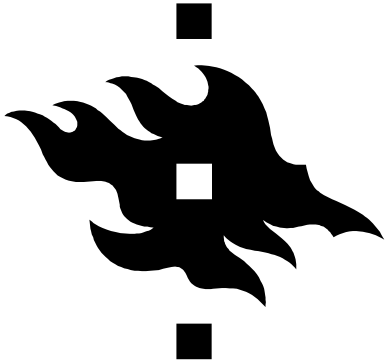

Latent stochastic models for comparing tumor samples of unknown purity

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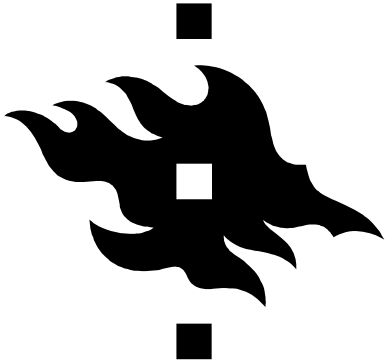
Introduction

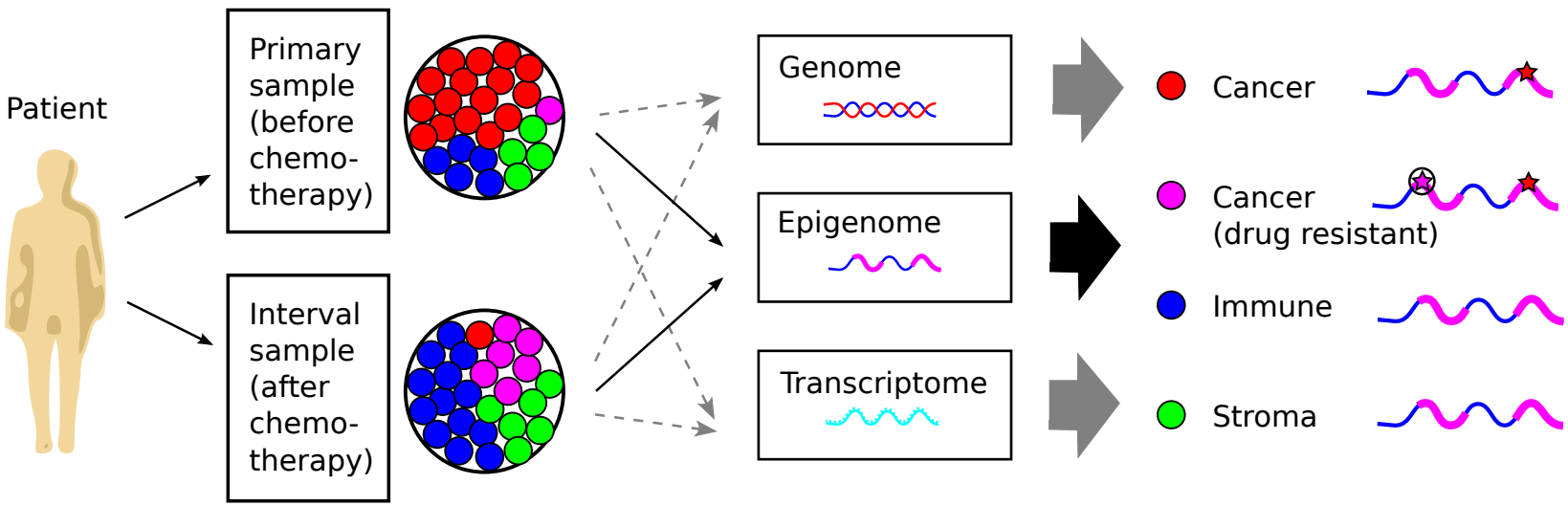
- We work on understanding the development of chemoresistance in ovarian cancer using a systems biology approach
  - $>$ 40,000 OVCA-caused deaths per year in Europe alone [1]
  - $>$ 50% of patients die within 5 years from diagnosis [1, 2]
  - Alterations driving it are heterogeneous [2]
- As part of their treatment, tumor samples are collected from the OVCA patients at multiple stages of the disease progression
  - Primary sample (at diagnosis)
  - Interval samples (during treatment)
  - Typically the patient response is good, but the cancer recurs
  - Which aberrations drive the development of chemoresistant cancer cell subpopulations?

