PhyDOSE: Design of Follow-up Single-cell Sequencing Experiments of Tumors

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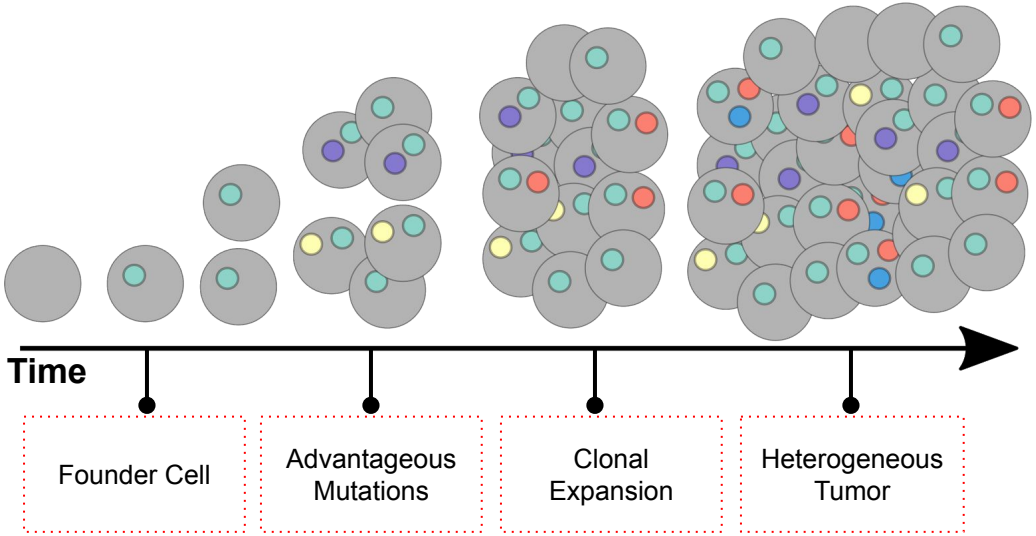
*These authors contributed equally to this work

Cancer is an evolutionary process



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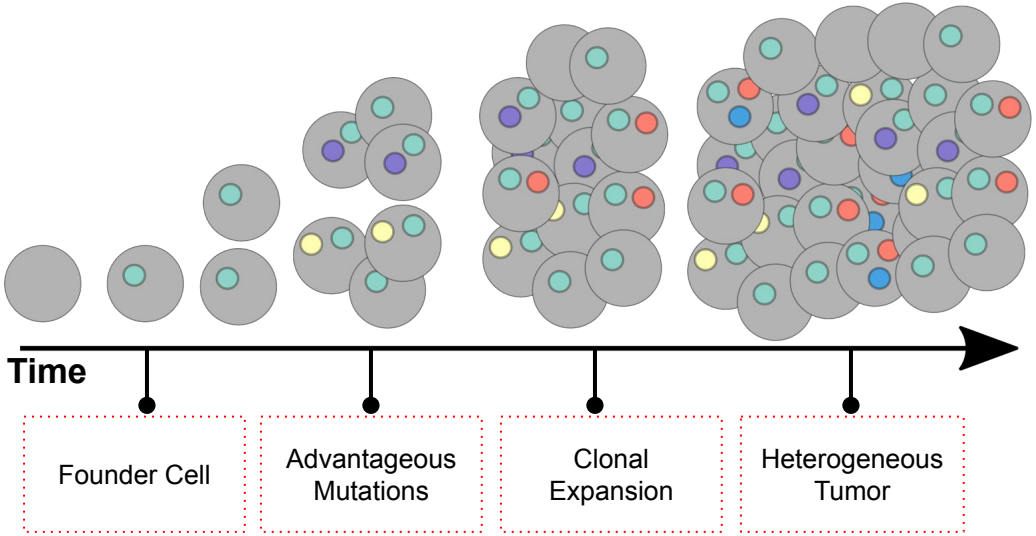
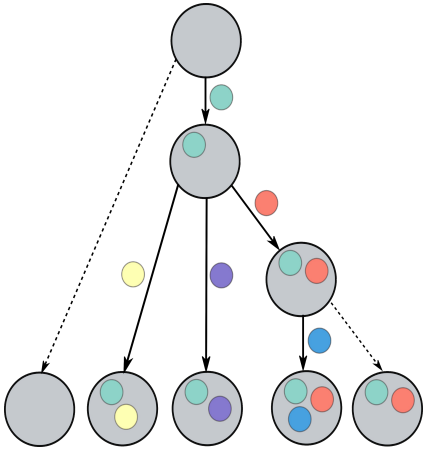




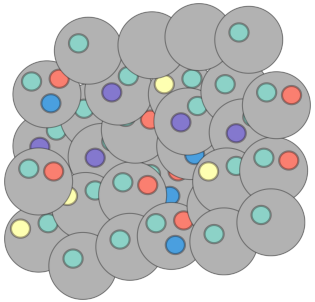
Phylogenetic Tree

Identify treatment targets	Understand metastatic development	Compare evolutionary patterns across patients

DNA sequencing of tumors

Bulk DNA Sequencing ($)

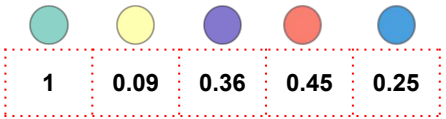
Single-cell DNA Sequencing ($$$)

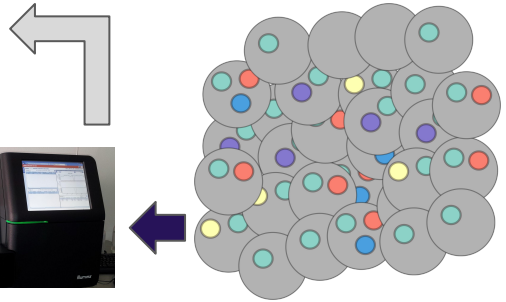


DNA sequencing of tumors

Bulk DNA Sequencing ($)

Cancer Cell Fractions



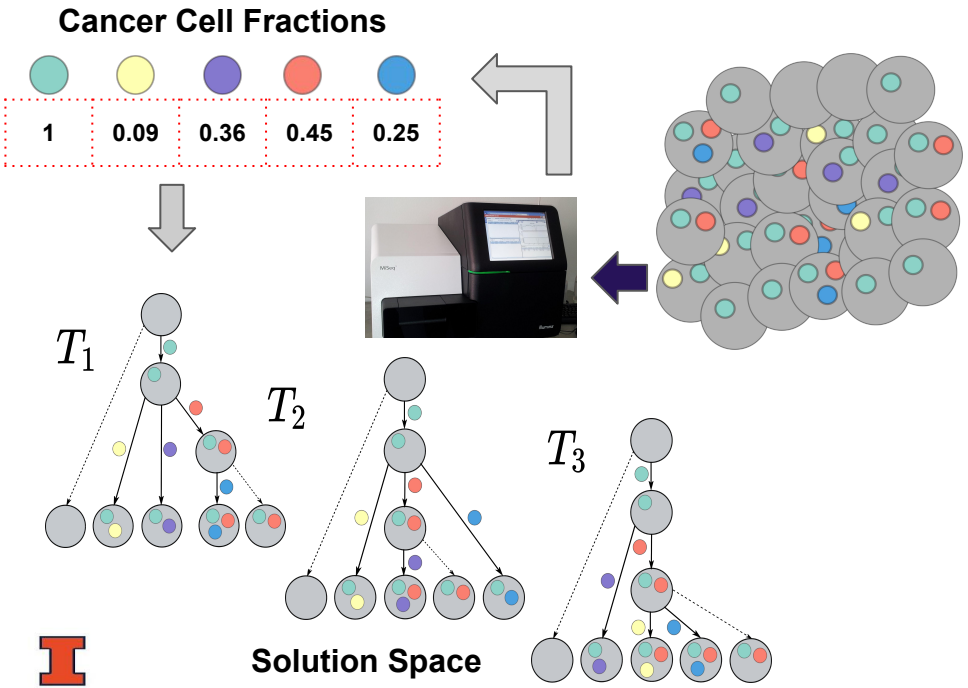


Single-cell DNA Sequencing ($$$)

DNA sequencing of tumors

Bulk DNA Sequencing ($)

