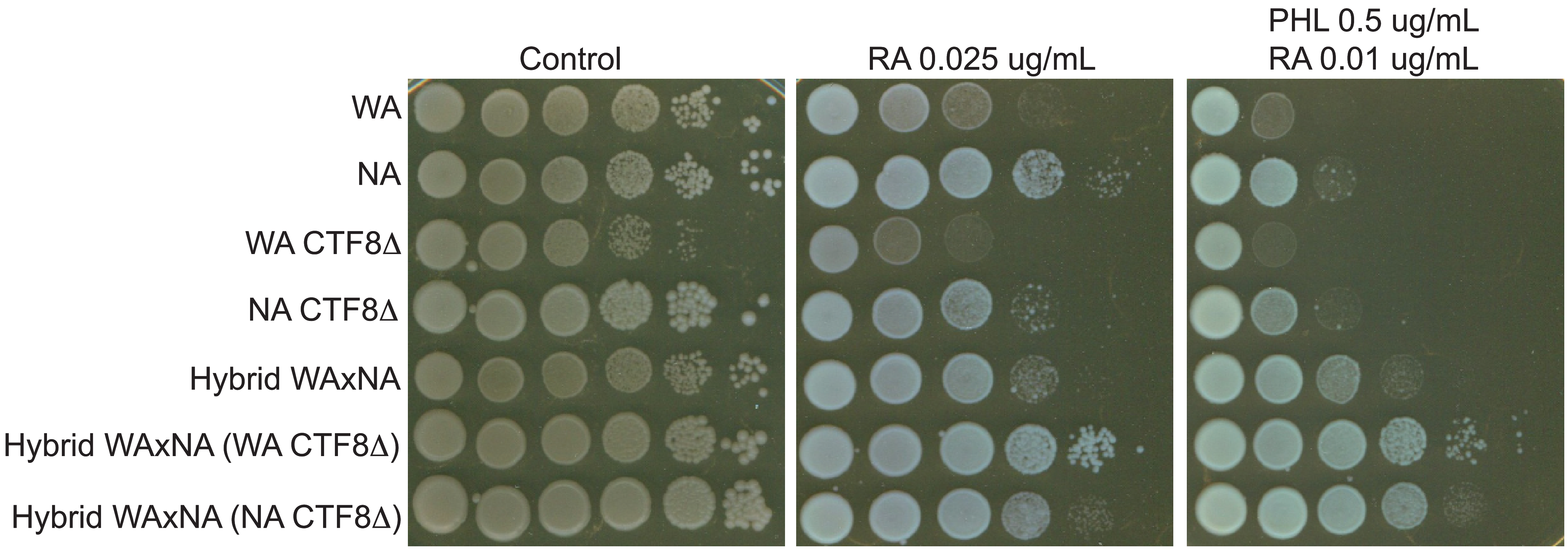

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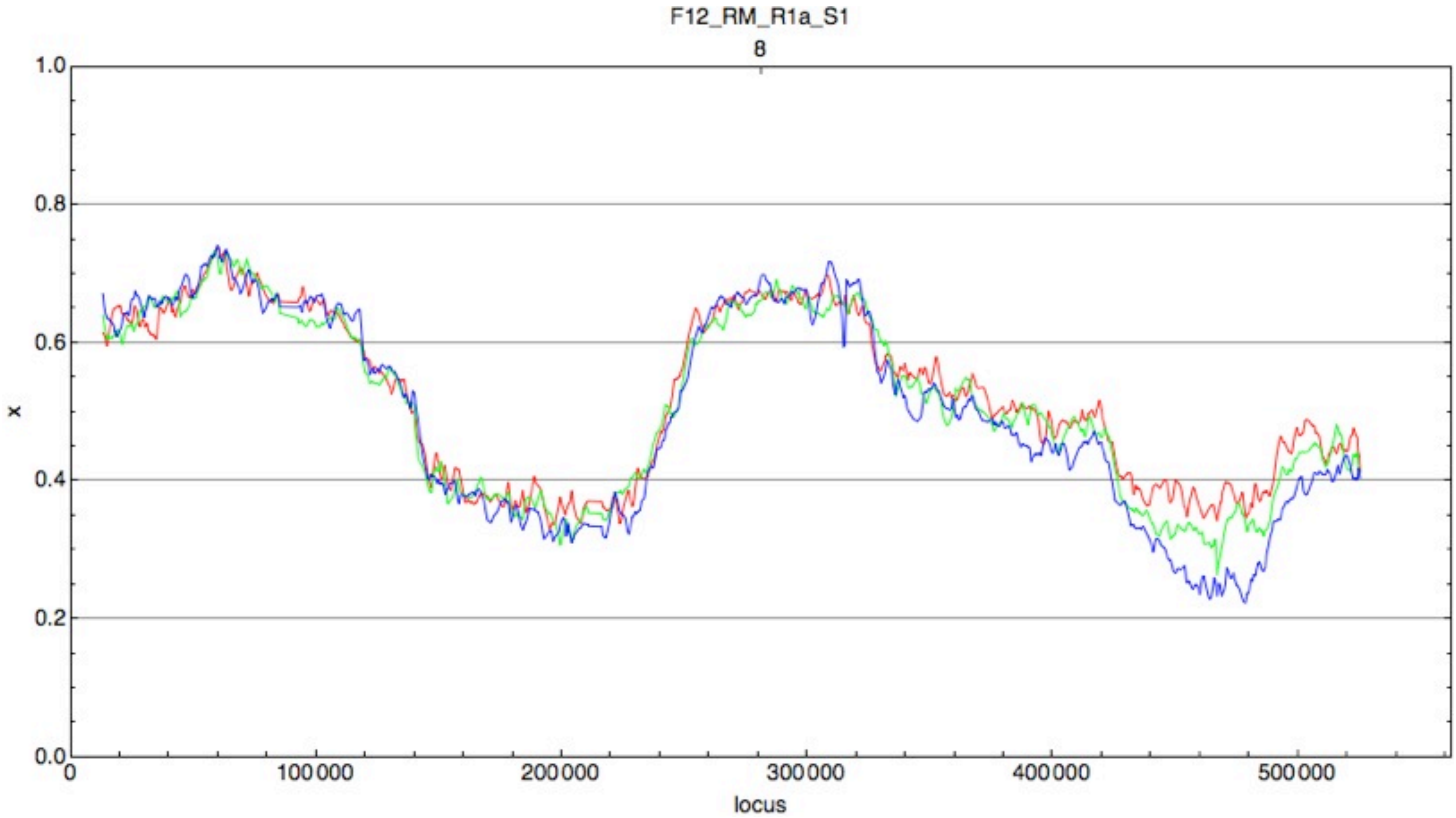

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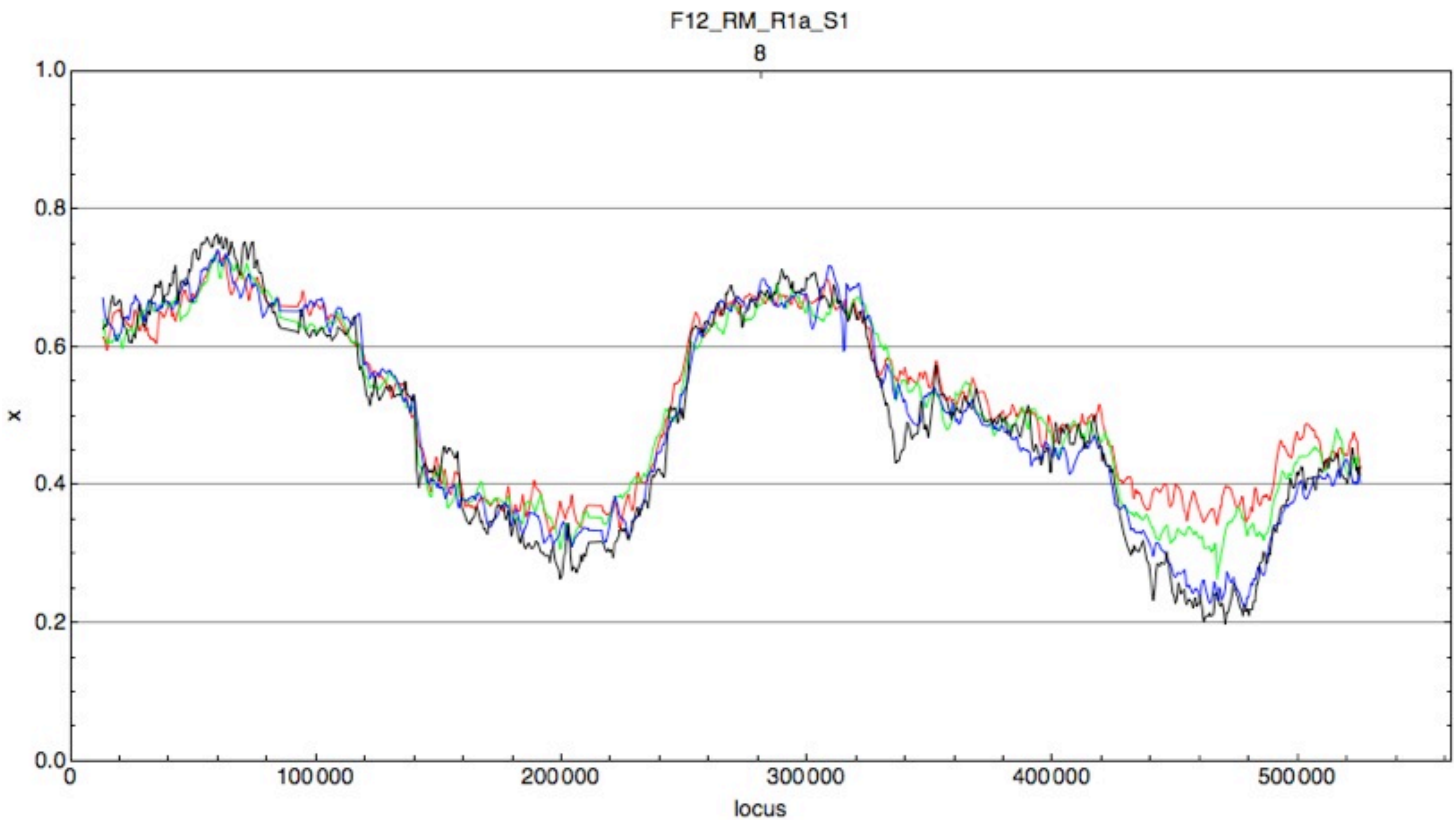

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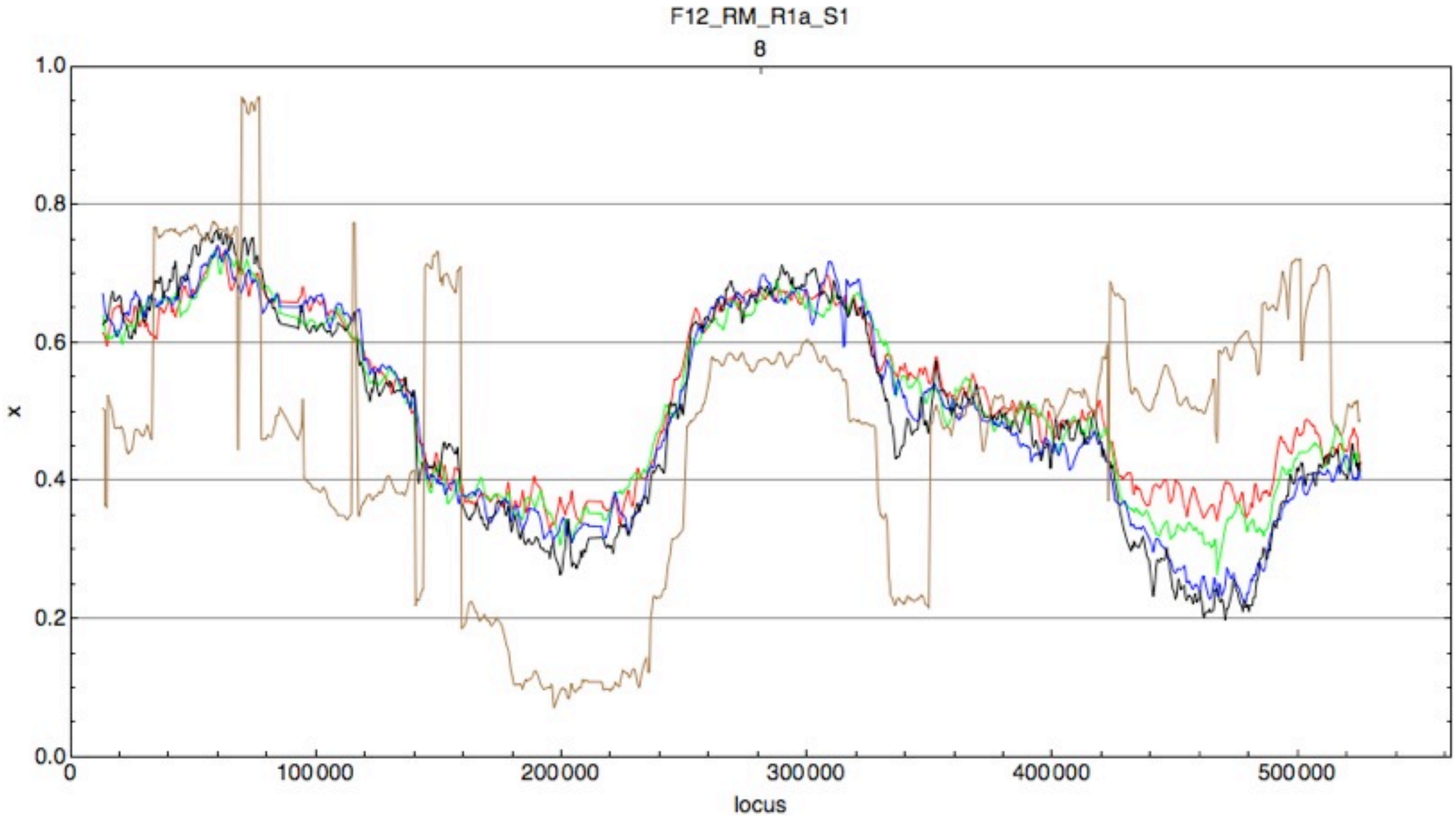

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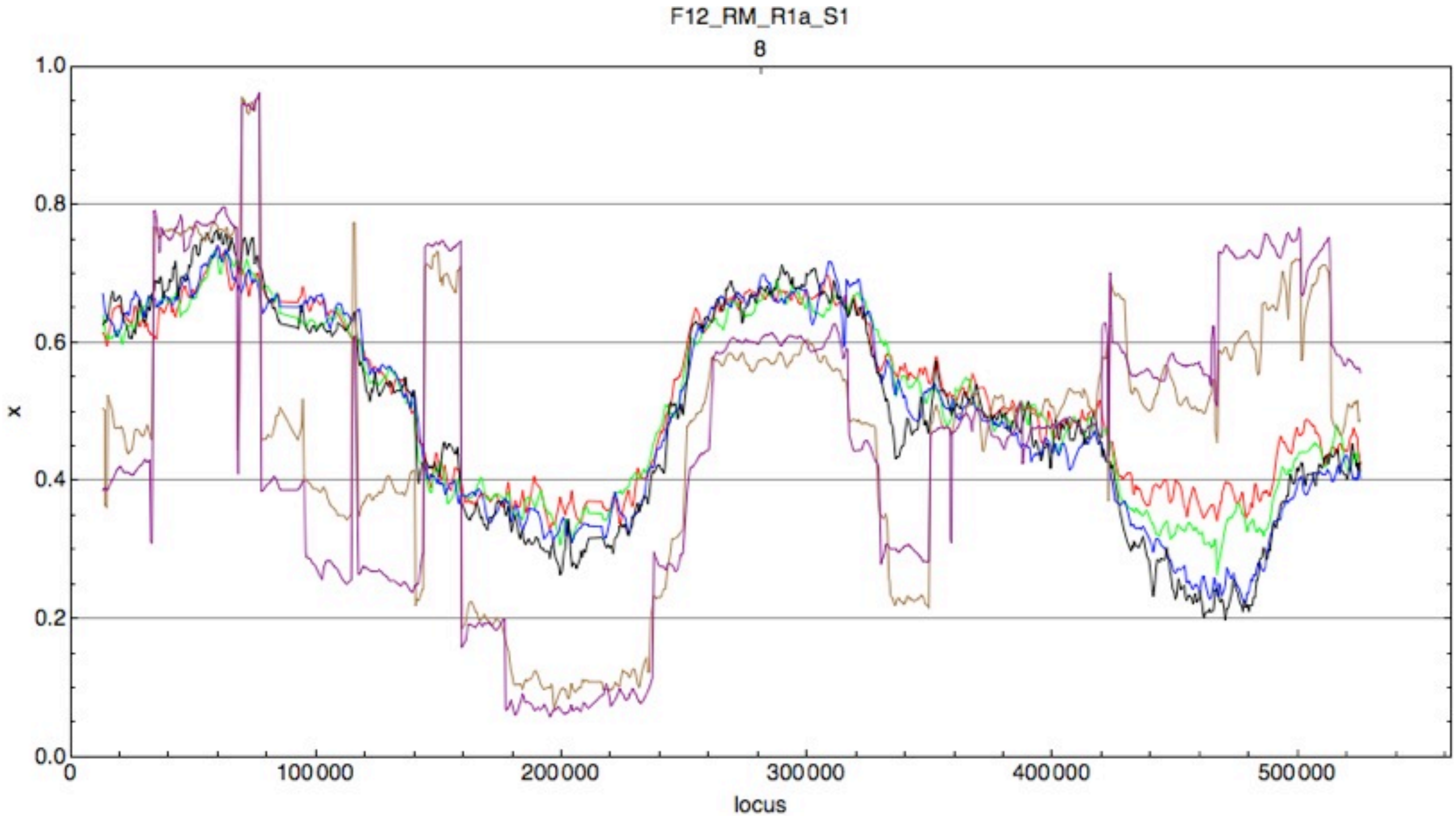