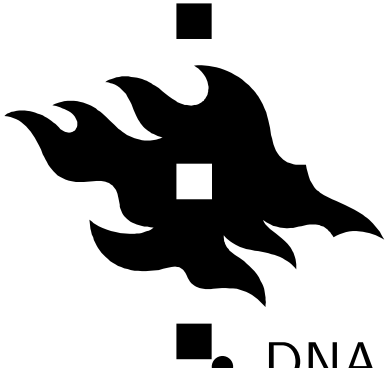


Introduction

- Clinical tumor samples contain various cell types in addition to the cancer cells: immune cells, stroma (blood cells, nerves), etc.
- Samples vary greatly in purity, some as low as 30% [3]; public resources like TCGA discard low purity samples ($< 60\%$) [4]
- Dilutes comparison at best–i.e. differences represent those between normal cells and not between the cancer cells
- Injects systematic bias at worst
- Problematic when comparing primary vs. interval as the treatment affects the tumor composition
- The mixed signal confounds subsequent analysis of active biological processes in cancer cells (DNA repair, apoptosis, etc.)

Introduction

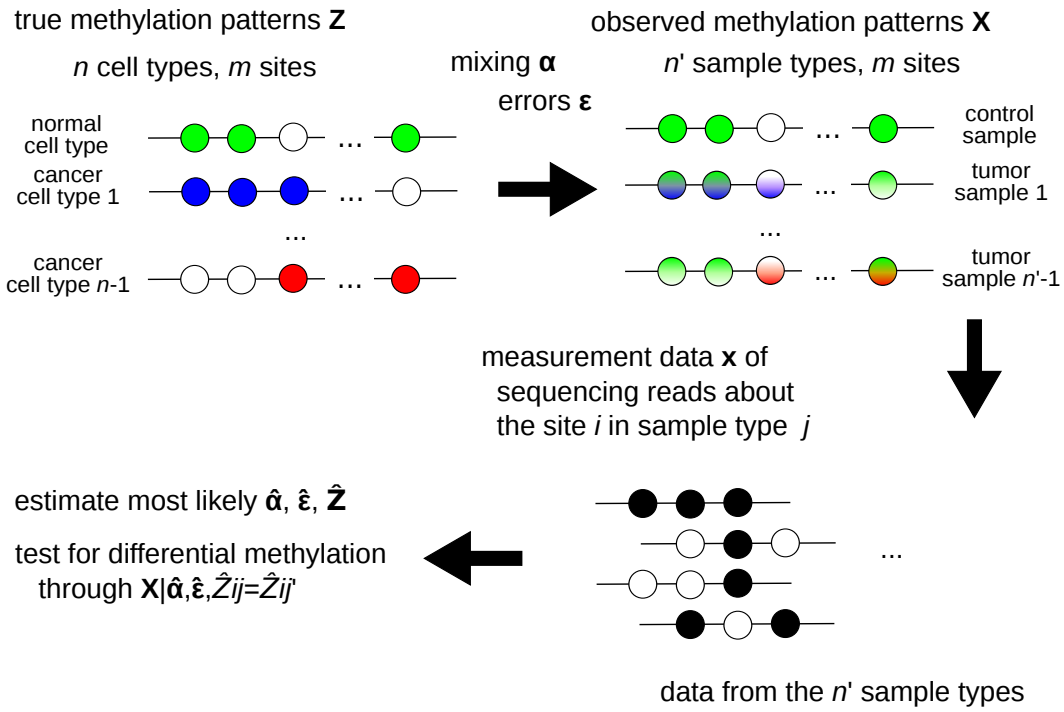




Methylation: background

- DNA methylation is an epigenetic modification [5]
  - Aberrations observed in most cancer types
  - Modulates expression at regulatory areas e.g. silencing of tumor suppressor genes
  - General lack of methylation causes genomic instability
- Standard methods assume pure samples, only few recent ones control but require normal cell profiles
  - Most techniques use linear regression [6, 7]
    - Suitable only for large number of replicates
    - Purity must be obtained by other means (e.g. IHC)
  - Can we do this without control and purity estimates?
    - Reliable information cannot be obtained from all patients