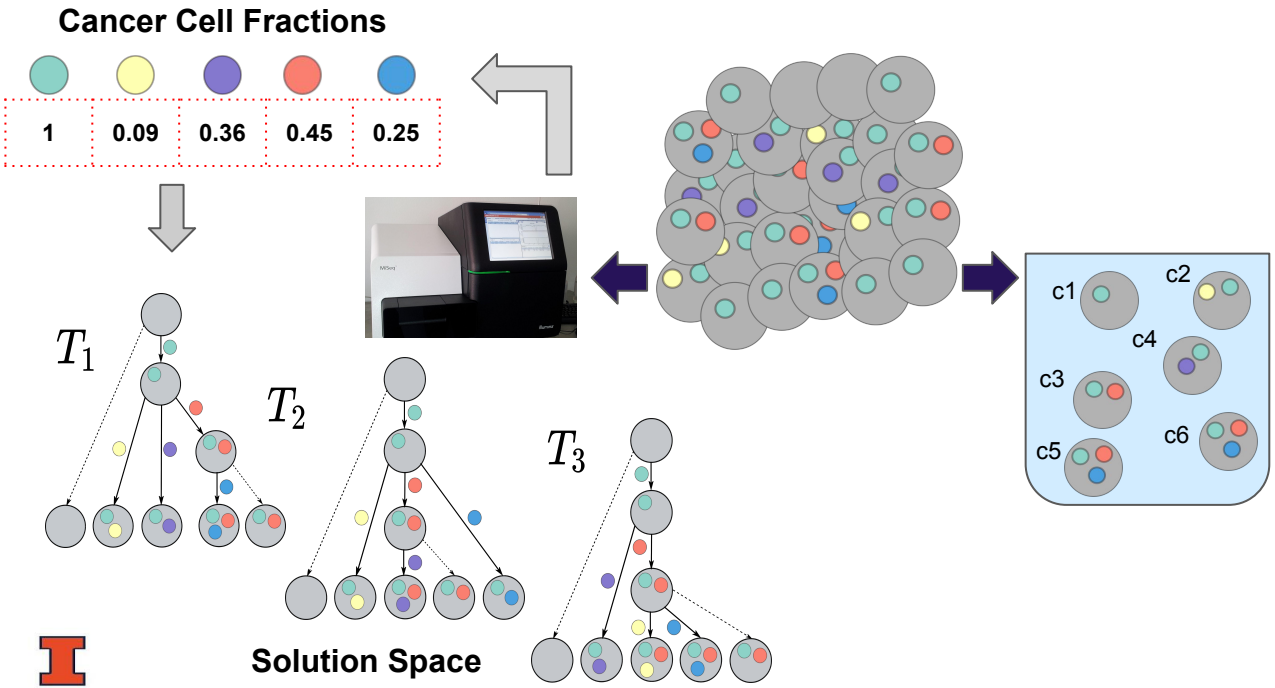
Single-cell DNA Sequencing ($$$)



DNA sequencing of tumors

Bulk DNA Sequencing ($)

Single-cell DNA Sequencing ($$$)



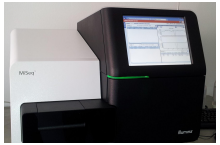
DNA sequencing of tumors

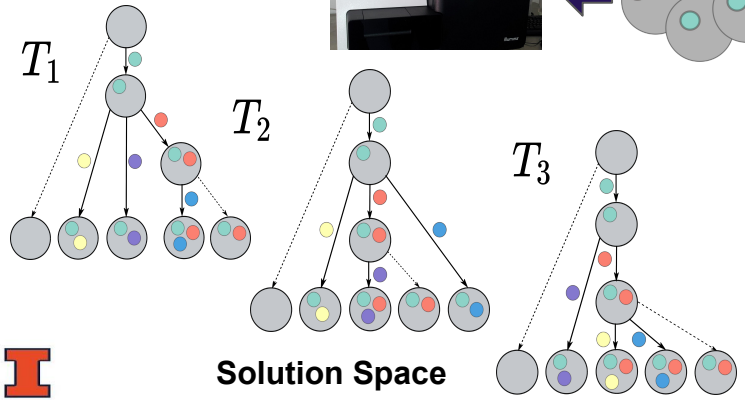
Bulk DNA Sequencing ($)

Single-cell DNA Sequencing ($$$)

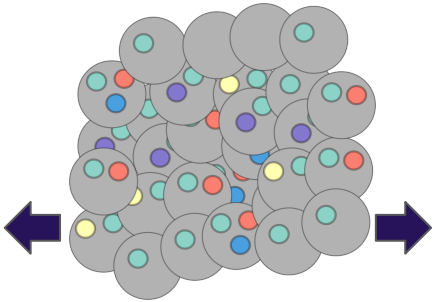
Cancer Cell Fractions

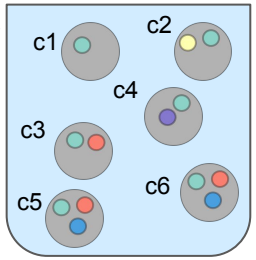
1	0.09	0.36	0.45	0.25





Solution Space





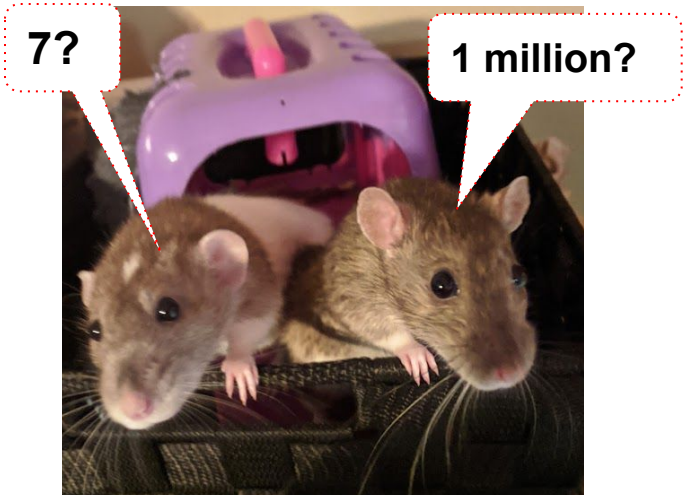
c1	1	0	0	0	0
c2	1	1	0	0	0
c3	0	0	0	1	0
c4	1	0	0	1	0
c5	1	?	0	1	1
c6	1	0	0	1	0

0	False Negative

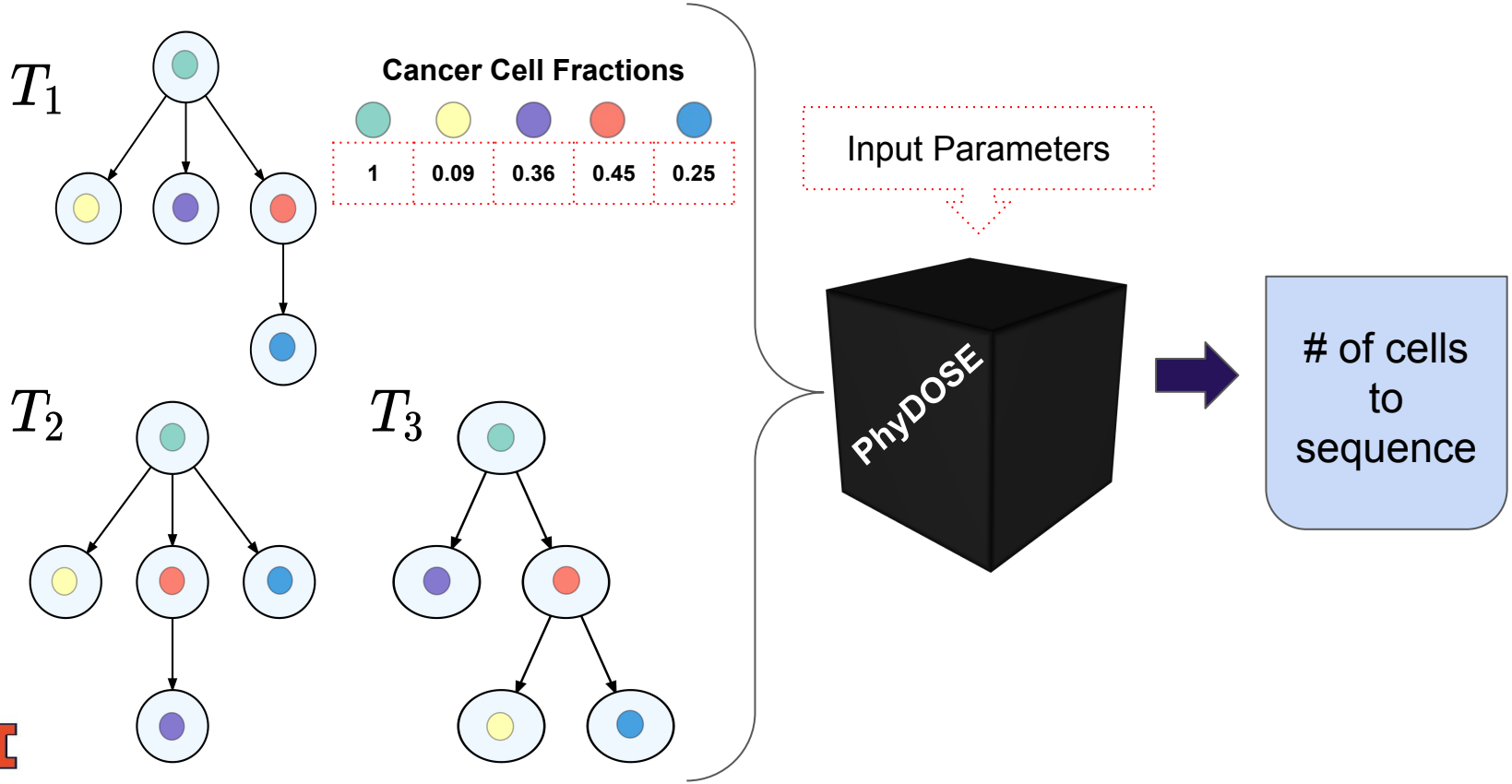
Phylogeny inference from DNA sequencing

Method	Bulk Sequencing Data	Single-cell Data
SCITE [Jahn et al., 2016]		X
OncoNEM [Ross & Markowetz, 2017]		X
SPhyR [El-Kebir, 2018]		X
SiCloneFit [Zafar et al., 2019]		X
PhiSCS [Malikic et al., 2019a]	X	X
B-SCITE [Malikic et al. 2019b]	X	X

How many single-cells should you sequence to minimize costs?