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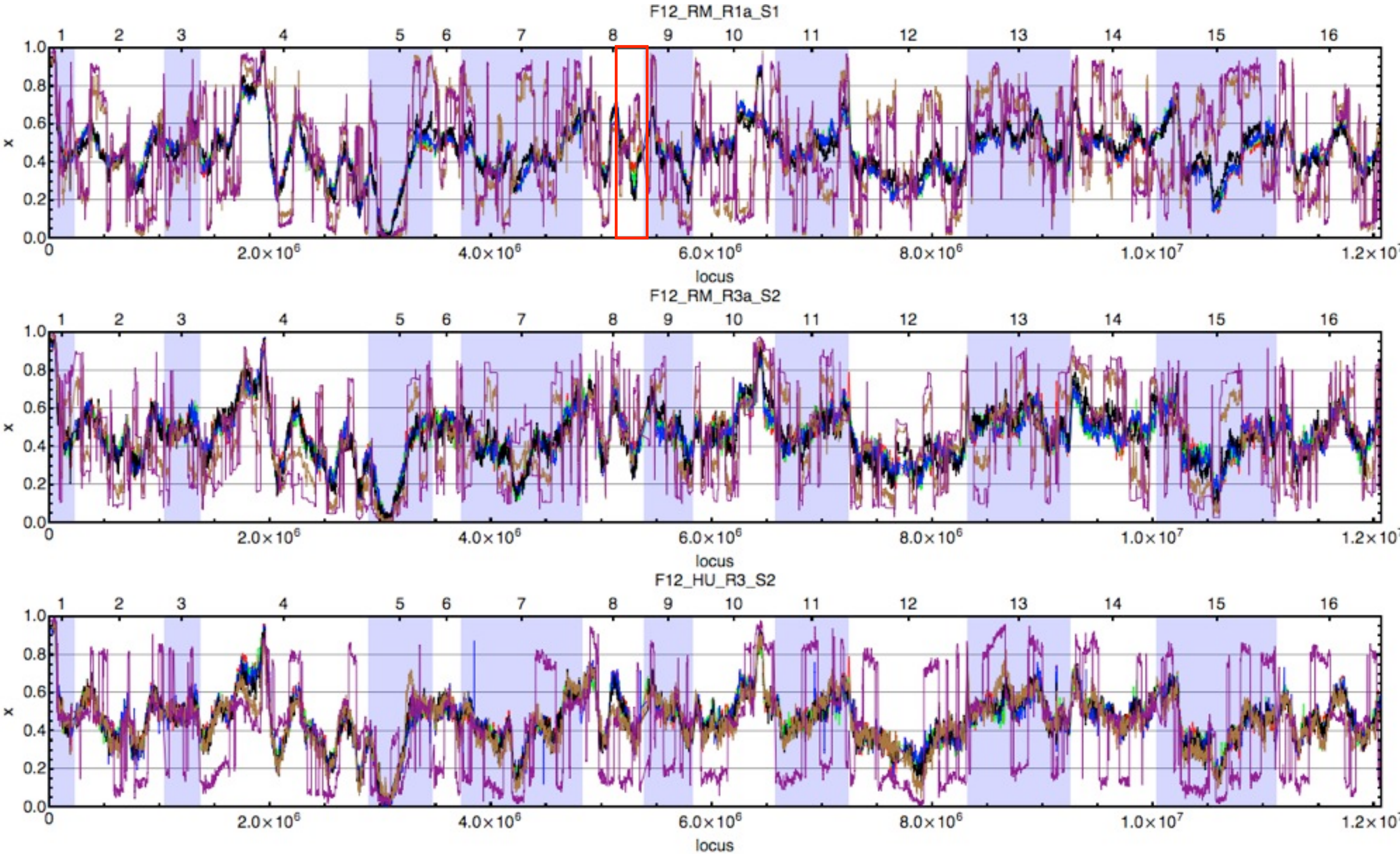
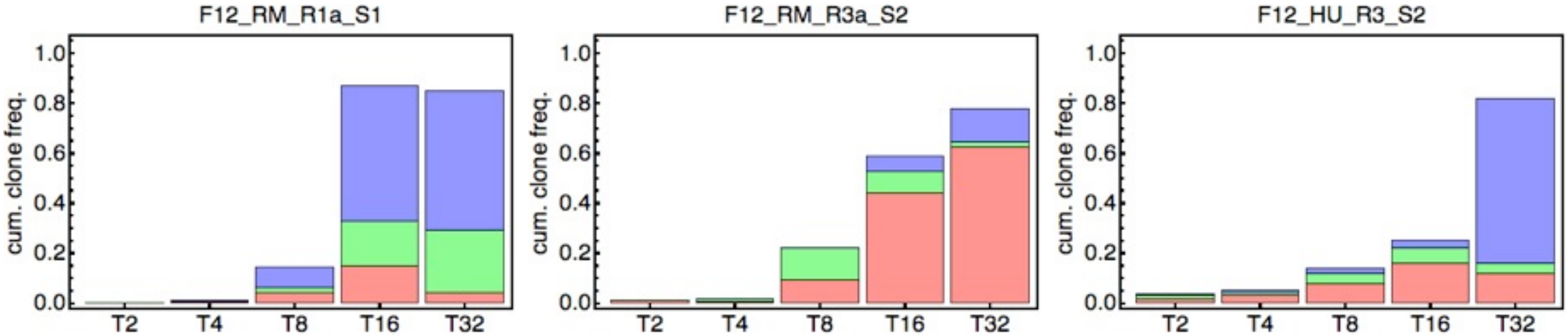


Understanding the clones using cloneHD



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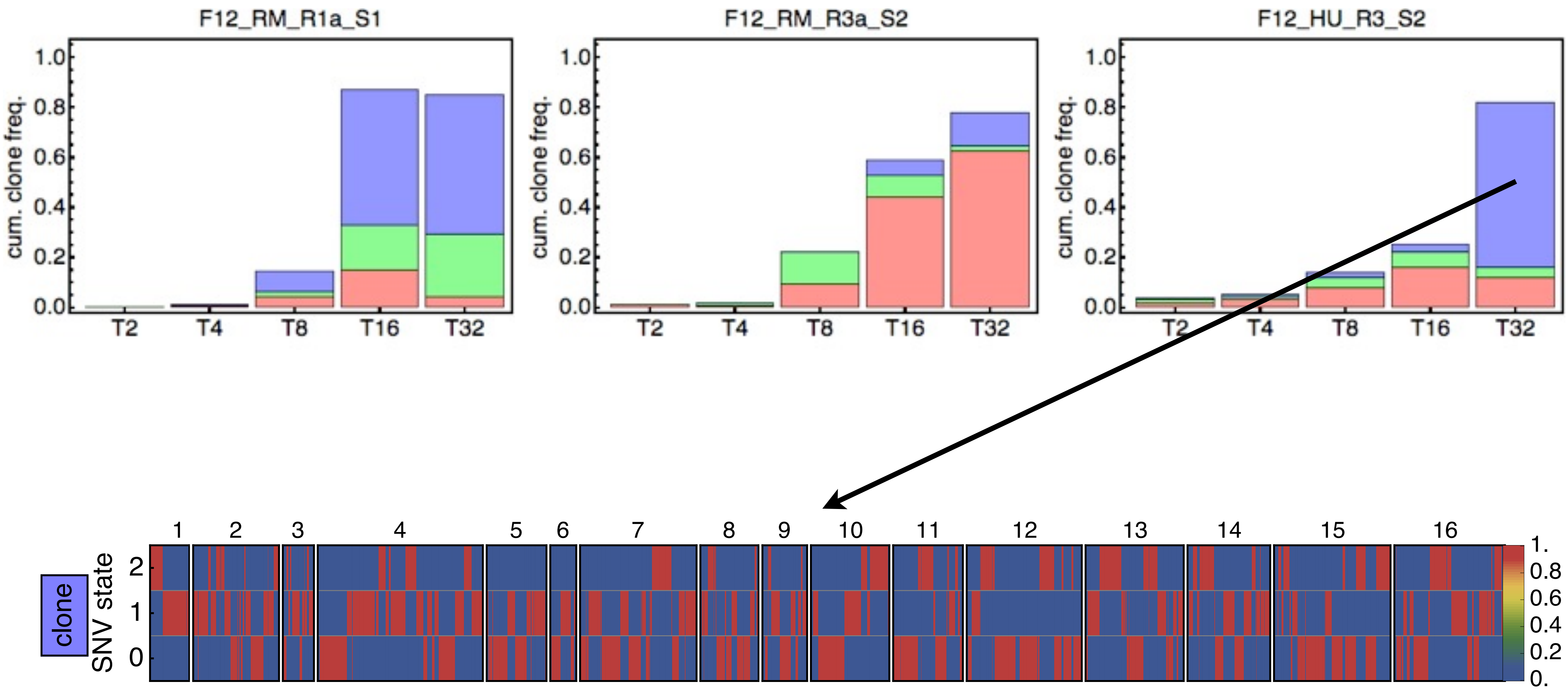

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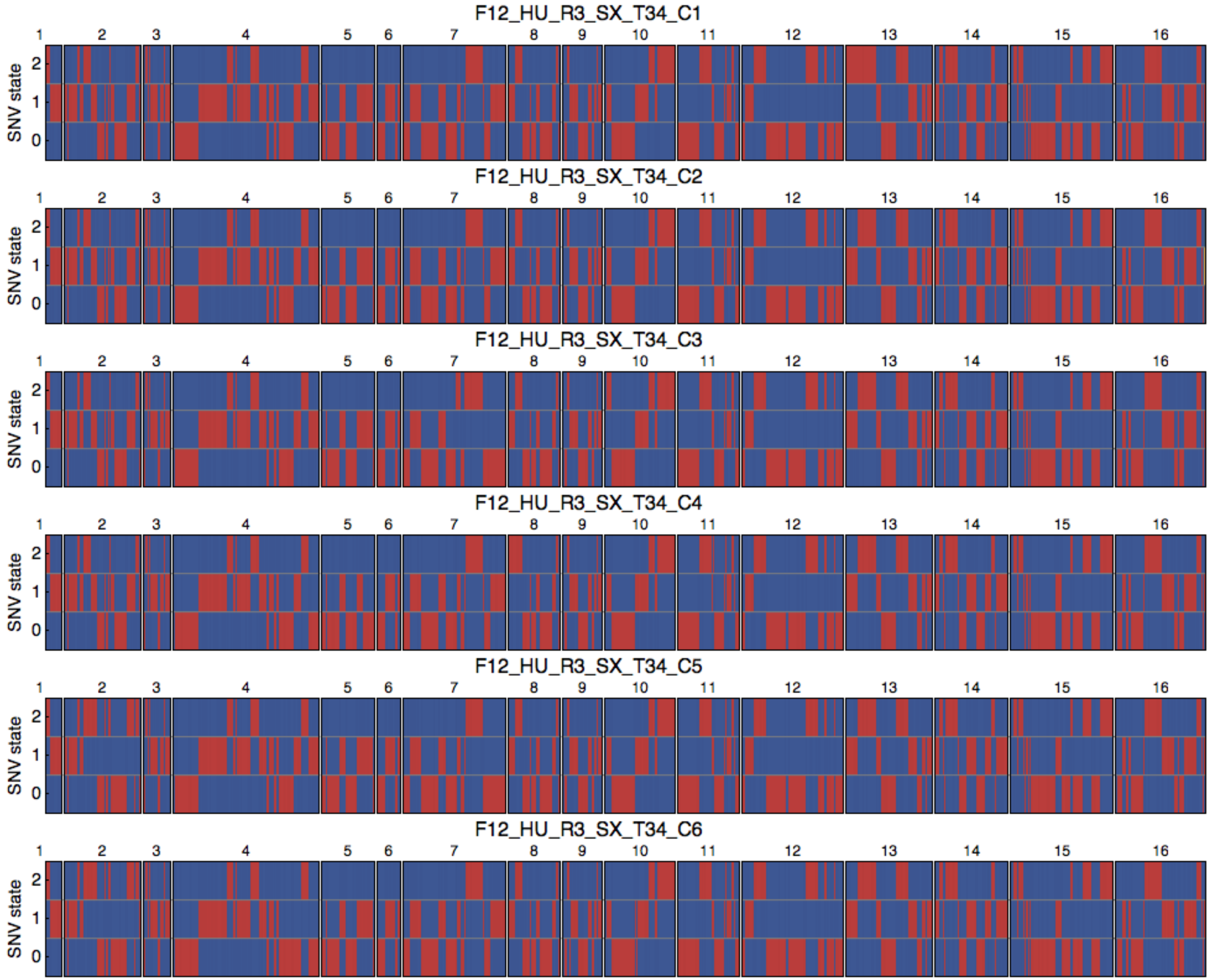

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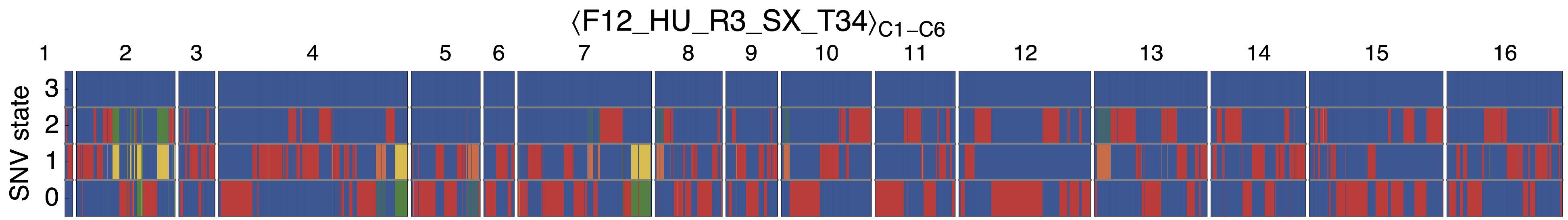