Methylation: models



$$\mathbb{P}\left[X_{i,1} | Z_{i,0}, Z_{i,1}, \epsilon_1, \alpha_1 \right]$$

	$(Z_{i,0}, Z_{i,1}) = (0,0)$
$X_{i,1} = 0$	$1 - \epsilon_1$
$X_{i,1} = 1$	ϵ_1

	$(Z_{i,0}, Z_{i,1}) = (1,0)$
$X_{i,1} = 0$	$(1-\alpha_1)\,\epsilon_1 + \alpha_1\,(1-\epsilon_1)$
$X_{i,1} = 1$	$(1-\alpha_1)\,(1-\epsilon_1) + \alpha_1\,\epsilon_1$

$$\ldots$$

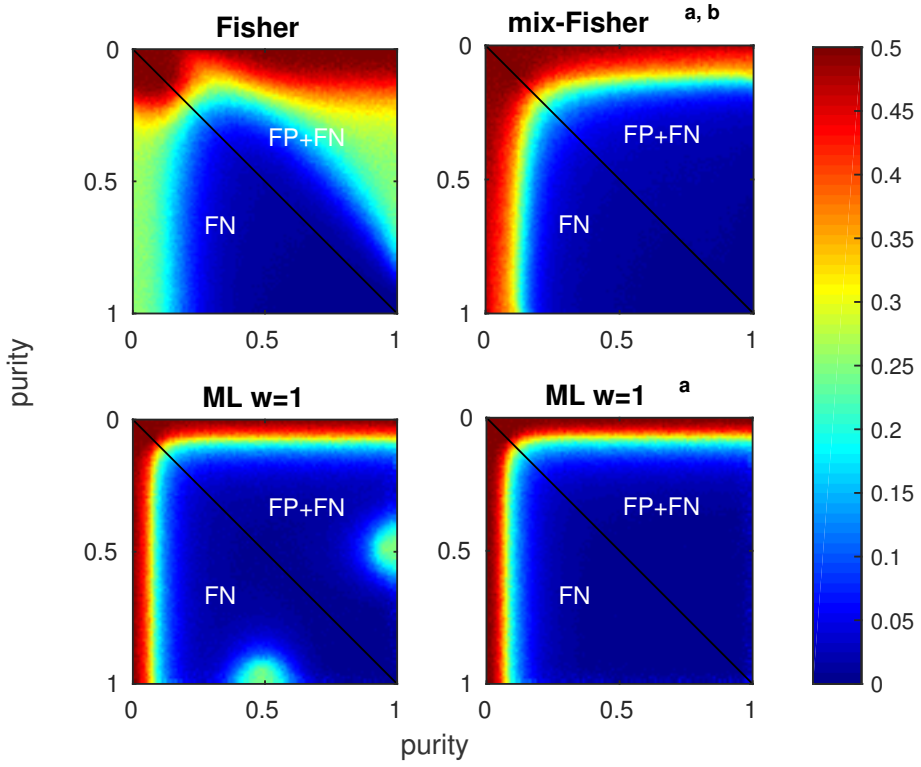
EM-algorithm:

$$\mathbb{P}[\mathbf{Z}|\mathbf{X},\,\theta] = \frac{\mathbb{P}[\mathbf{X},\,\mathbf{Z}|\,\theta]}{\mathbb{P}[\mathbf{X}|\,\theta]} = \frac{\mathbb{P}[\mathbf{X},\,\mathbf{Z}|\,\theta]}{\sum_{\mathbf{Z}} \mathbb{P}[\mathbf{X},\,\mathbf{Z}|\,\theta]}$$