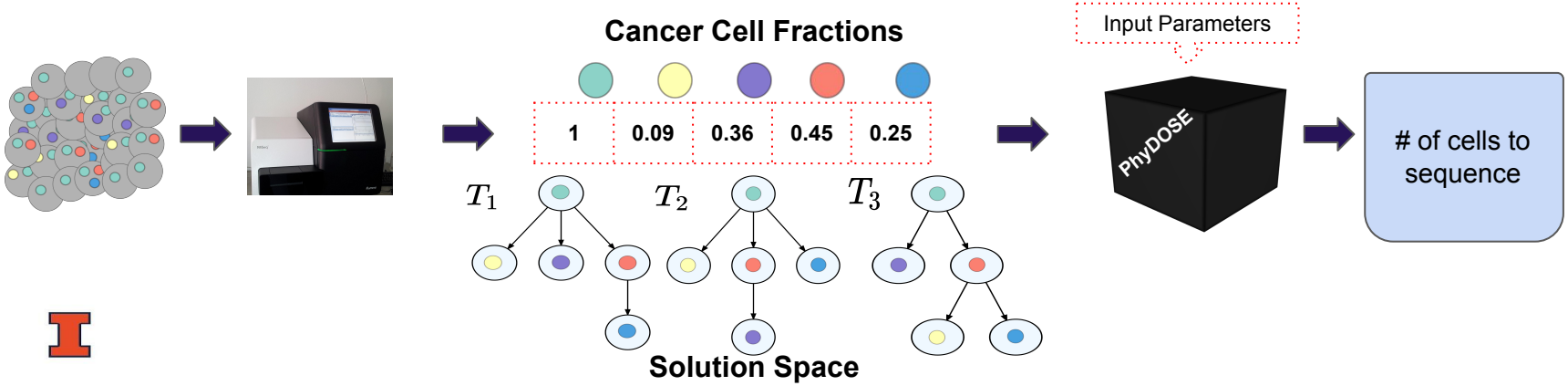


Key idea: Design a cost-effective single-cell sequencing experiment using bulk DNA data

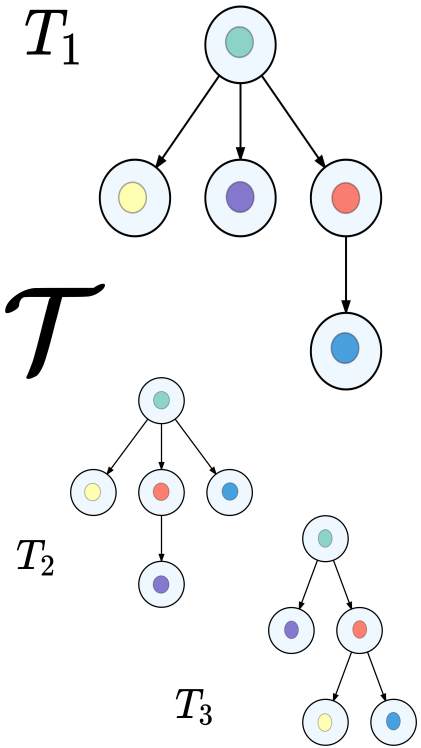


Outline

- Problem statement
- Methods
- Complexity
- Simulation study
- Application to real data
- Conclusions and future work



Key idea: Bulk data guides cost effective single-cell experiment design



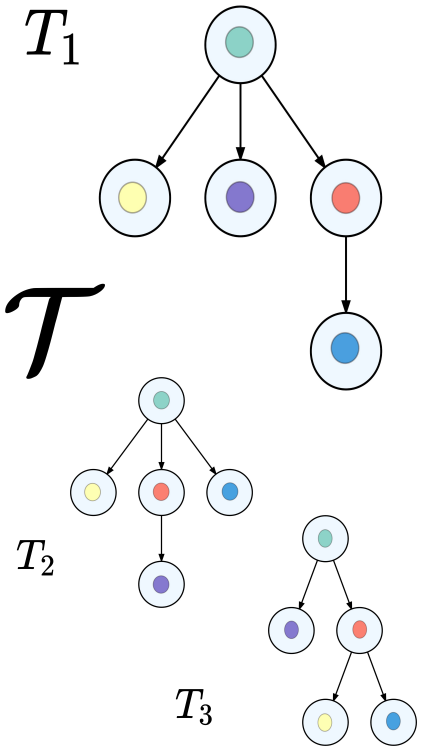
SINGLE-CELL SEQUENCING POWER CALCULATION (SCS-PC)

Given a set $\mathcal{T}$ of candidate phylogenies, frequencies **f**

Cancer Cell Fractions f

1	0.09	0.36	0.45	0.25

Key idea: Bulk data guides cost effective single-cell experiment design



SINGLE-CELL SEQUENCING POWER CALCULATION (SCS-PC)

Given a set $\mathcal{T}$ of candidate phylogenies, frequencies $\mathbf{f}$ and confidence level γ,

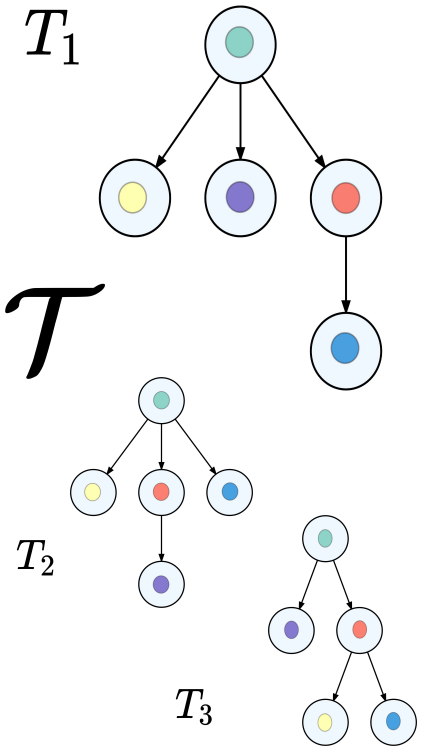
Cancer Cell Fractions $\mathbf{f}$

1	0.09	0.36	0.45	0.25

Confidence Level

$$\gamma = 0.95$$

Key idea: Bulk data guides cost effective single-cell experiment design



Single-cell Sequencing Power Calculation (SCS-PC)

Given a set $\mathcal{T}$ of candidate phylogenies, frequencies $\mathbf{f}$ and confidence level γ, find the minimum number k^* of single cells needed to determine the true phylogeny T among $\mathcal{T}$ with probability at least γ.