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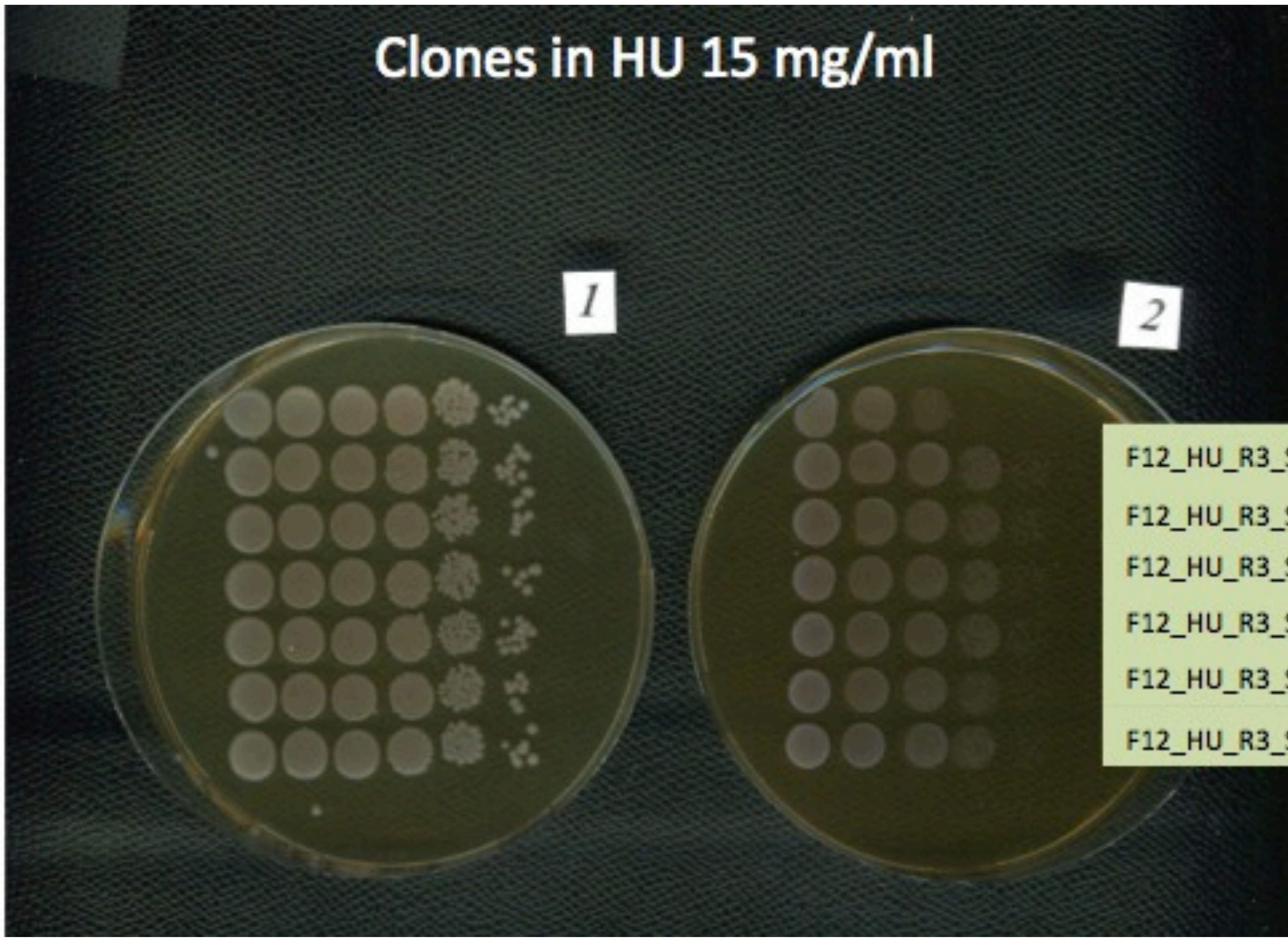

F12_HU_R3_S2_C1 – RNR2 Tyr169His

F12_HU_R3_S2_C2 – RNR2 Tyr169His

F12_HU_R3_S2_C3 – RNR2 Tyr169His

F12_HU_R3_S2_C4 – RNR2 Tyr169His

F12_HU_R3_S2_C5 – RNR2 Tyr169His

F12_HU_R3_S2_C6 – RNR2 Tyr169His

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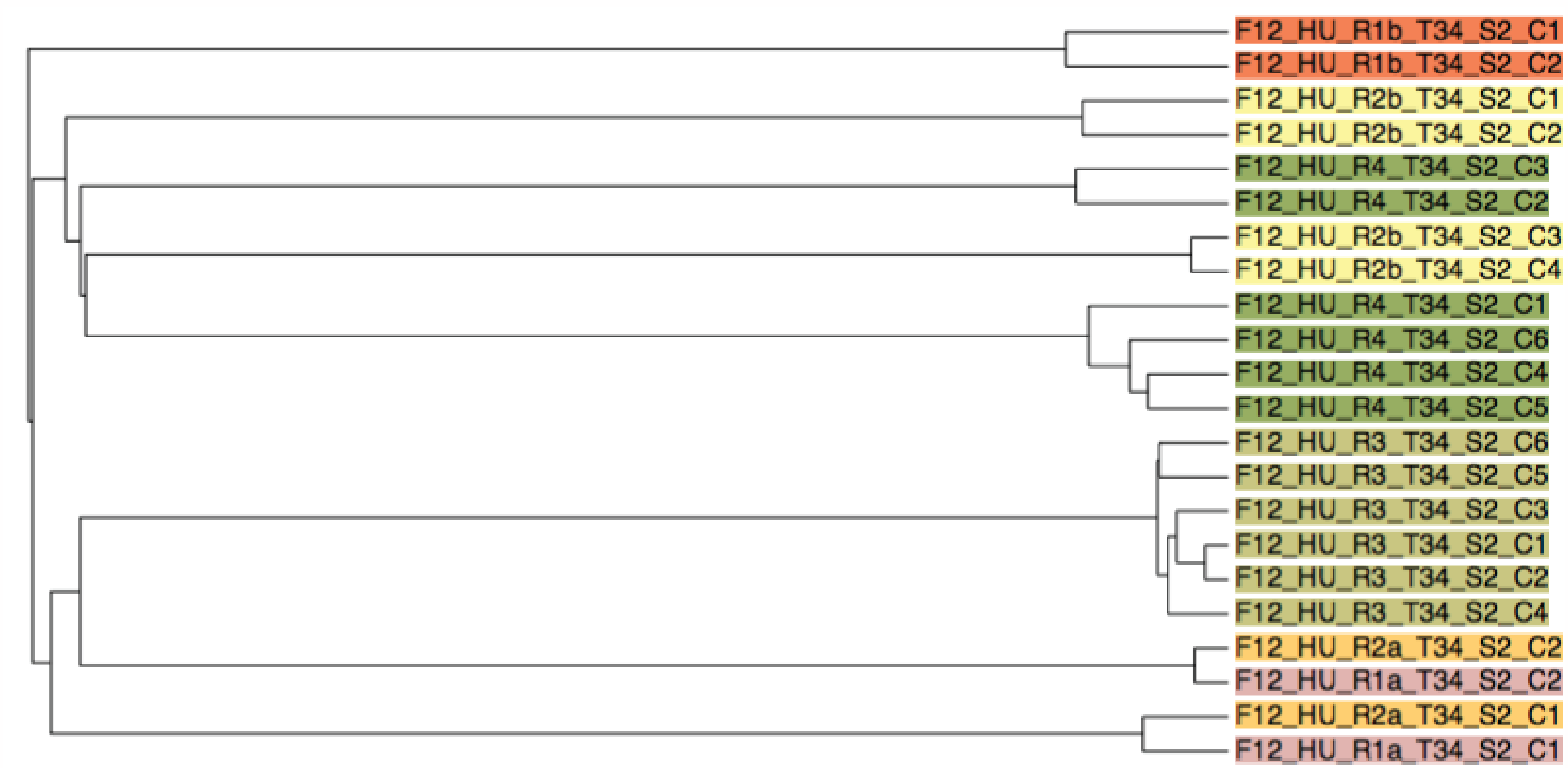
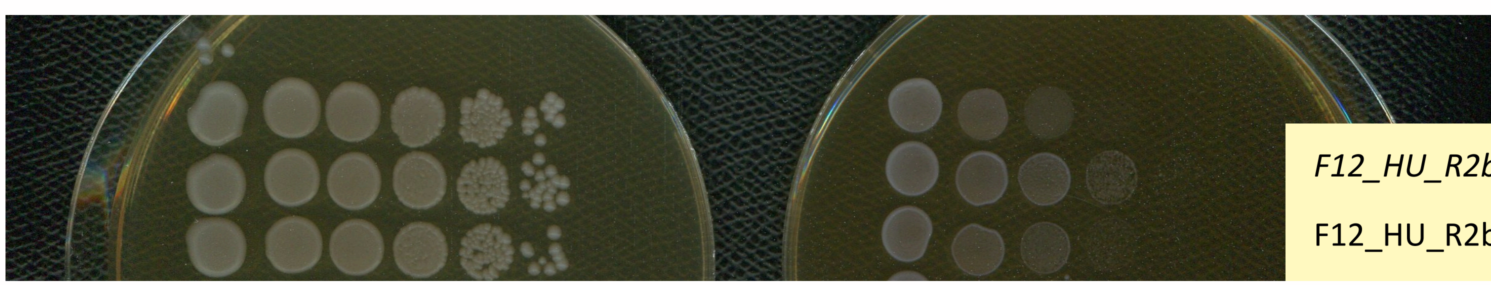




F12_HU_R2b_S2_C1 – RNR2 Thr206Ile

F12_HU_R2b_S2_C2 – RNR2 Thr206Ile

Conclusions Part 1

- We propagated heterogenous yeast populations under cancer drugs and monitored how the genomic composition of the population changed.
- Early time-points allowed for identification of driver events, e.g., CTF8 is validated as a Rapamycin resistance enabling gene (in a complex and interesting way).
- There are other candidates identified with our driverscan algorithm.
- Late time-points show a striking mode of adaptation with emergence of clones.
- We developed an algorithm, cloneHD, to analyse the clones and their dynamics from bulk sequencing data.
- We identified some of the mutations driving resistance phenotype, e.g. RNR2, RNR4, FKB1.
- Evidence for extensive, ongoing, diversification via LOH within the leading clone of HU.

Acknowledgments

- Mustonen Group

- Gianni Liti Lab, Institute of Research on Cancer and Ageing of Nice
  - Francisco Salinas
  - Anders Bergström, Jordi Tronchoni, Agnès Llored, Benjamin Barré, Johan Hallin
- Jonas Warringer Group, Gothenburg
  - Sebastian Ibstedt

Wellcome Trust for funding

Part II: High-definition reconstruction of clonal composition in cancer

Acknowledgments

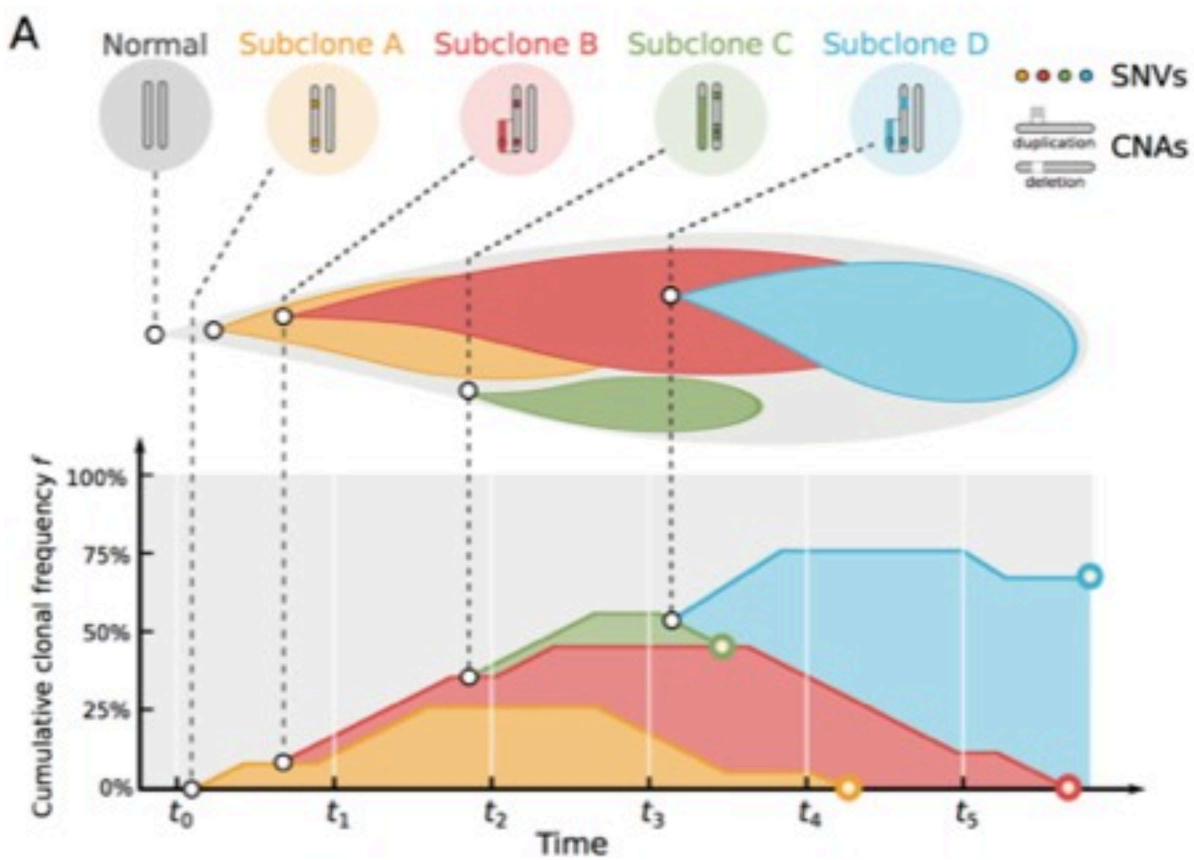
- Mustonen Group

- Chris Illingworth (Cambridge)

- Gianni Liti Group, Institute of Research on Cancer and Ageing of Nice
- Sanger CGP: Peter Van Loo, David Wedge, Peter Campbell
- C. Greenman (TGAC), I. Tomlinson (Oxford), O. Krijgsman (NKI)

Wellcome Trust for funding

Tumours are not genetically homogeneous



- naive sample extraction strategies will lead to an underestimation of real tumour heterogeneity.
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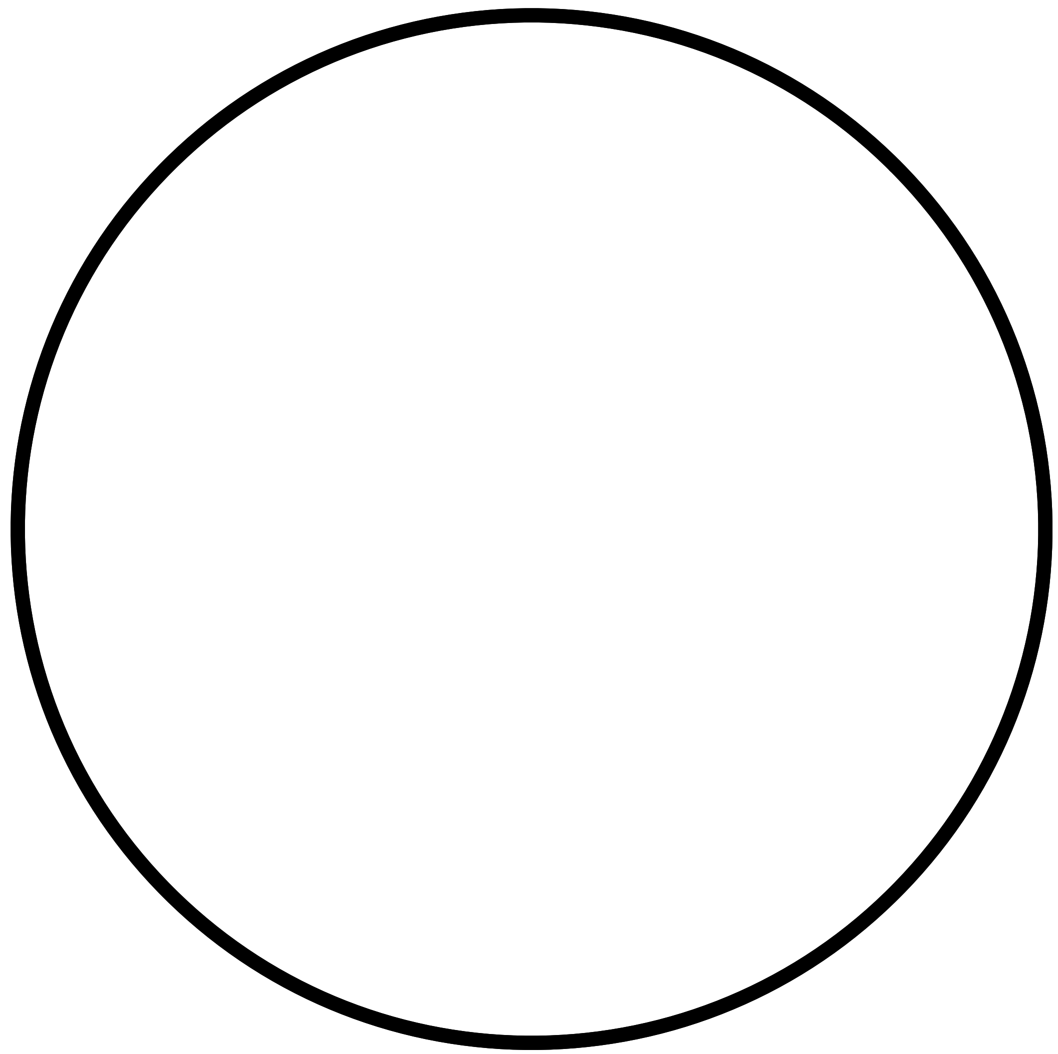
What do we mean by subclones