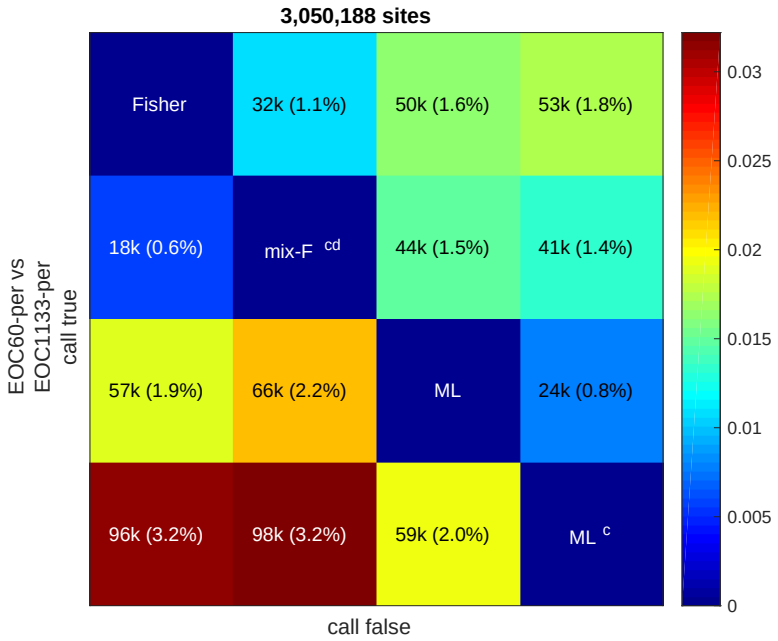
$$\theta^{(r+1)} = \underset{\theta}{\operatorname{argmax}}\, \mathbb{E}\left[\log \mathbb{P}[\mathbf{X}|\mathbf{Z},\,\theta] \,\middle|\, \mathbf{Z} = \mathbf{Z}^{(r)} | \mathbf{X},\,\theta^{(r)} \right]$$

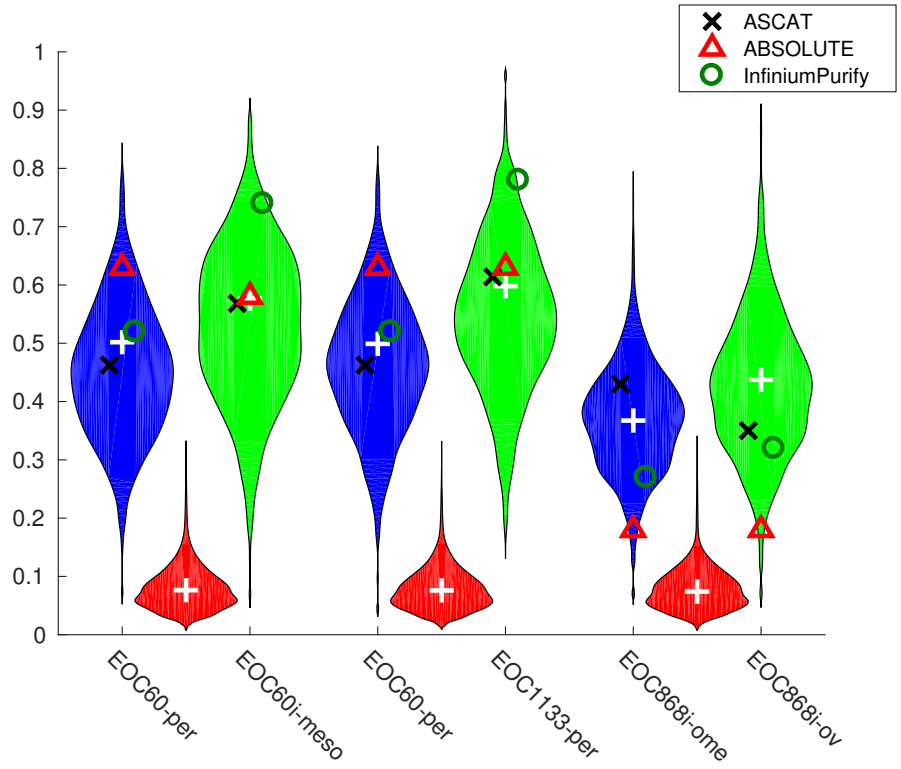
$$= \underset{\theta}{\operatorname{argmax}} \sum_{\mathbf{Z}} \log \mathbb{P}[\mathbf{X}|\mathbf{Z},\,\theta]\; \mathbb{P}\left[\mathbf{Z} = \mathbf{Z}^{(r)} | \mathbf{X},\,\theta^{(r)} \right]$$