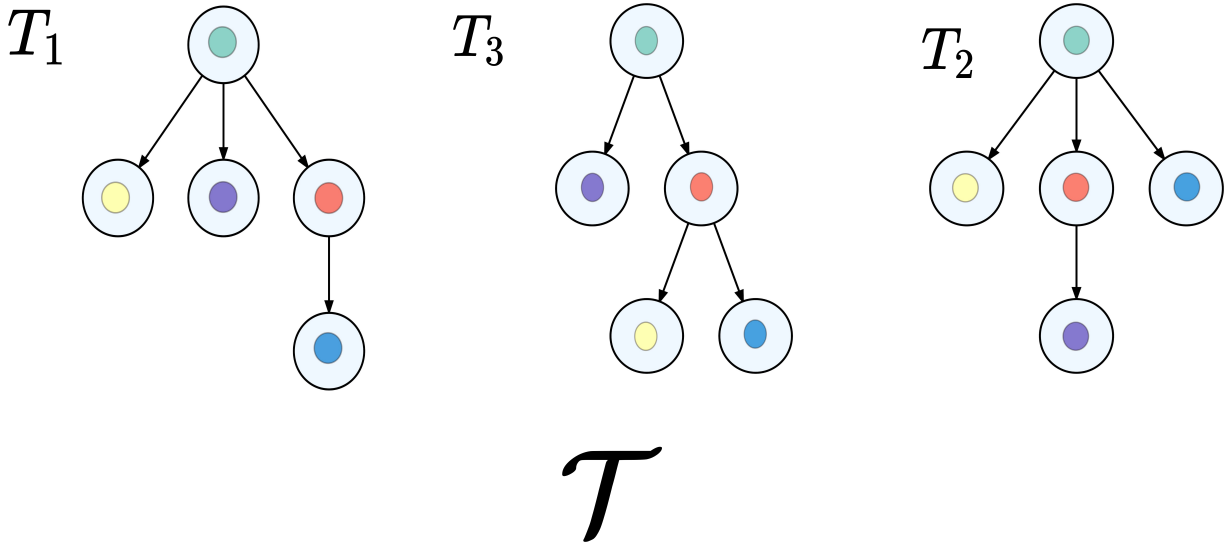
Cancer Cell Fractions $\mathbf{f}$

1	0.09	0.36	0.45	0.25

Confidence Level

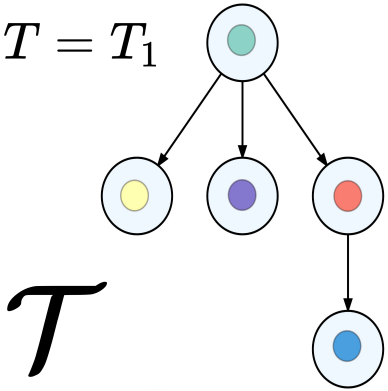
$\gamma = 0.95$ → k^*

Solving the SCS-PC



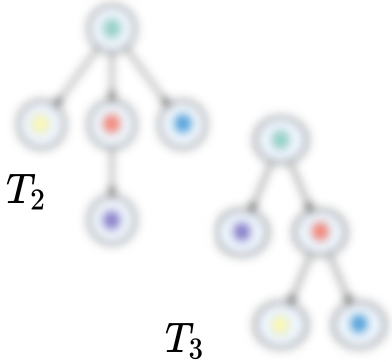
True phylogeny unknown

Key idea: condition on each tree being the true tree and solve SCS-PC



SCS Power Calculation for Phylogeny T (T-SCS-PC)

Given a set $\mathcal{T}$ of candidate phylogenies and a phylogeny $T \in \mathcal{T}$, frequencies $\mathbf{f}$ and confidence level γ,



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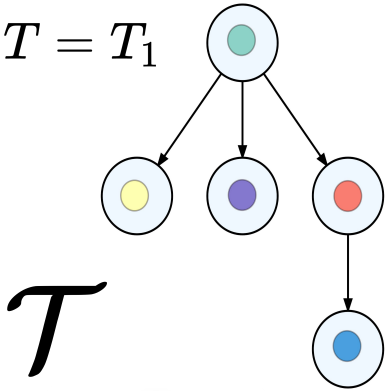
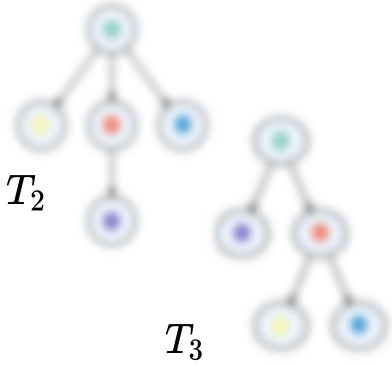




SCS Power Calculation for Phylogeny T (T-SCS-PC)

Given a set $\mathcal{T}$ of candidate phylogenies and a phylogeny $T \in \mathcal{T}$, frequencies $\mathbf{f}$ and confidence level γ, find the minimum number k^* of single cells needed such that the probability of a successful SCS experiment is greater than or equal to γ.

Cancer Cell Fractions $\mathbf{f}$

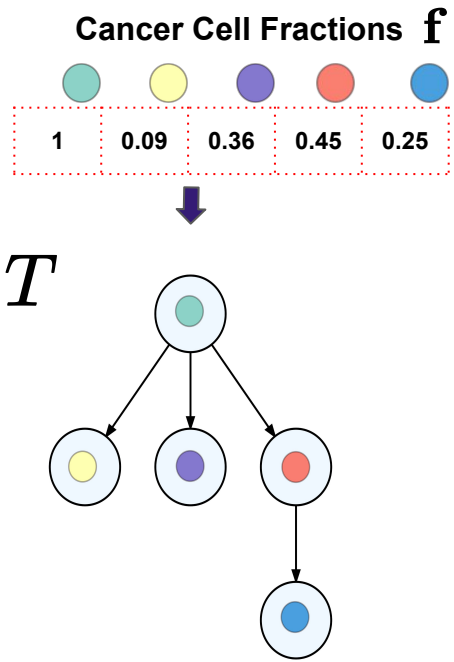
1	0.09	0.36	0.45	0.25

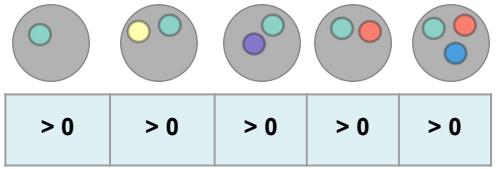
Confidence Level

$\gamma = 0.95$ → k^*

$$k^* = \arg\min_{k} P(Success \mid T, \mathcal{T}, k, \mathbf{f}) \geq \gamma$$

What is a successful experiment given T?





SCOPIT
[Davis et al. 2019]

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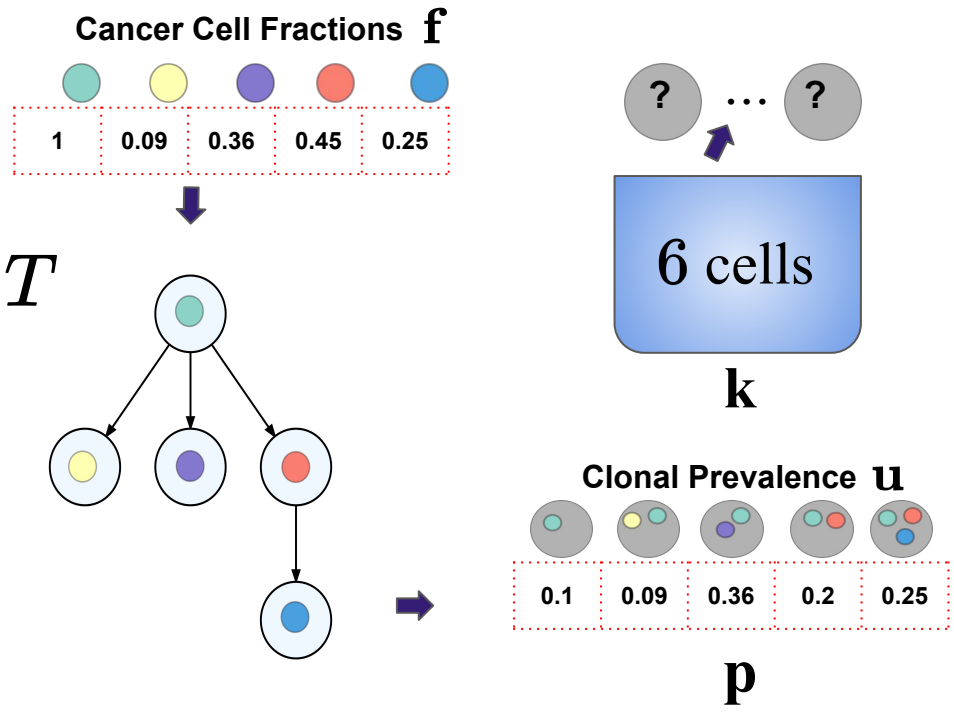