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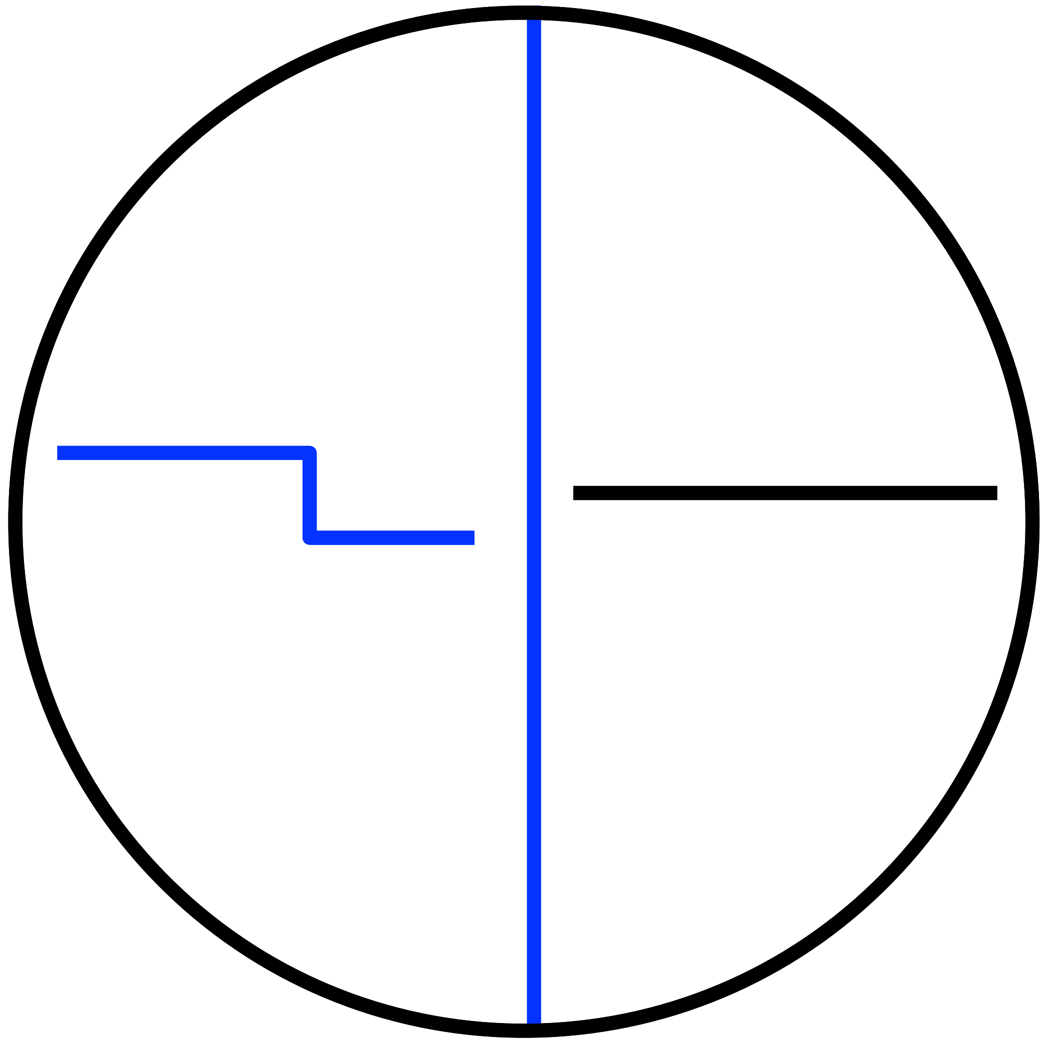

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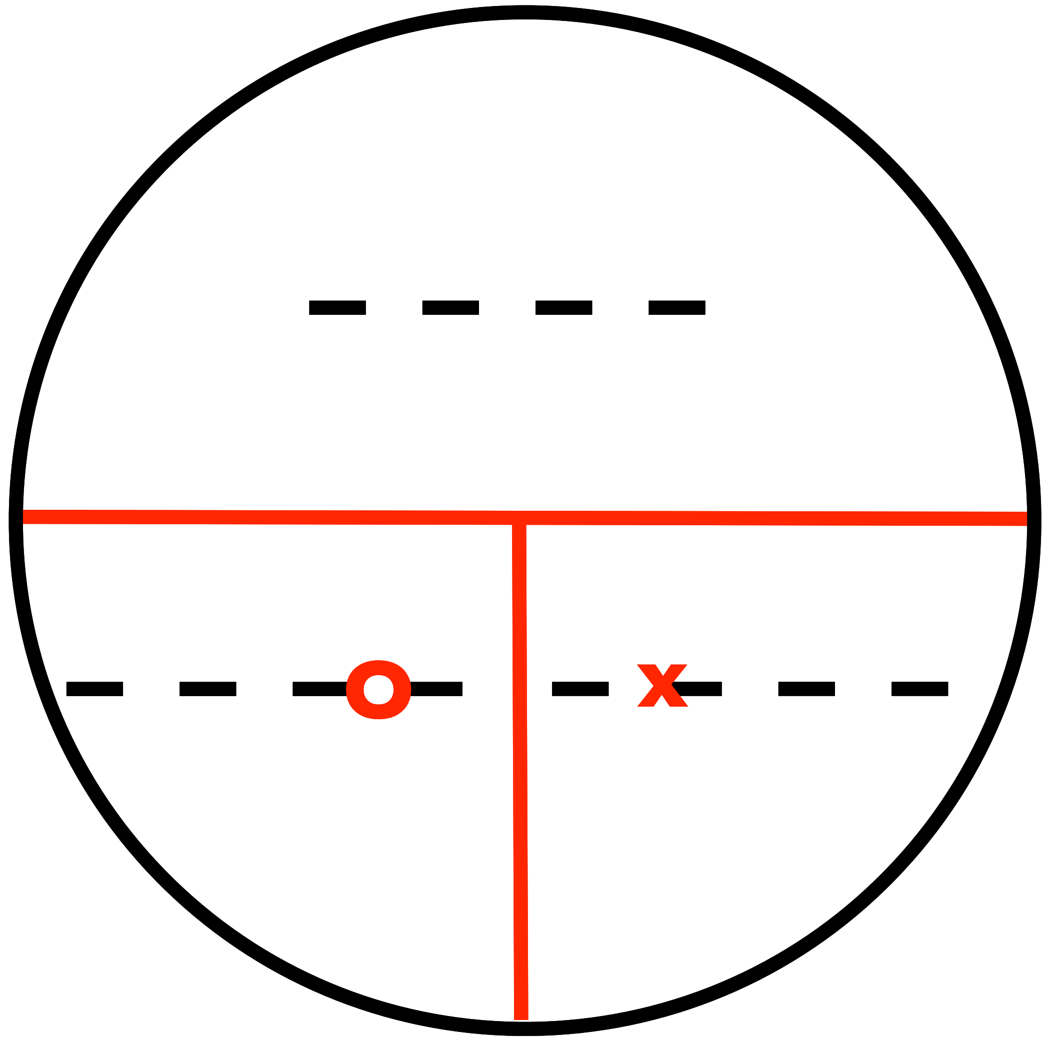

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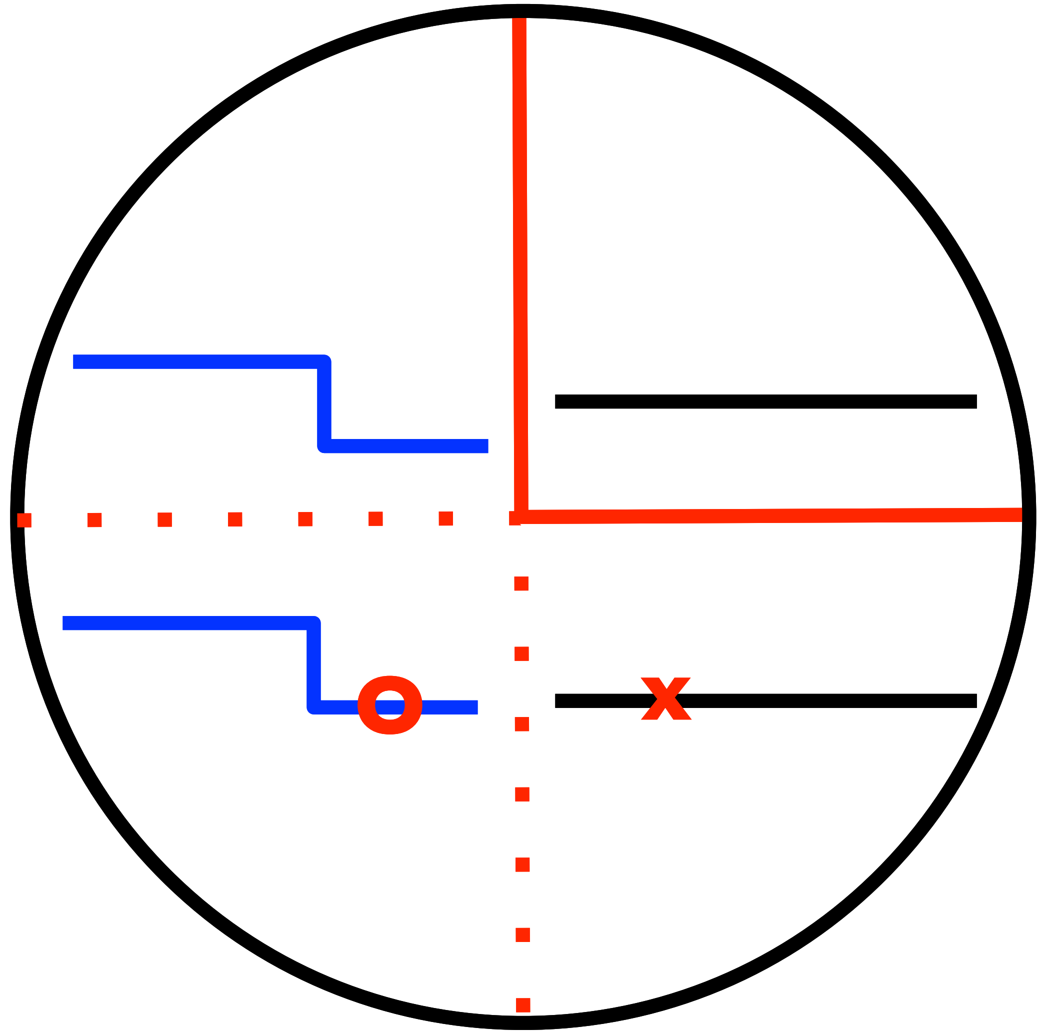

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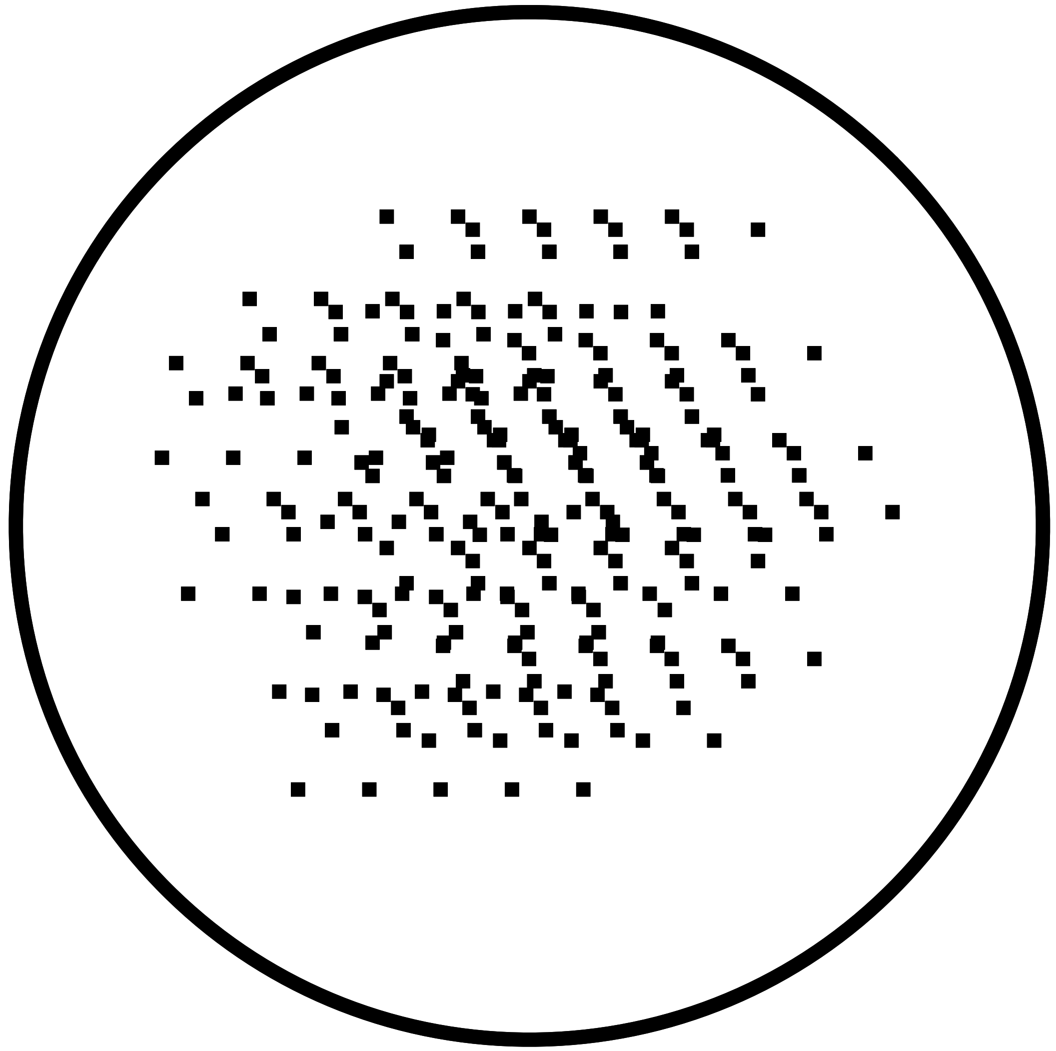
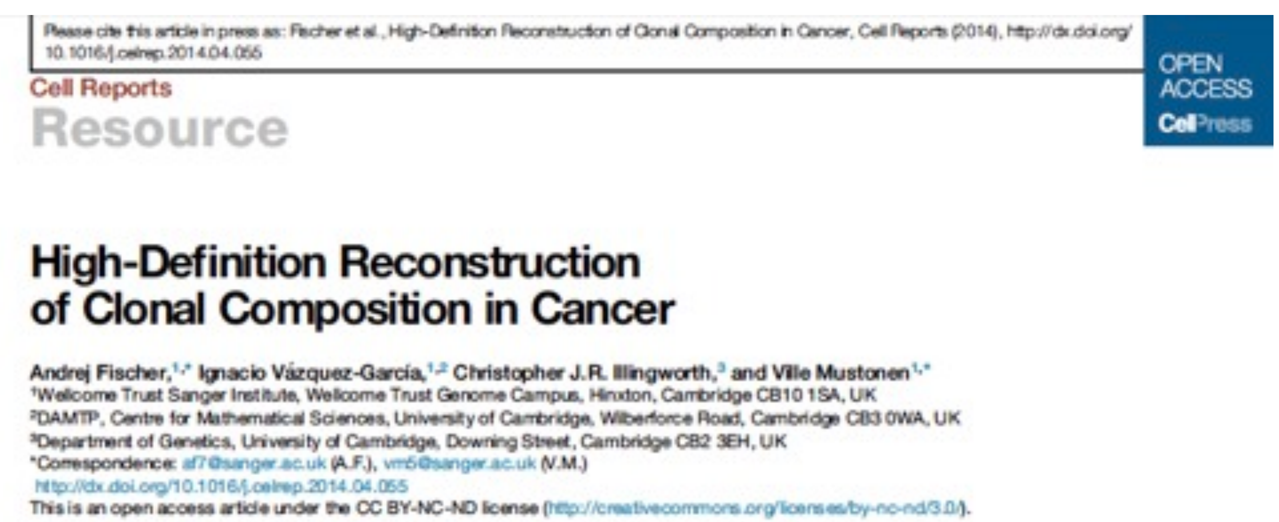


cloneHD: overview

- Fully probabilistic algorithm that exploits correlations
  - across time (longitudinal data).
  - across space (multi region and/or metastatic samples).
  - along genomes caused by events such as copy number changes.
- Operates at the level of read depth counts, B-allele counts and somatic SNVs counts.
- Computationally powerful. Can utilize a large number of variants per sample (tens of thousands) and several measurements per tumour.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High-Definition Reconstruction of Clonal Composition in Cancer

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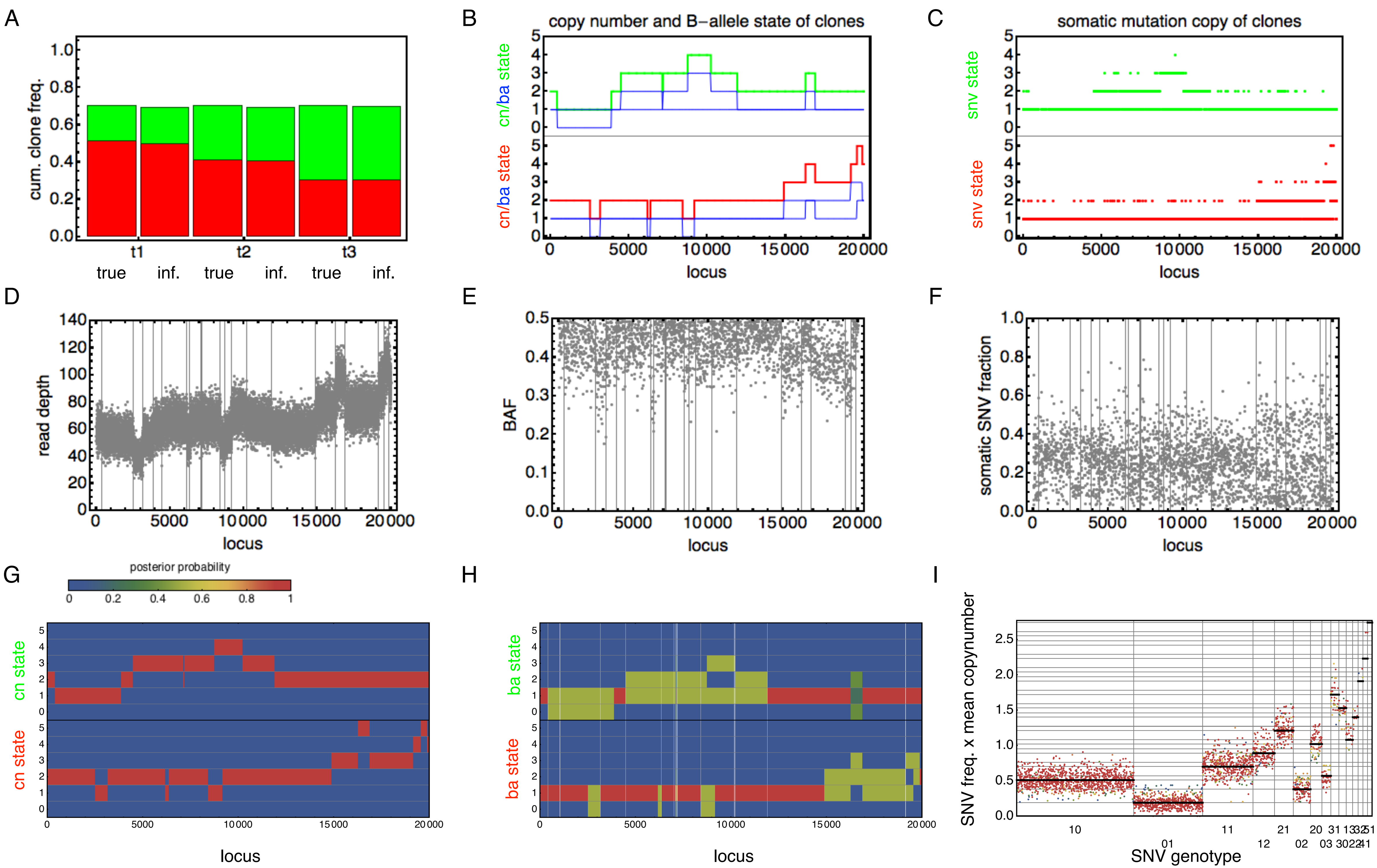
*Correspondence: af7@sanger.ac.uk (A.F.), vm5@sanger.ac.uk (V.M.)
http://dx.doi.org/10.1016/j.celrep.2014.04.055

Benchmarking with simulations



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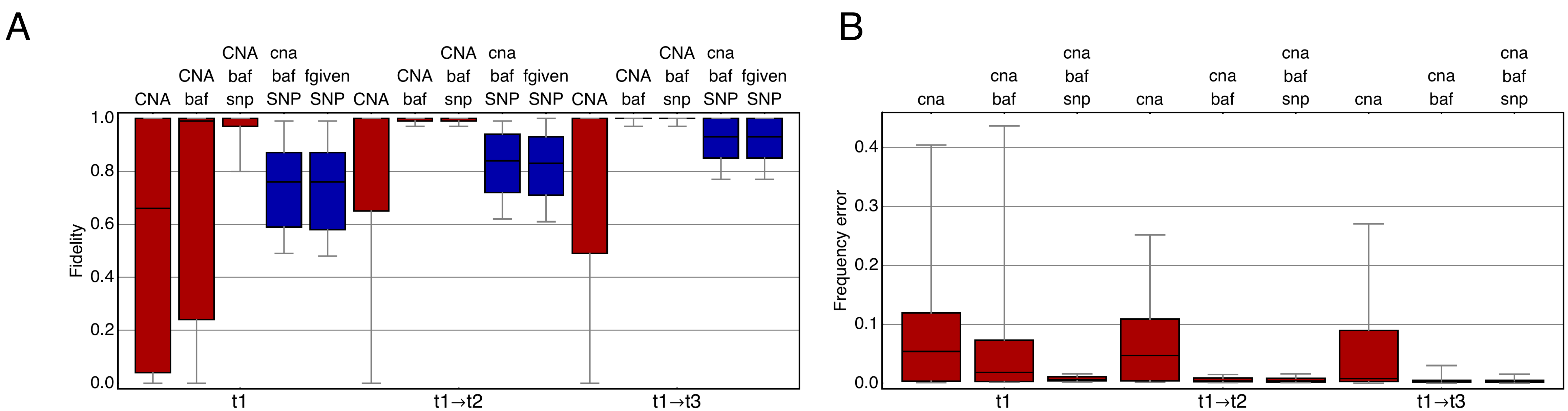


Case study of CLL

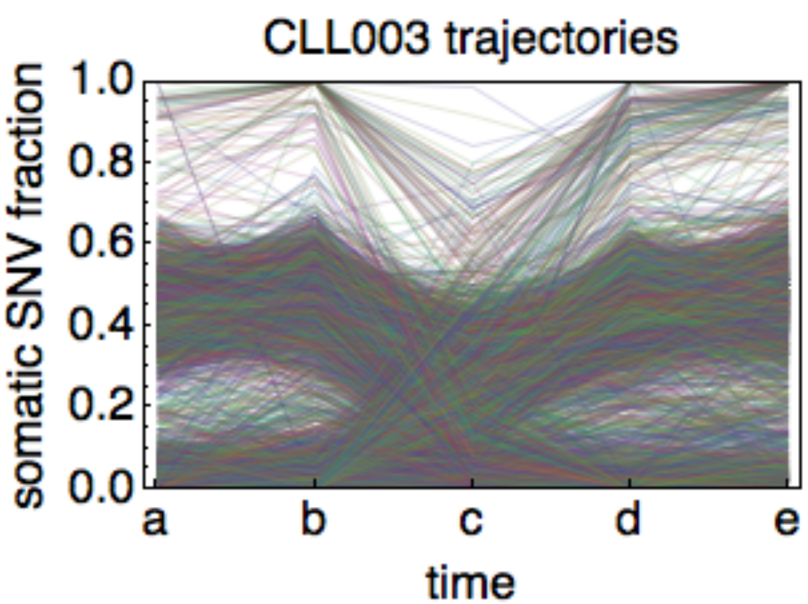
Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns

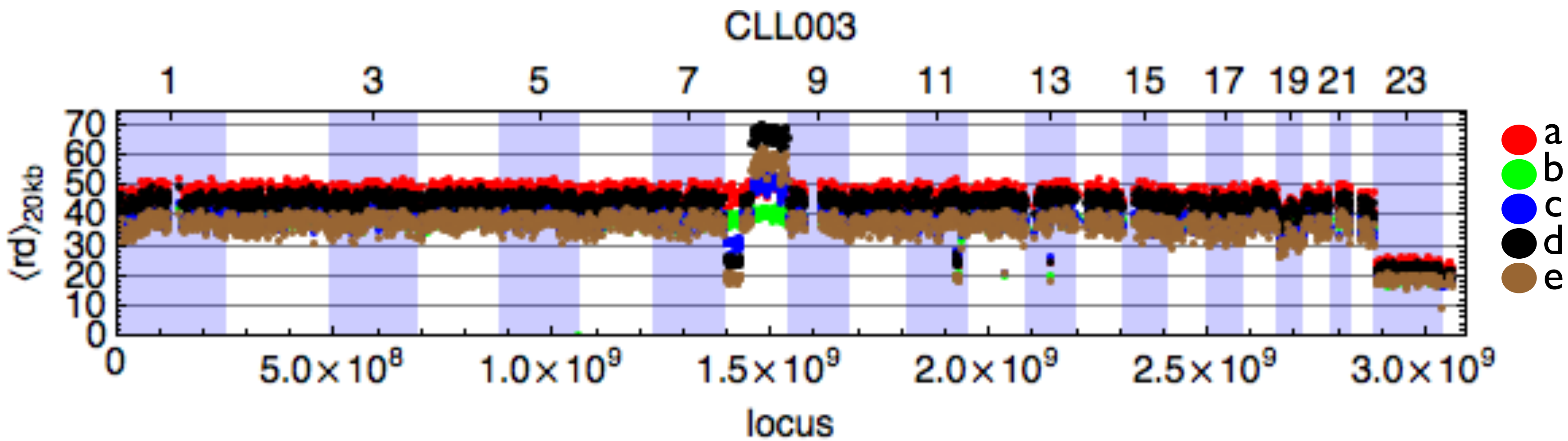
Anna Schuh,[1] Jennifer Becq,[2] Sean Humphray,[2] Adrian Alexa,[2] Adam Burns,[1] Ruth Clifford,[1] Stephan M. Feller,[3] Russell Grocock,[2] Shirley Henderson,[1] Irina Khrebtukova,[4] Zoya Kingsbury,[2] Shujun Luo,[4] David McBride,[2] Lisa Murray,[2] Toshi Menju,[3,5] Adele Timbs,[1] Mark Ross,[2] Jenny Taylor,[1] and David Bentley[2]

[1]Oxford National Institute of Health Research (NIHR) Biomedical Research Centre, University of Oxford, Oxford, United Kingdom; [2]Illumina Cambridge Ltd, Saffron Walden, United Kingdom; [3]Biologic Systems Architecture Group, Department of Oncology, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom; [4]Illumina Inc, Hayward, CA; and [5]Department of Thoracic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

- Three chronic lymphocytic leukemia patients sequenced at 5 time points for each.
- Thanks to Anna Schuh, Jennifer Becq and J-B. Cazier for help with data access.

Clones for CLL003 using cloneHD





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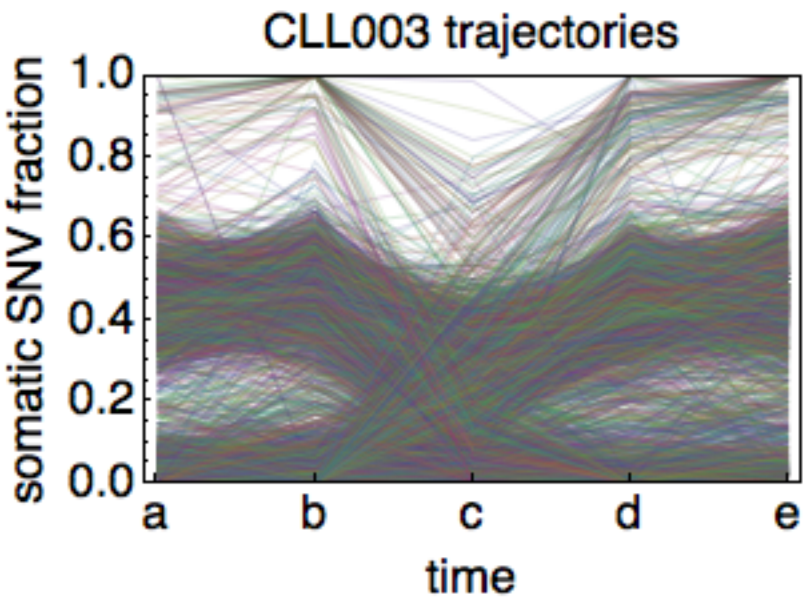

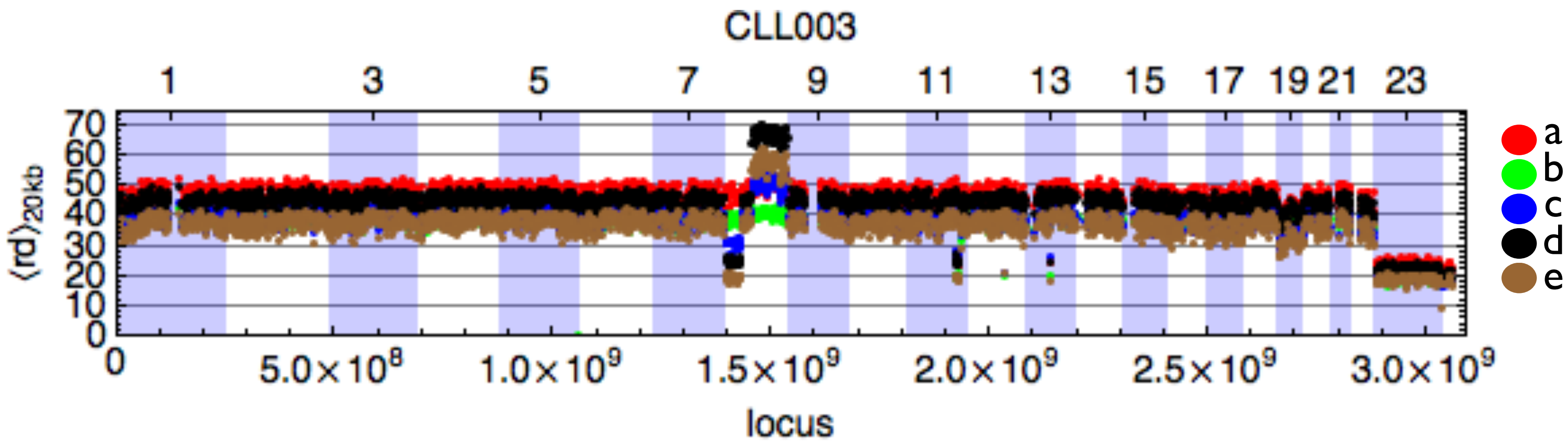

cloneHD

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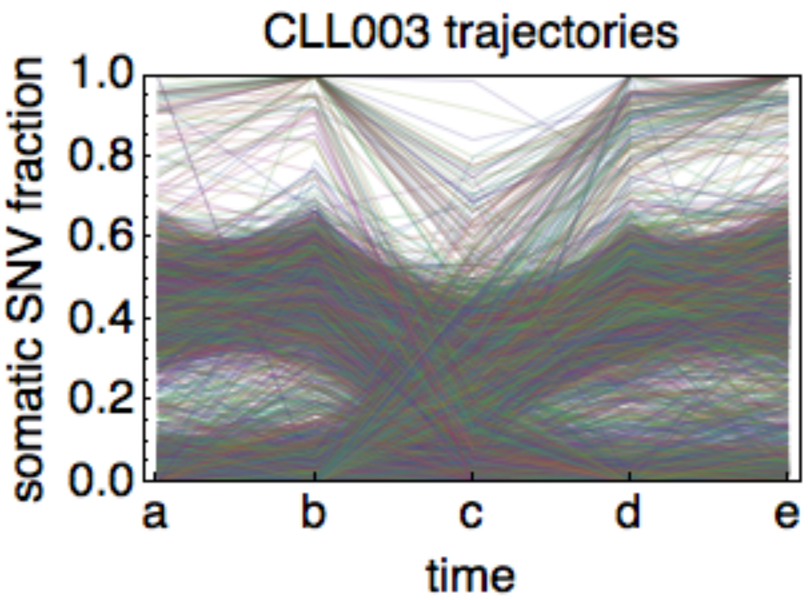