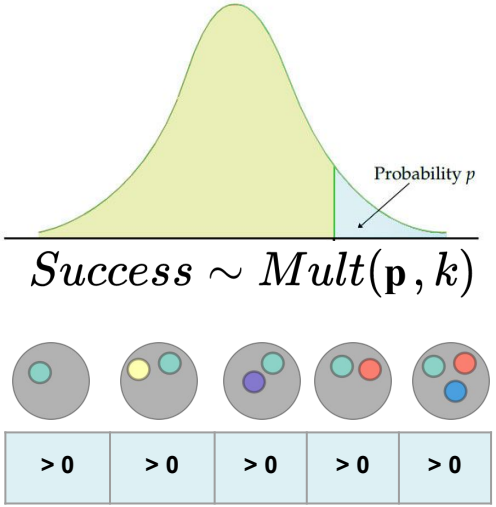

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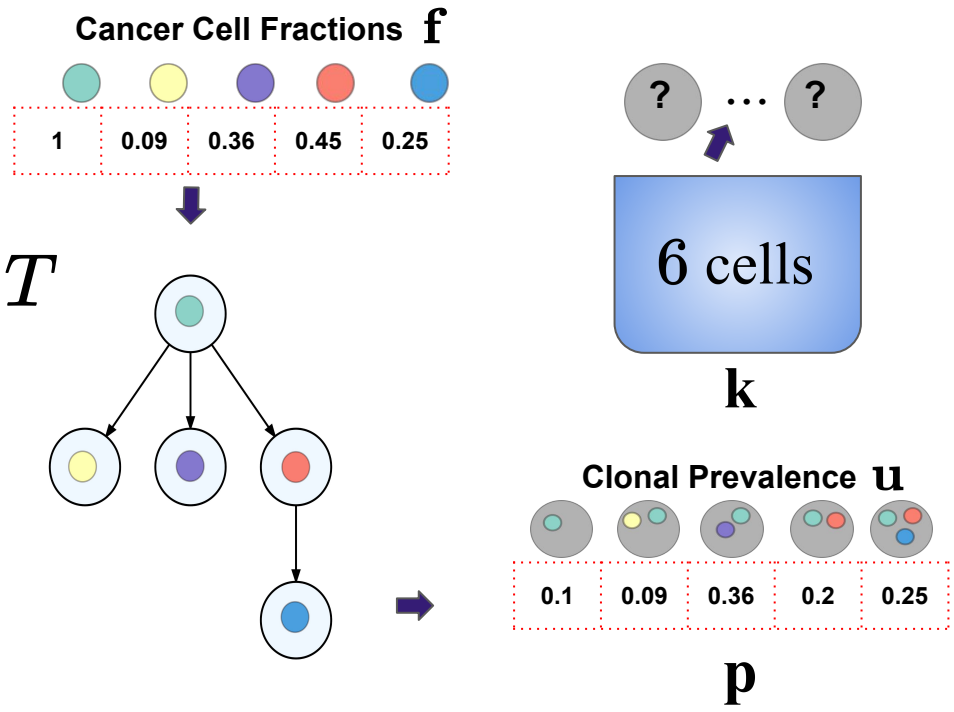
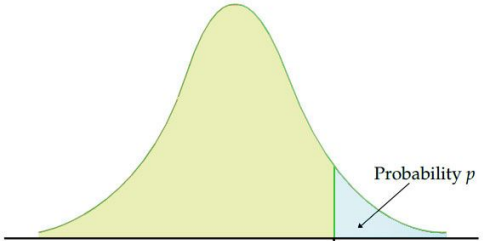
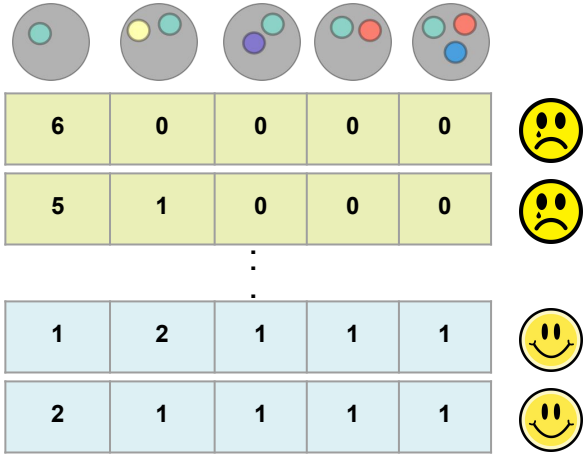


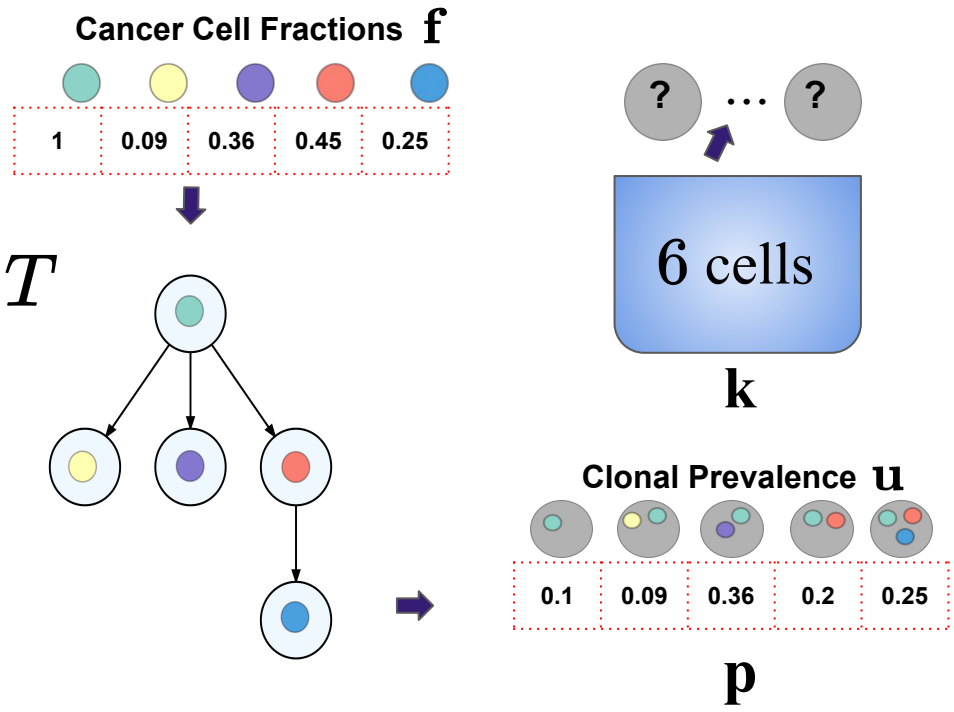


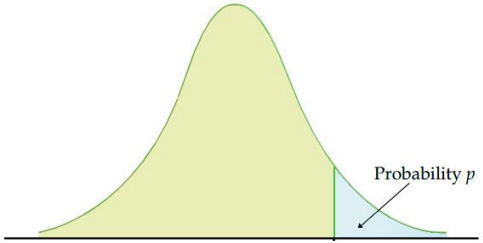
$$Success \sim Mult(\mathbf{p}, k)$$



SCOPIT
[Davis et al. 2019]

What is a successful experiment given T?





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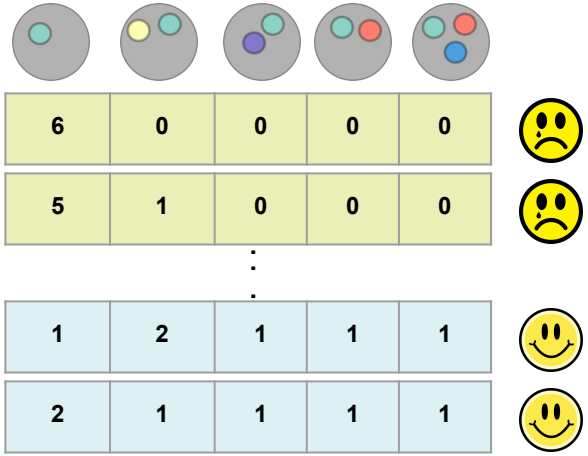
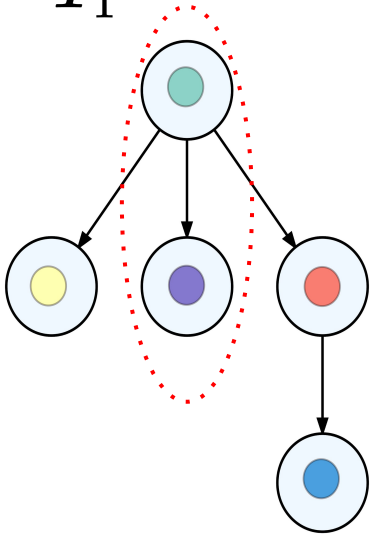


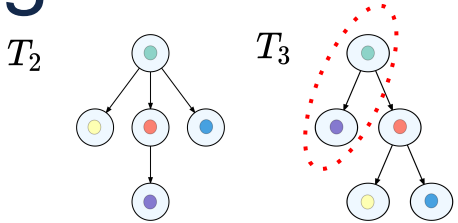
But we don't always need to observe all clones for a successful experiment!

SCOPIT
[Davis et al. 2019]

Key idea: distinguishing feature

$T = T_1$

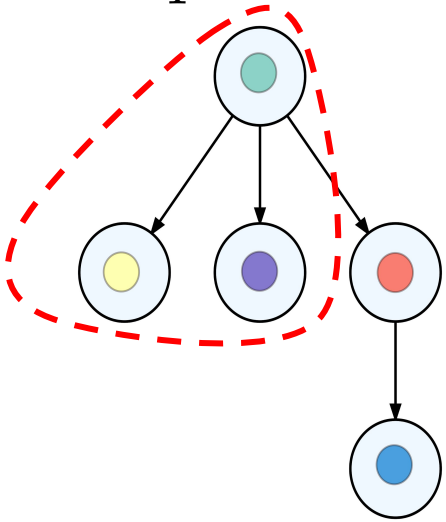




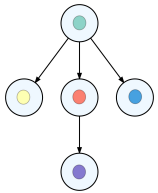
Featurette /clone in T	T_2	T_3
	+	+
	+	+
	-	+
	+	-
	-	+

Key idea: distinguishing feature

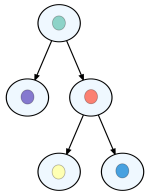
$T = T_1$



T_2



T_3



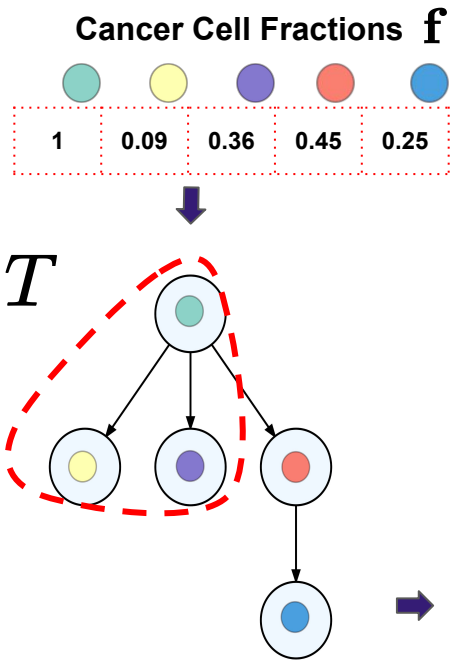
Featurette /clone in T

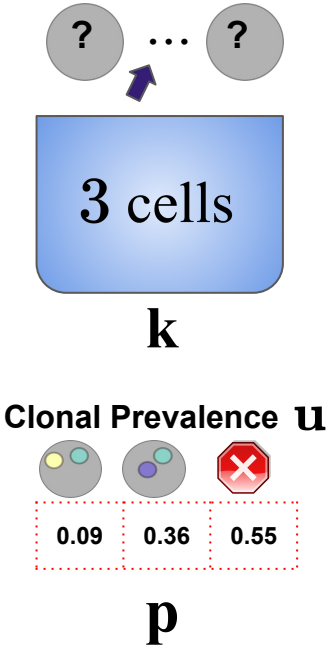
	+	+
	+	+
	-	+
	+	-
	-	+

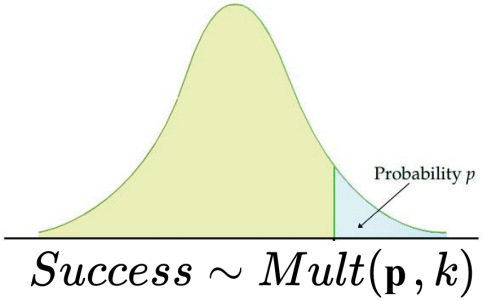
Distinguishing feature

Success is defined as observing a distinguishing feature.

Probabilistic model







0	0	3
0	1	2
⋮		
1	1	1
1	2	0
2	1	0

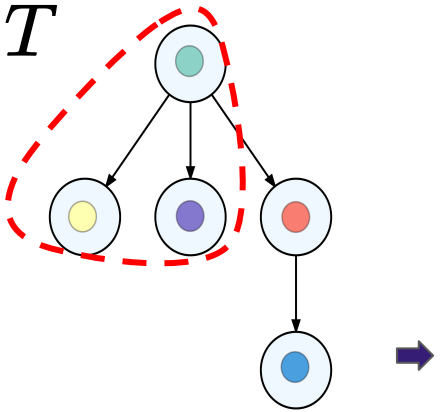
Success is defined as observing a distinguishing feature.

Probabilistic model

Cancer Cell Fractions $\mathbf{f}$

1	0.09	0.36	0.45	0.25

T



? … ?

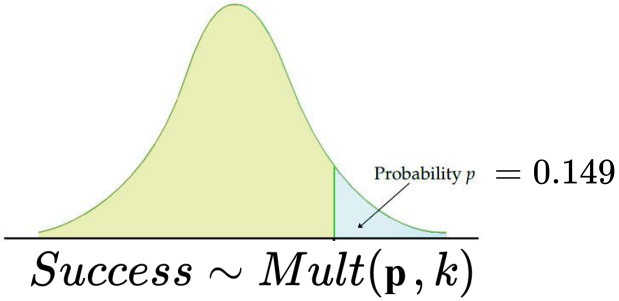
3 cells

$\mathbf{k}$

Clonal Prevalence $\mathbf{u}$

0.09	0.36	0.55

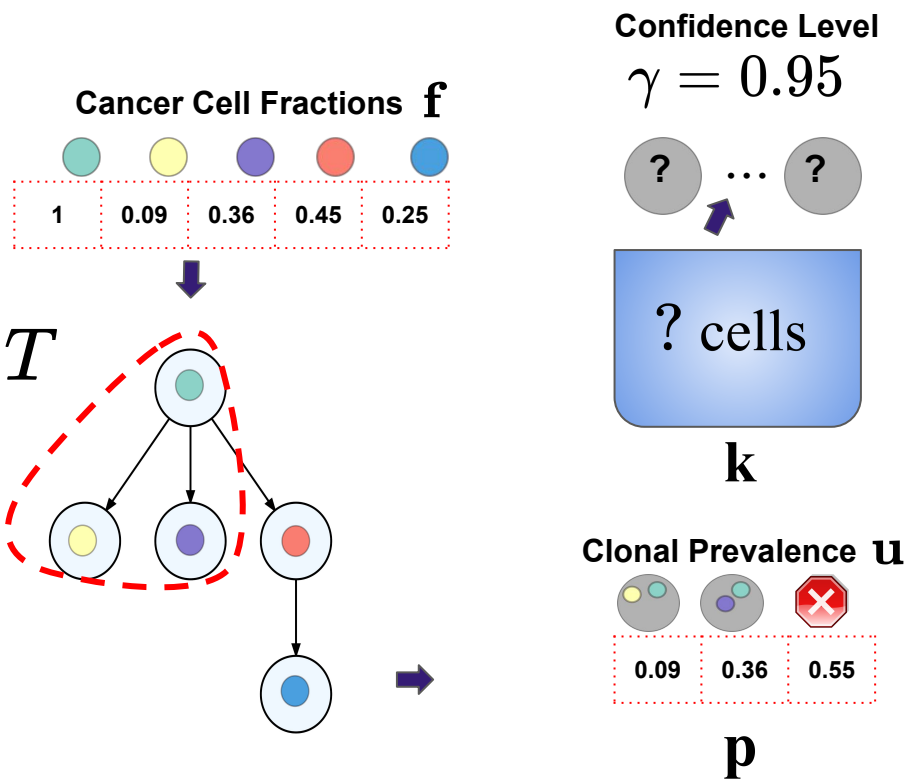
$\mathbf{p}$



			prob.
0	0	3	0.0
0	1	2	0.0
⋮			
1	1	1	0.11
1	2	0	0.03
2	1	0	0.009

Success is defined as observing a distinguishing feature.

Power calculation for fixed tree T

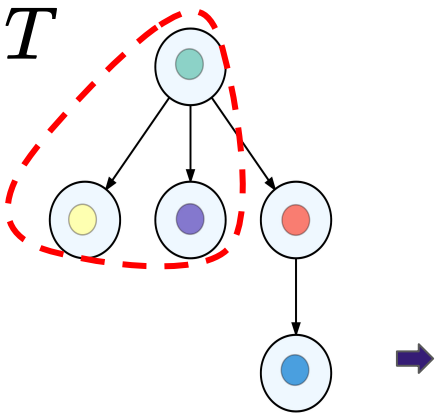


$$k^* = \arg\min_k P(Success \mid T, \mathcal{T}, k, \mathbf{f}) \geq \gamma$$

Power calculation for fixed tree T

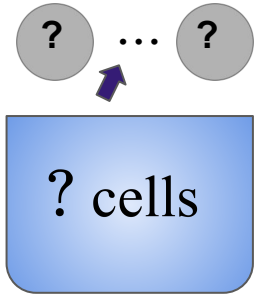
Cancer Cell Fractions $\mathbf{f}$

1	0.09	0.36	0.45	0.25



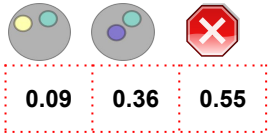
Confidence Level

$$\gamma = 0.95$$

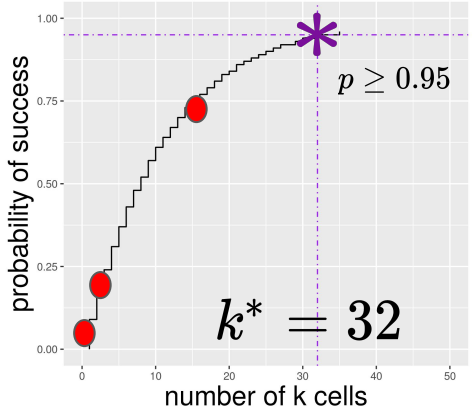


$\mathbf{k}$

Clonal Prevalence $\mathbf{u}$



$\mathbf{p}$



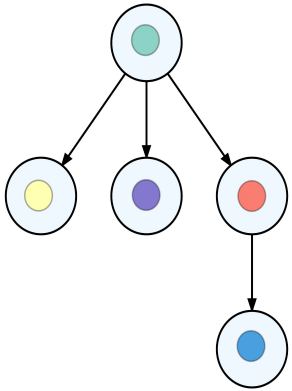
$$k^* = \arg\min_k P(Success \mid T, \mathcal{T}, k, \mathbf{f}) \geq \gamma$$

k	prob.
3	0.15
4	0.25
⋮	
15	0.75
⋮	
32	0.95

k*= 32 is the solution to the T-SCS-PC problem.