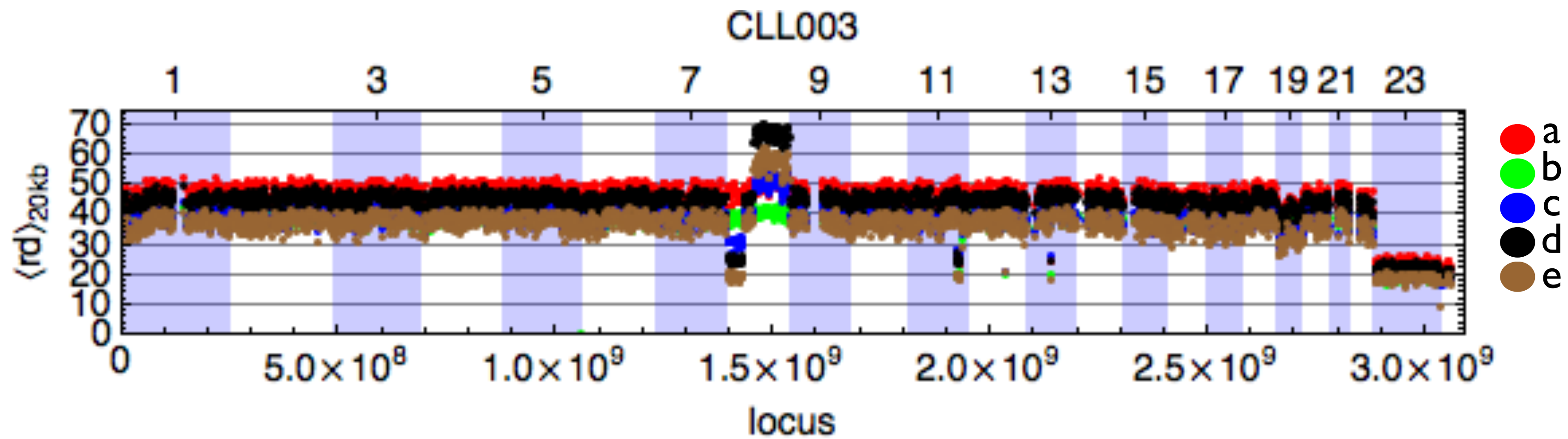

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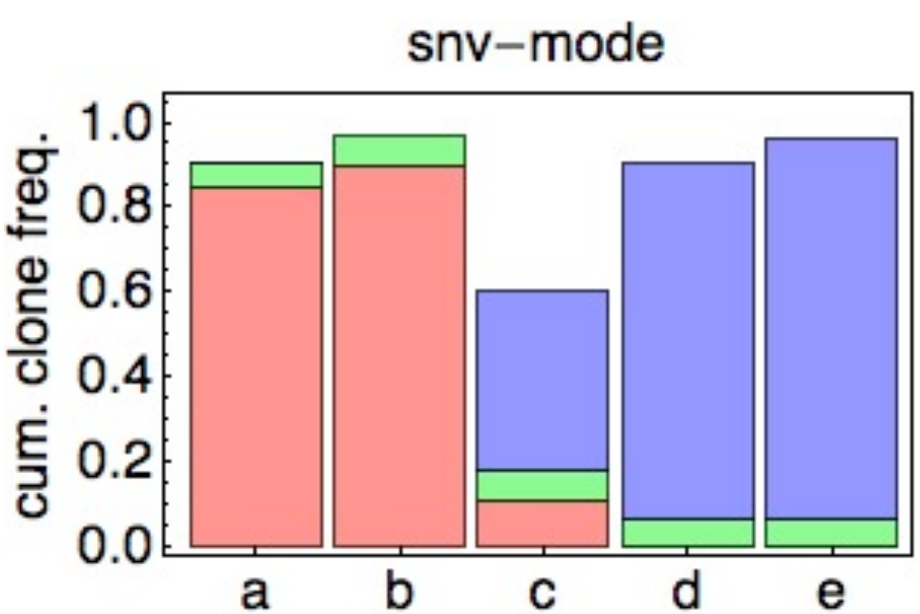

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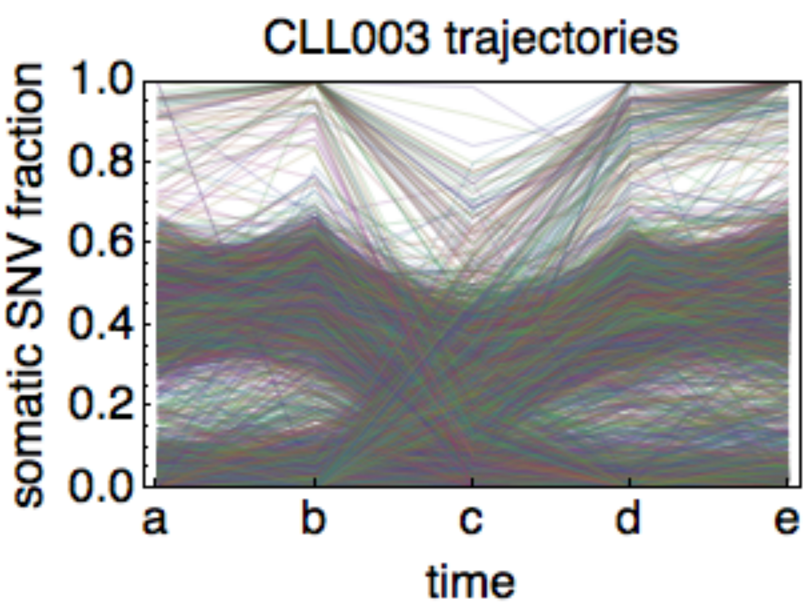

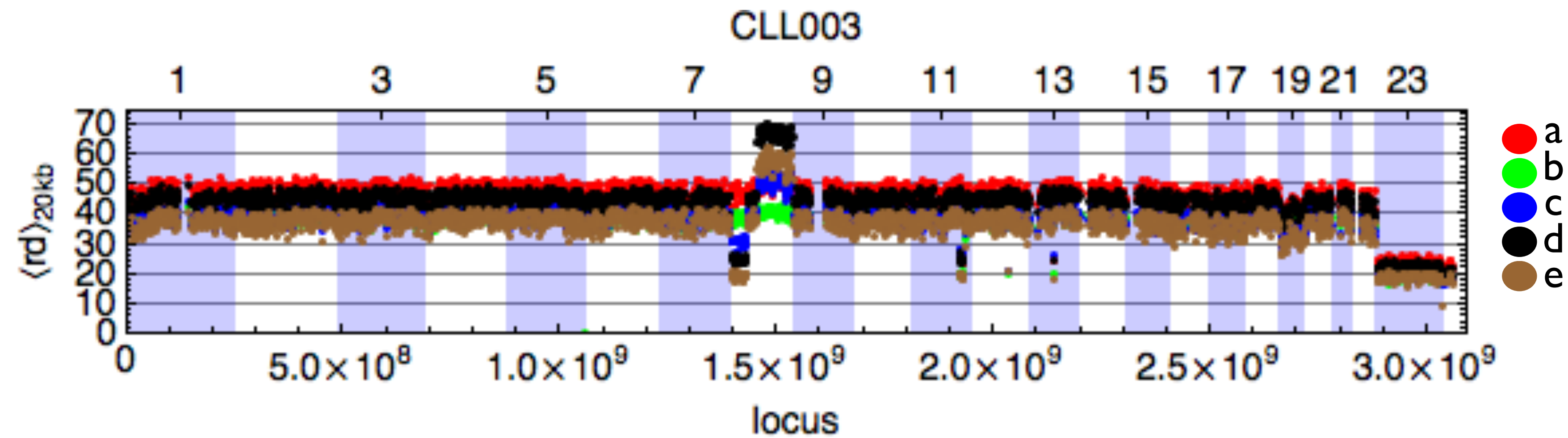

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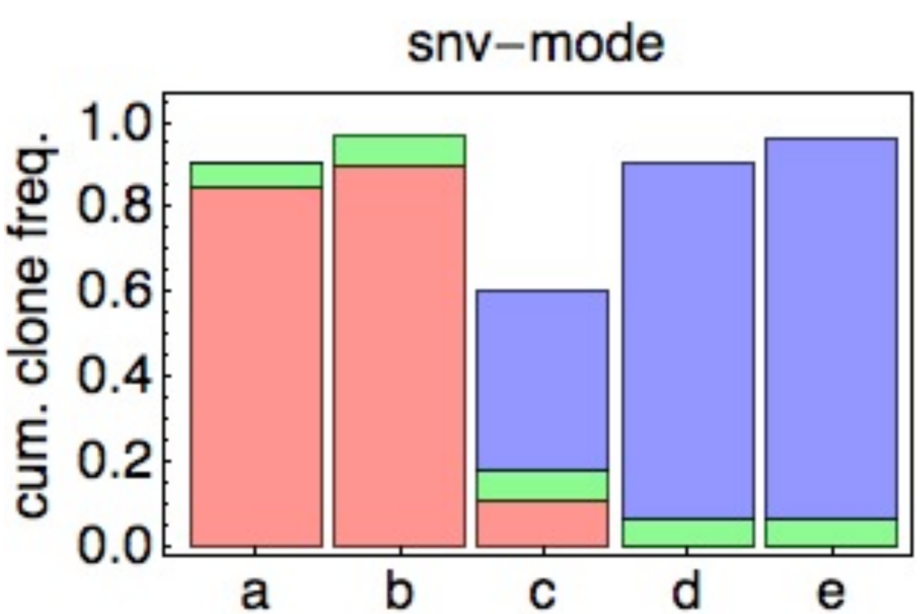

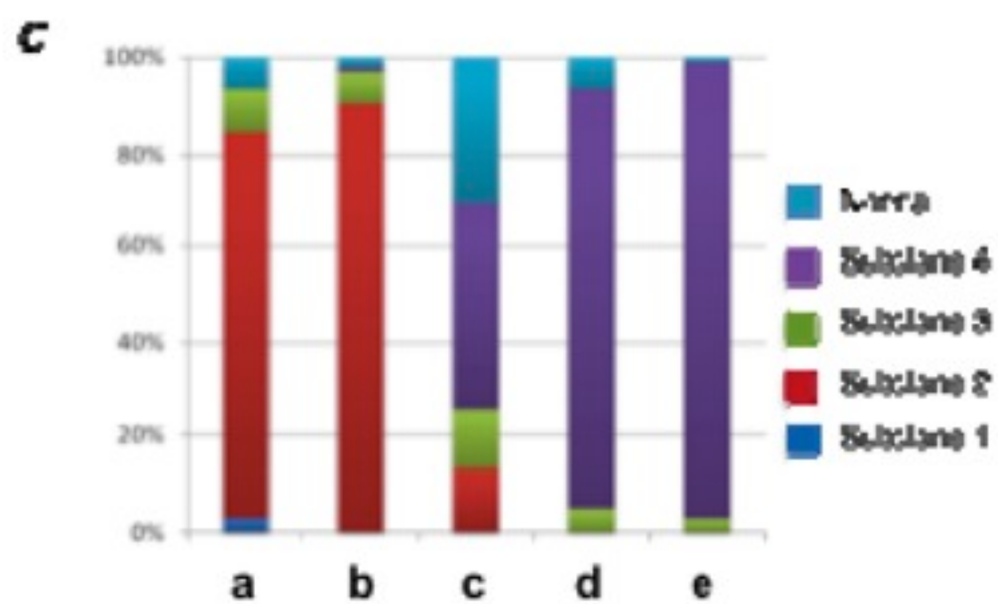

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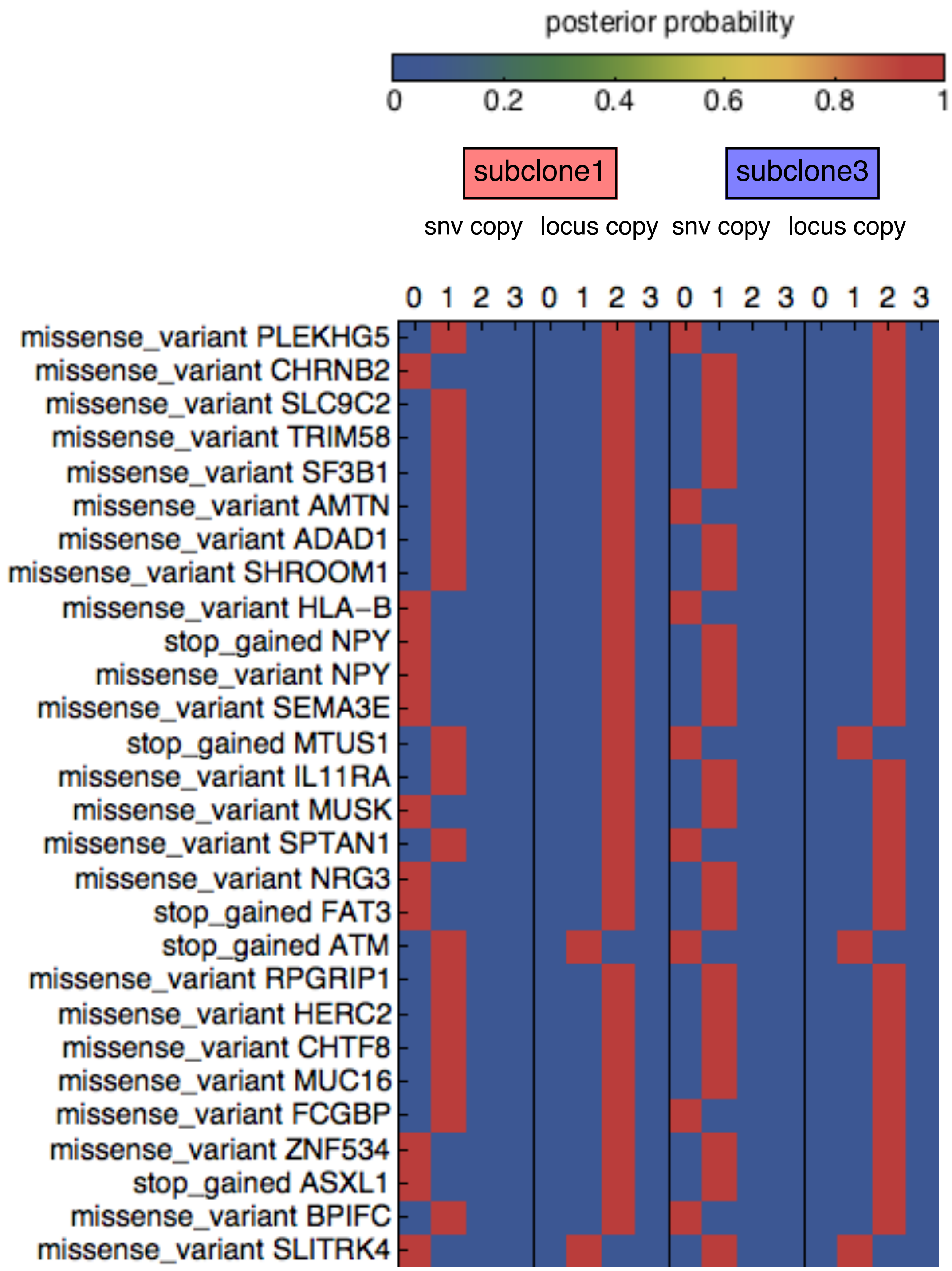

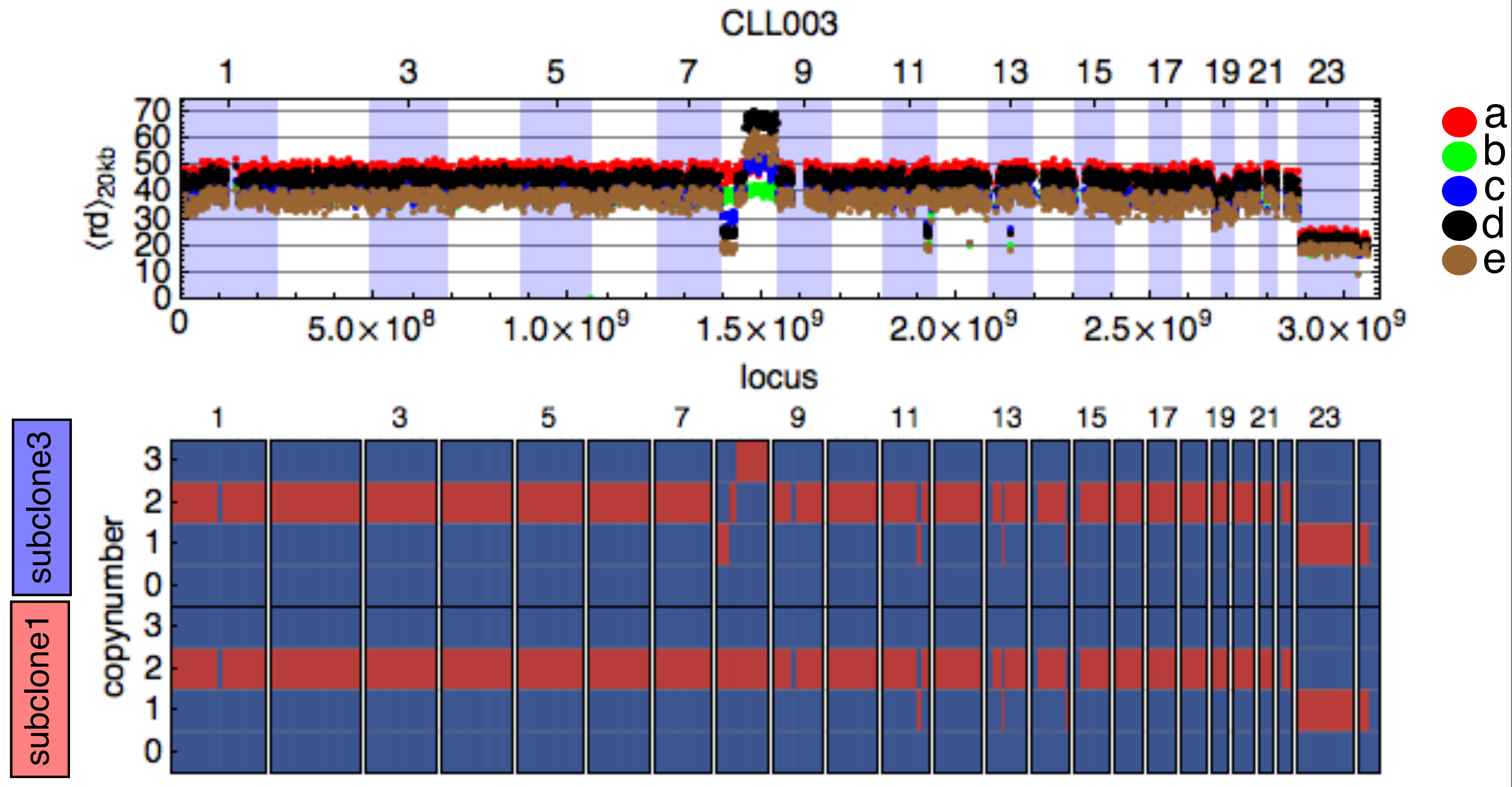

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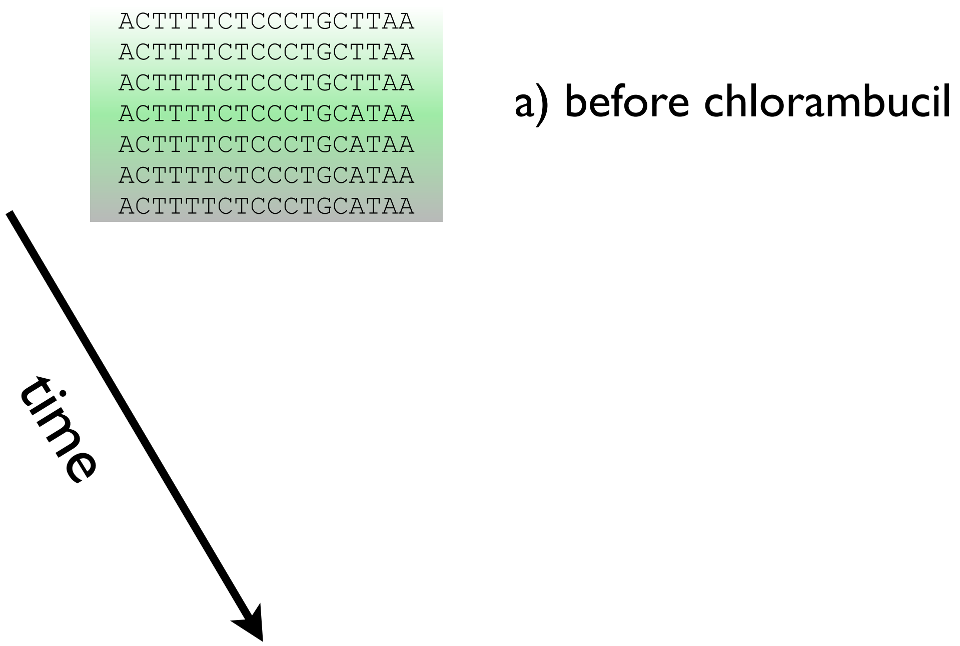
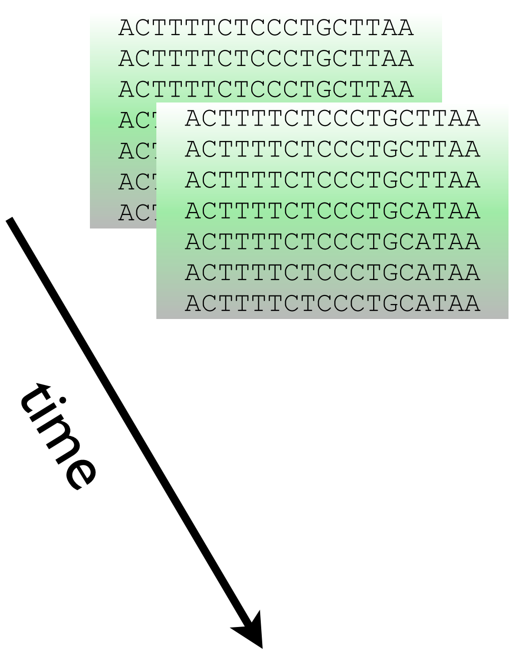


High-definition but retrospective...

- Good for learning about tumour evolution.
- Not so good for gauging potential value for guiding treatment decisions.
- Let’s try partial inference to mimic a real-time scenario.



a) before chlorambucil

b) before fludarabine, cyclophosphamide, rituximab

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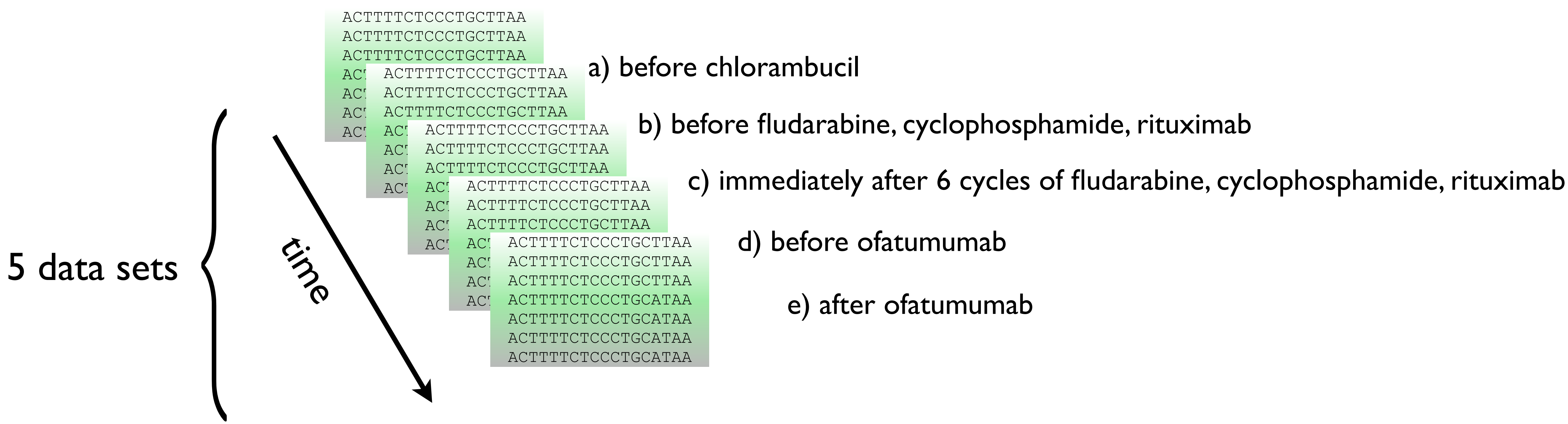
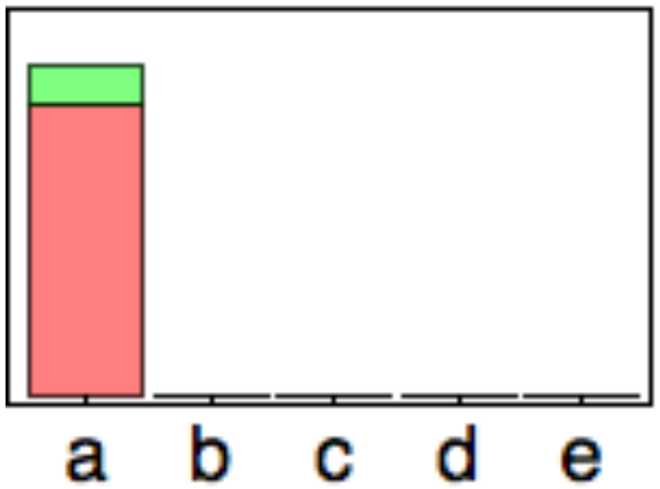
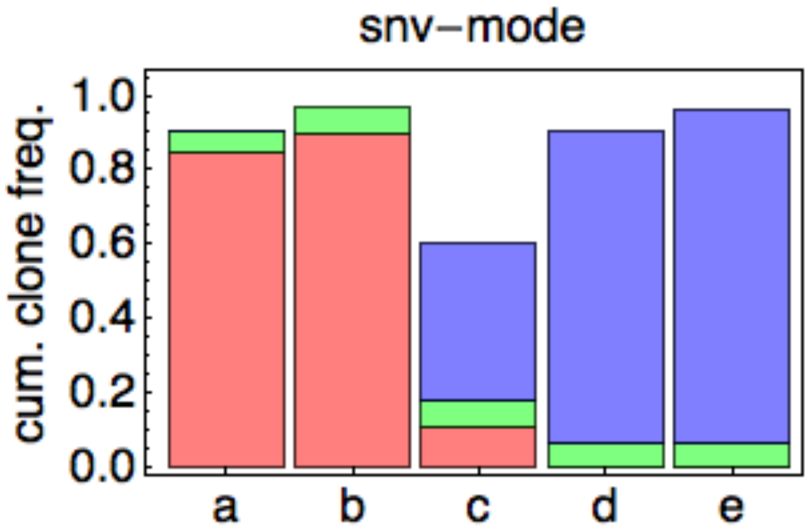


Inference using partial data





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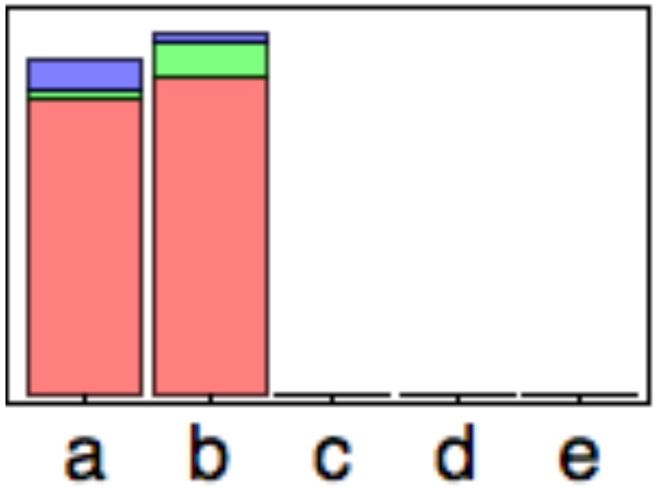

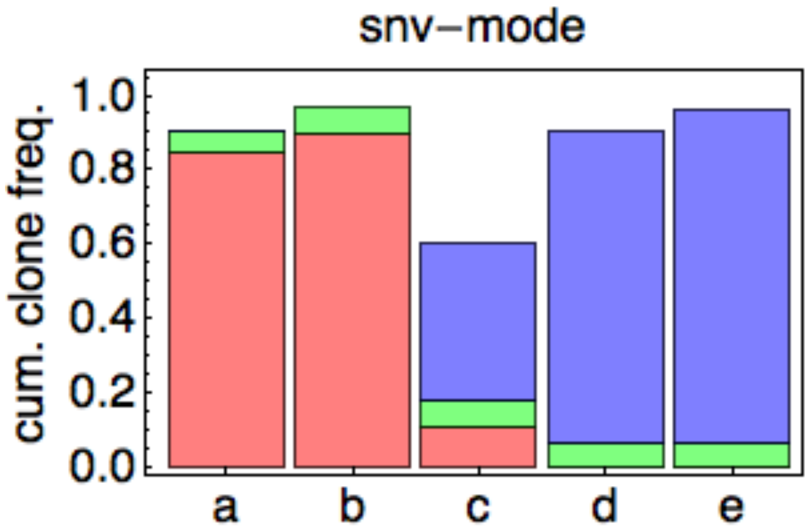

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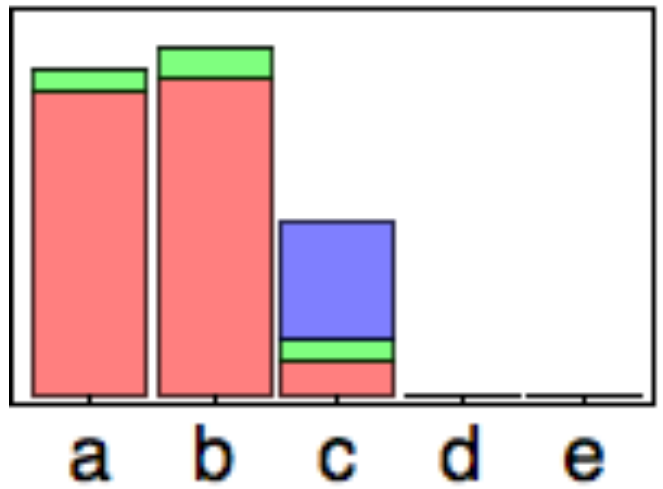
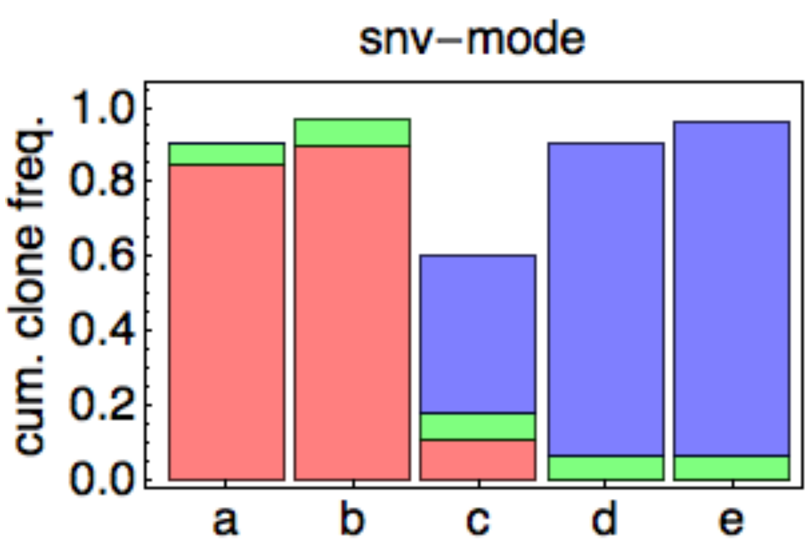
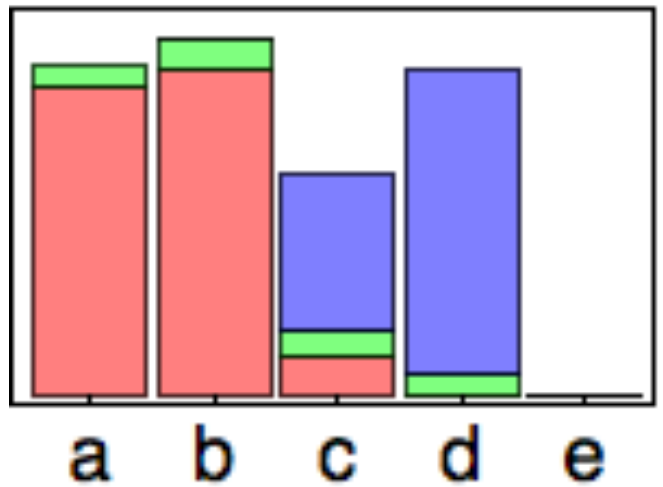
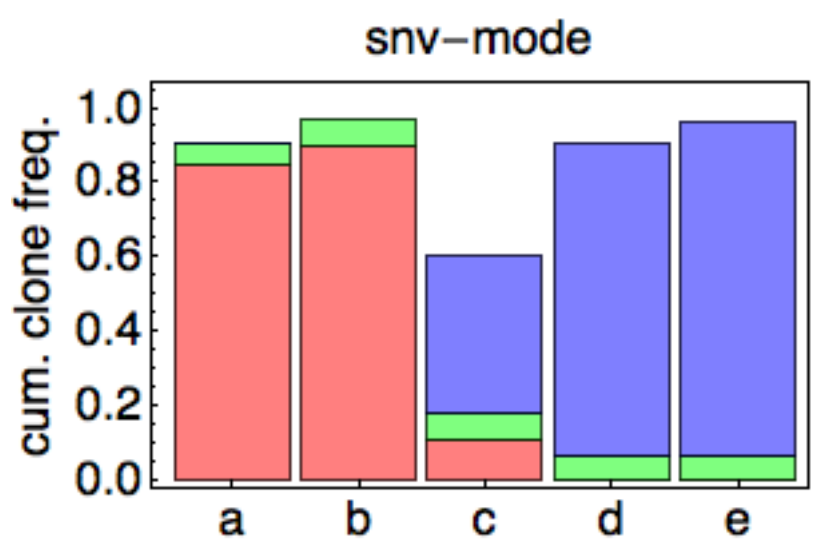