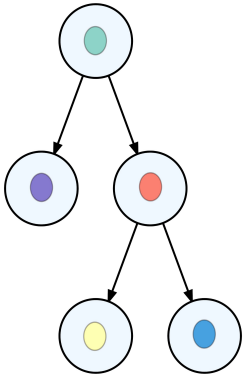
Solving the SCS-PC

T_1



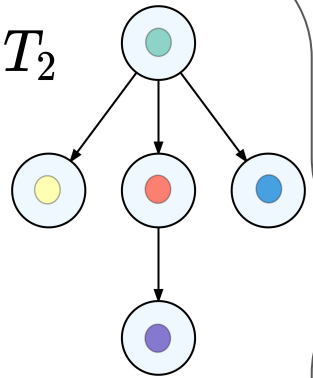
$k^* = 32$

T_3



$k^* = 32$

T_2



$k^* = 4$

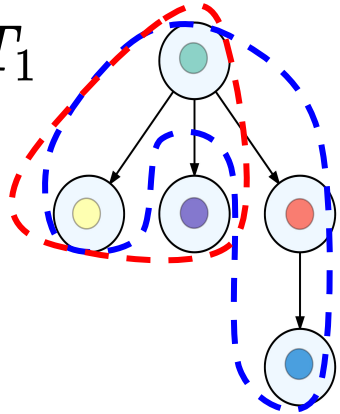
Taking the maximum yields and upper bound

$k^* = 32$

k*= 32 is the solution to the SCS-PC problem.

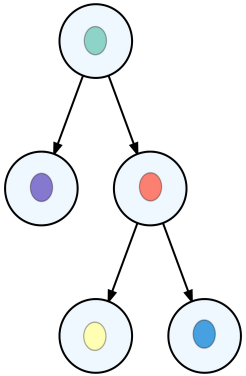
Solving the SCS-PC

T_1



$k^* = 32$

T_3



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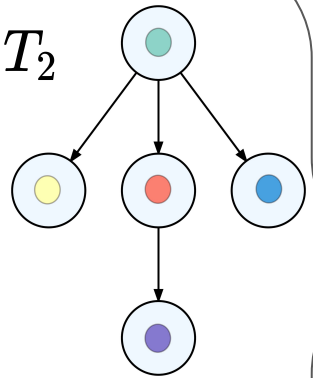
T_2



$k^* = 4$

Taking the maximum yields and upper bound

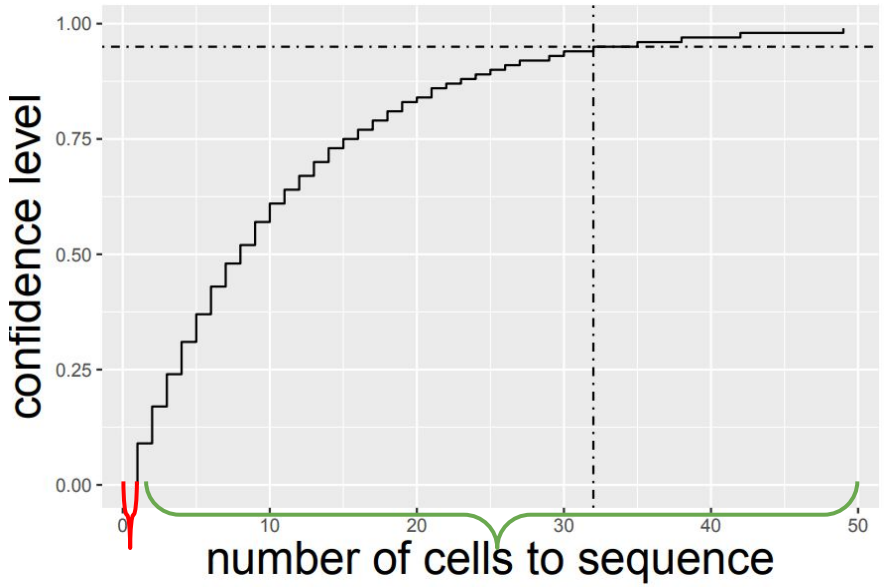
$k^* = 32$

Account for multiple distinguishing features

Adjust for false negatives

k*= 32 is the solution to the SCS-PC problem.

T-SCS-PC is NP-hard by reduction from Set Cover



Probability of success is 0

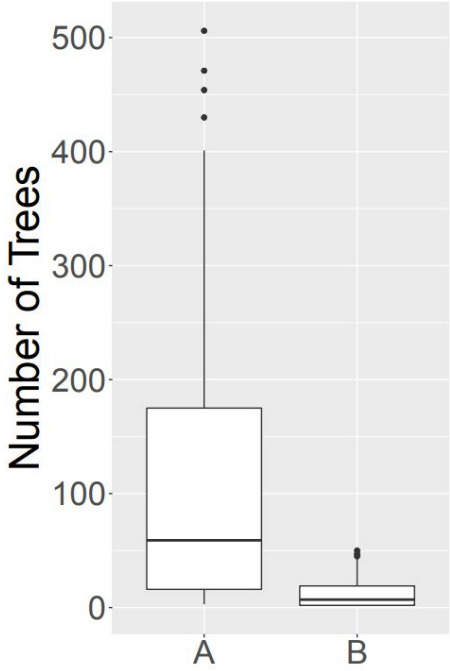
Probability of success is > 0

Set Cover ⇔ T-SCS-PC

Lemma: Let $(\mathcal{T}, T_0, \mathbf{f}, \gamma = \epsilon)$ be the *T*-SCS-PC instance corresponding to Set Cover instance $(U, \mathcal{F})$. A minimum cover has size k^* if and only if k^* is the smallest integer such that

$\cdot \Pr(Y_{k^*} \mid u(T_0, \mathbf{f})) \geq \gamma$

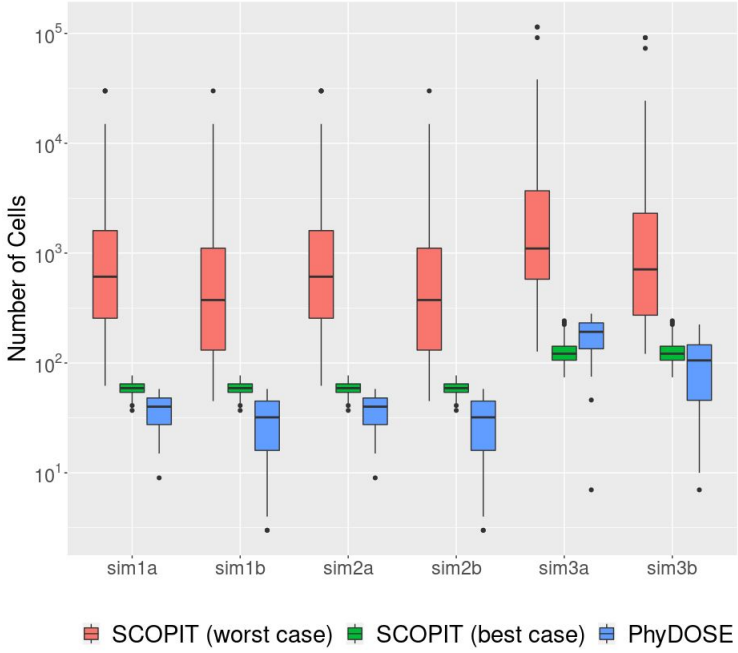
Simulation design



ID	% of Trees	Clones	Mutations	Prevalence Noise	FNR β	Doublet δ
sim1a	100%	7	7	0%	0	0
sim1b	10%	7	7	0%	0	0
sim2a	100%	7	7	5%	0	0
sim2b	10%	7	7	5%	0	0
sim2c	100%	7	7	20%	0	0
sim3a	100%	7	7	5%	0.2	0.1
sim3b	10%	7	7	5%	0.2	0.1
sim4a	100%	10	100	5%	0.2	0.1

- 100 replications
- SCOPIT comparison
- SPhyR phylogeny inference
- $\gamma = 0.95$

SCOPIT comparison



ID	% of Trees	Clones	Mutations	Prevalence Noise	FNR β	Doublet δ
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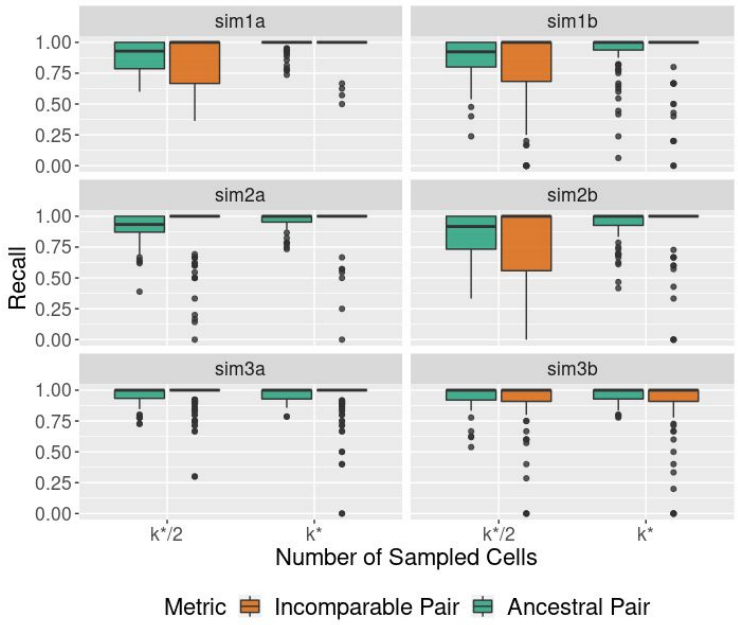
sim2b	10%	7	7	5%	0	0
sim2c	100%	7	7	20%	0	0
sim3a	100%	7	7	5%	0.2	0.1
sim3b	10%	7	7	5%	0.2	0.1
sim4a	100%	10	100	5%	0.2	0.1

- 100 replications
- SCOPIT comparison
- SPhyR phylogeny inference
- $\gamma = 0.95$

Phylogeny inference with SPhyR



ID	% of Trees	Clones	Mutations	Prevalence Noise	FNR β	Doublet δ
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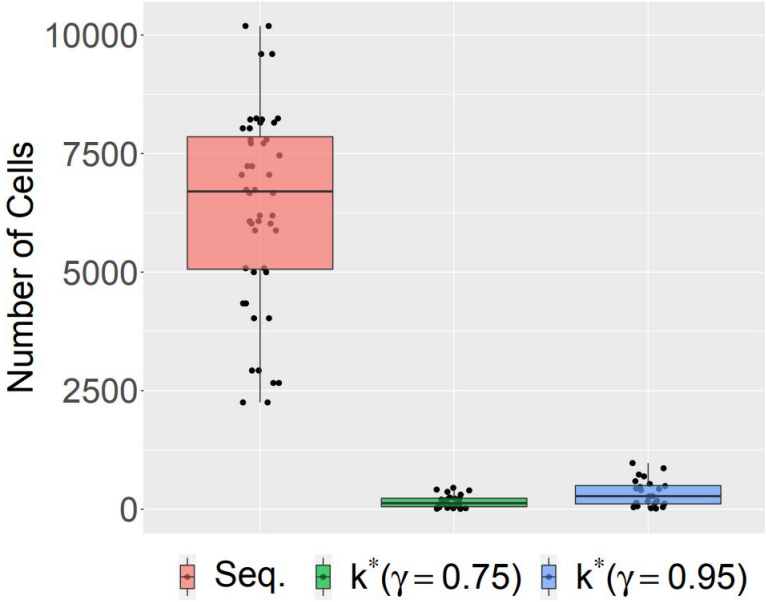
sim1b	10%	7	7	0%	0	0
sim2a	100%	7	7	5%	0	0
sim2b	10%	7	7	5%	0	0
sim2c	100%	7	7	20%	0	0
sim3a	100%	7	7	5%	0.2	0.1
sim3b	10%	7	7	5%	0.2	0.1
sim4a	100%	10	100	5%	0.2	0.1

- 100 replications
- SCOPIT comparison
- SPhyR phylogeny inference
- $\gamma = 0.95$

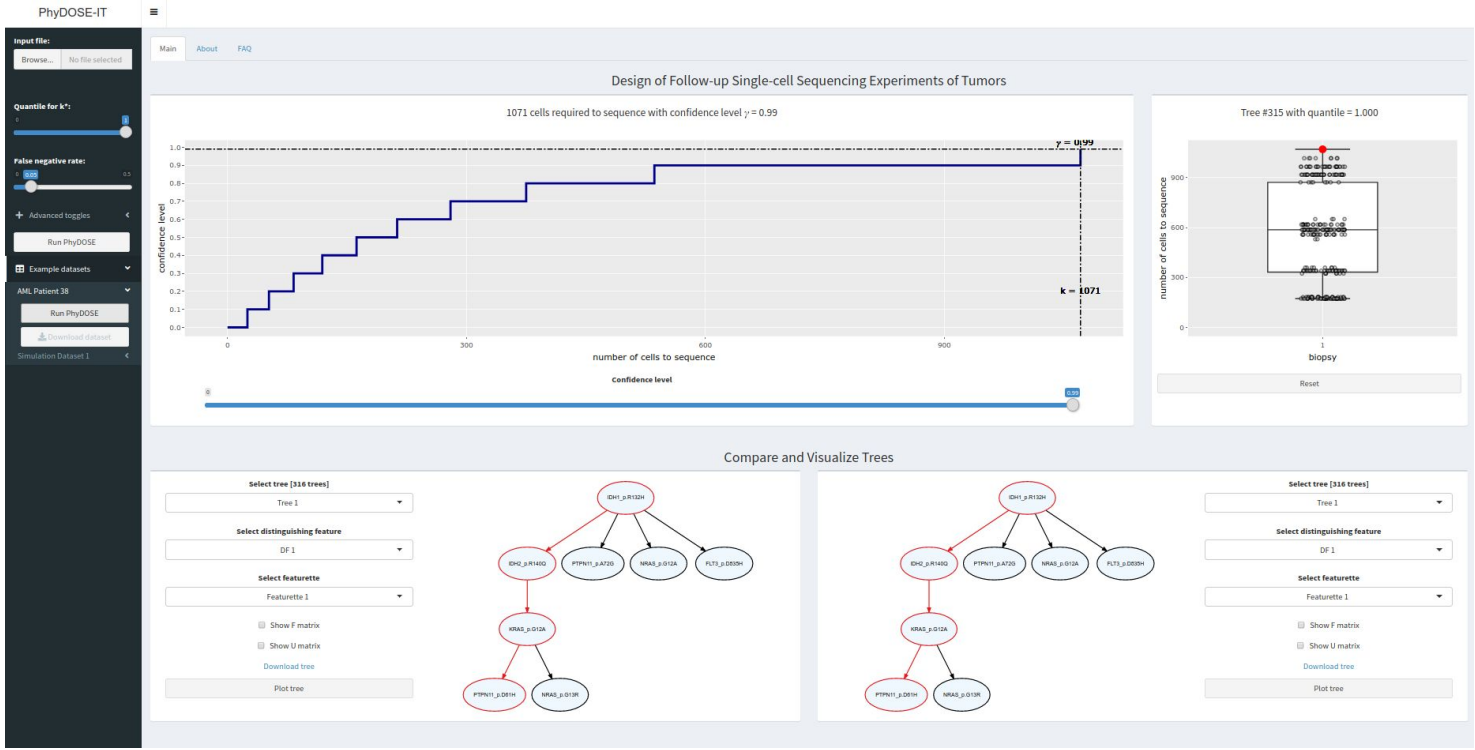
Acute myeloid leukemia (AML) cohort



PhyDOSE k compared with the original number of cells sequenced*

- Morita et al. (2020) performed high throughput targeted microfluidic single cell DNA sequencing on a cohort of 77 patients with AML.
- Based on the published variant allele frequencies, we enumerated between 2 and 316 candidate trees for 24 patients and used PhyDOSE to estimate k*.

PhyDOSE-IT and phydoser R package



https://phydose.shinyapps.io/PhyDOSE-IT/ https://github.com/elkebir-group/phydoser

Conclusions and future work

PhyDOSE Conclusions

- Proposes cost-efficient single-cell experiment design to yield high-fidelity phylogenies
- Agnostic to the type of single-cell sequencing technology used
- Available as both a web-application and an R package

Future Work

- Optimally determine the number of cells to sequence across multiple biopsies
- Explore evolutionary models beyond the infinite sites model
- Formulate and solve the RE-SCS-PC problem
  - Find out next time what it means to me...

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- Jackie Oh
- Chuanyi Zhang

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