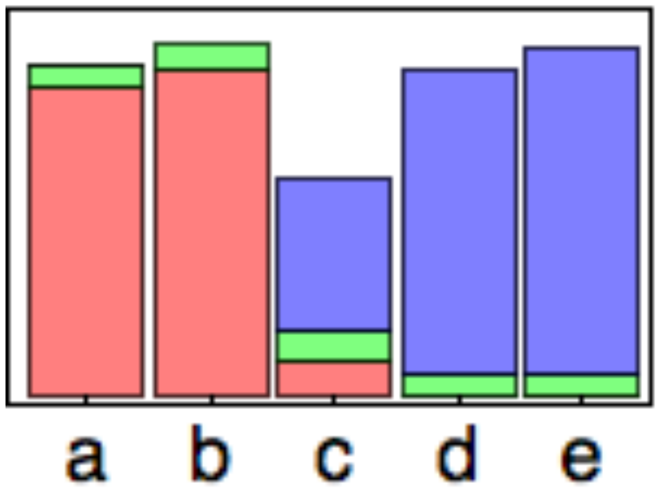


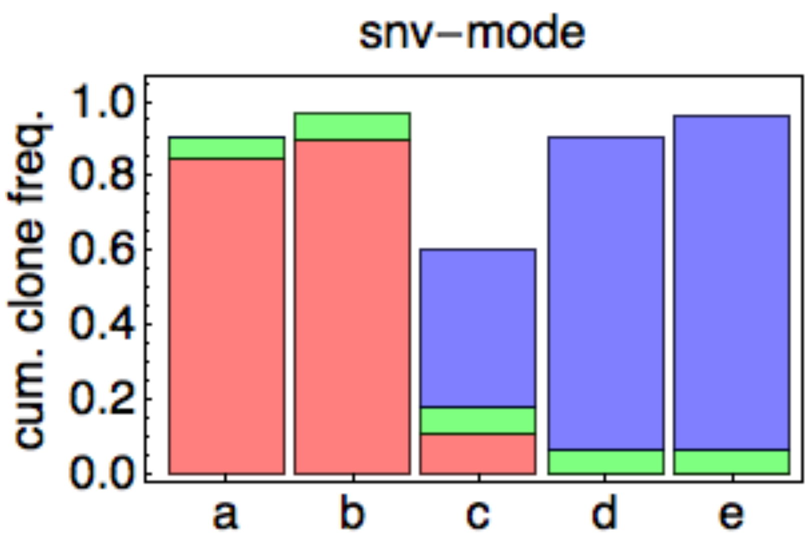
Inference using partial data



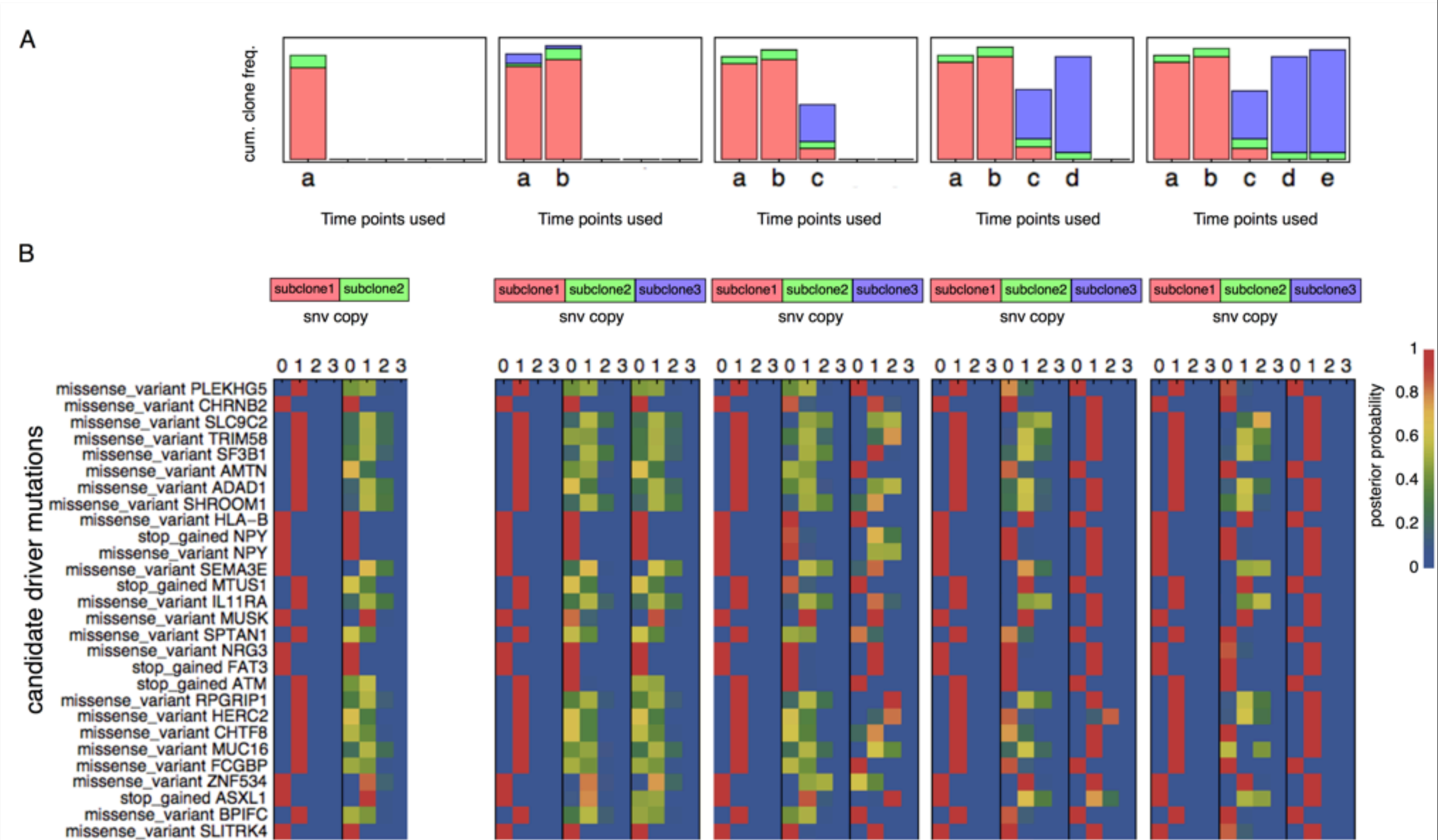


Inference using partial data





Inference using partial data



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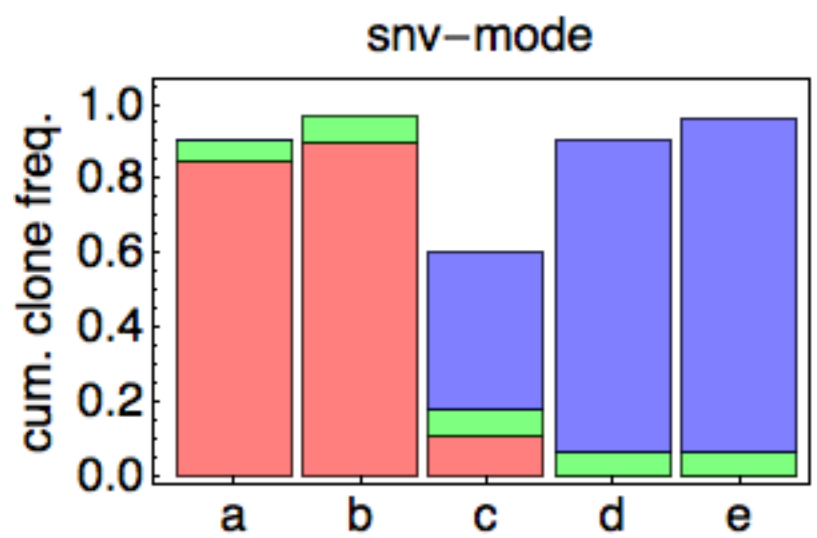
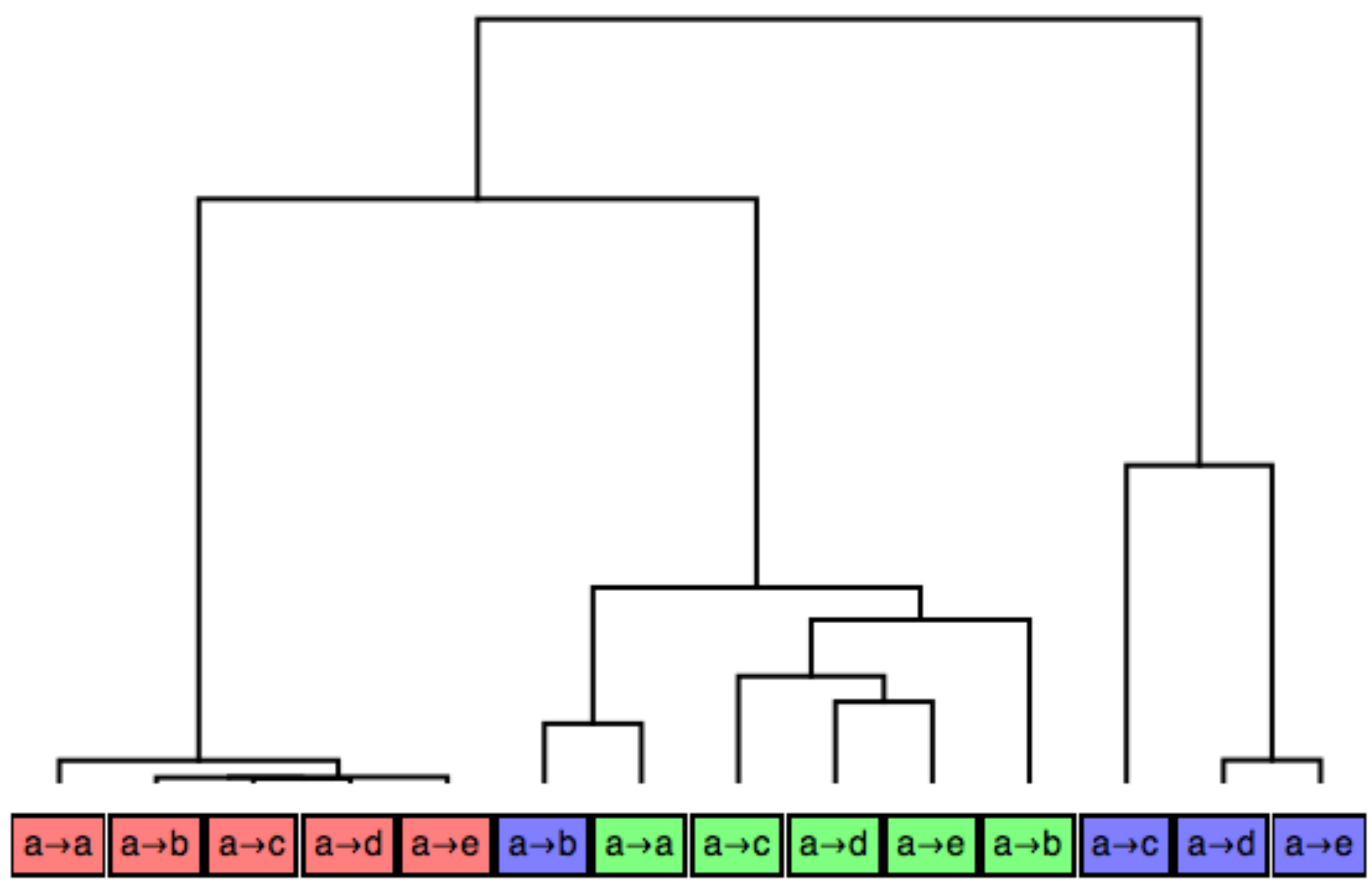
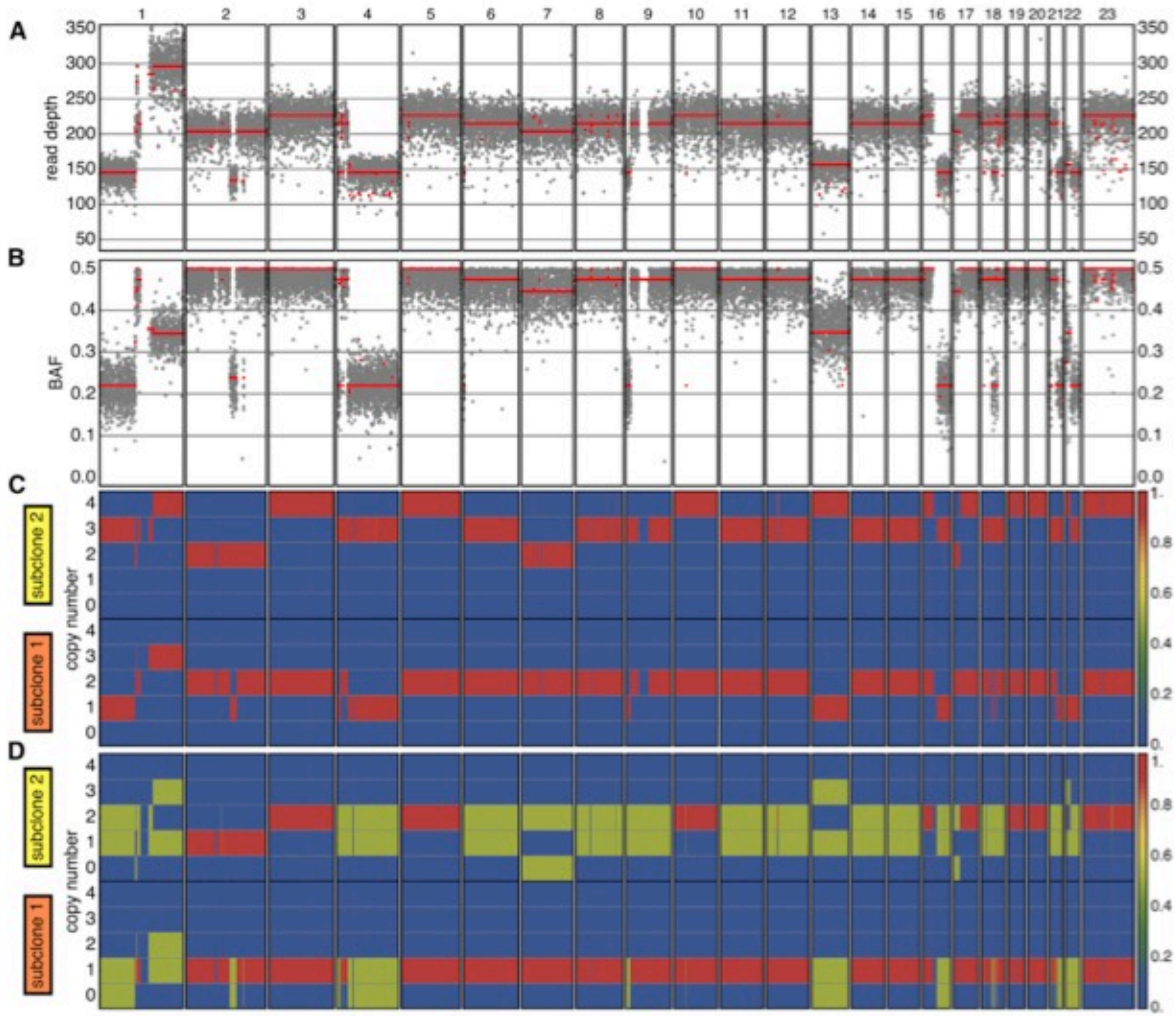




Other case studies

- Breast cancer PD4120a from Nik-Zainal *et al.* Cell 2012a,b



Conclusions Part II

- cloneHD algorithm reconstructs subclone fractions, their copynumber profiles and SNV genotypes using whole genome NGS data.
- It exploits correlations across space, time and along genomes.
- For reconstructing an evolutionary history numerous passenger mutations become an asset.
- Re-analysis of a CLL whole genome data recapitulated clonal evolution inferred from the targeted deep sequencing (exploiting the passengers).
- Once a large number of evolutions inferred we can start to think about dynamical models generating them.
- Mimicking a "real-time" monitoring scenario with CLL data looks promising.
- Subclone specific computational analysis is an interesting future direction.
- cloneHD facilitates monitoring that is needed to control evolving populations.