Methylation: results

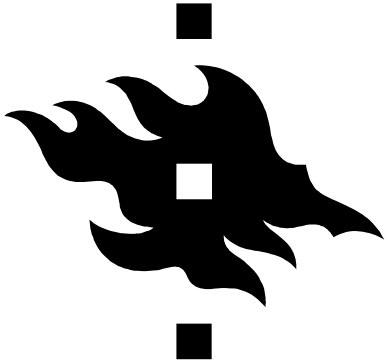


Methylation: results



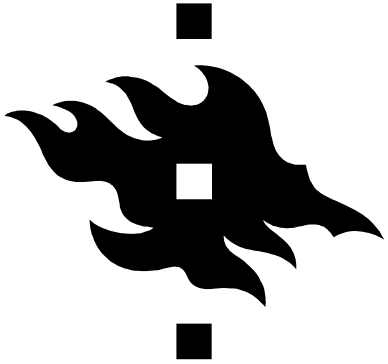


Sample A	Sample B	Sites	Corr -	$P_{-,0}$	Corr ML	$P_{ML,0}$	$P_{-,ML}$
EOC60-per	EOC60i-meso	F ∨ ML	−3.57%	0.04	−8.55%	8.44×10^{-7}	0.04
EOC60-per	EOC1133-per	F ∨ ML	−6.63%	9.74×10^{-27}	−10.48%	1.52×10^{-64}	9.23×10^{-6}
EOC868i-ome	EOC868i-ov	F ∨ ML	−1.88%	0.21	−12.04%	4.73×10^{-16}	1.22×10^{-6}



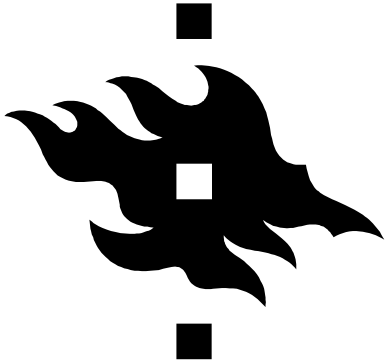
Methylation: results

- Reveals methylomes of cancer cells or compares them from two (or more) samples
- Generally outperforms other methods in all settings
- Suggests 5–10% new findings and false positives
- Predicts RNA expression better
- Purity estimates comparable to WGS methods
- Does not require normal cell control nor prior purity estimates
  - If these are available, used for increased accuracy



RNA-seq: background

- Cells harbor various alterations, only few drive tumor progression
- Alterations can affect the expression of the gene or its product
- RNA sequencing is widely employed: detect expression chages, discover splice variants, gene fusions, etc.
- There are methods of varying quality for decomposing the RNA-seq signal (e.g. from $\hat{\mathbf{W}} = \mathbf{X}^{\dagger}\,\mathbf{Y}$ to [8]):
  - Most are for microarray data (not NGS)
  - Based on libraries of known expression patterns for known cell types and deconvolution
    - No attempt to model heterogeneity
    - Cannot adapt to unknown cell types



RNA-seq: models

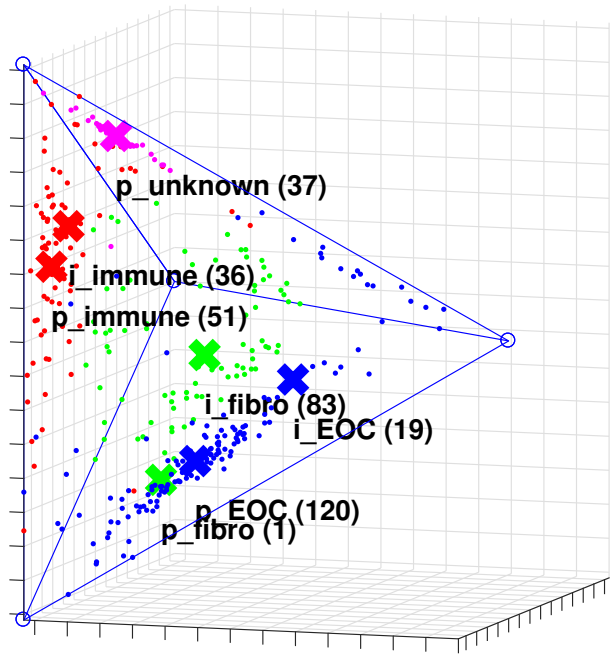
$\mathbf{X} \sim \mathcal{P}(\bar{\mathbf{U}}\,\mathbf{D}_U\,\bar{\mathbf{W}}\,\mathbf{D}_W)$
$[\mathbf{W}]_{:,j} \sim \mathcal{D}(\boldsymbol{\alpha}_j)$

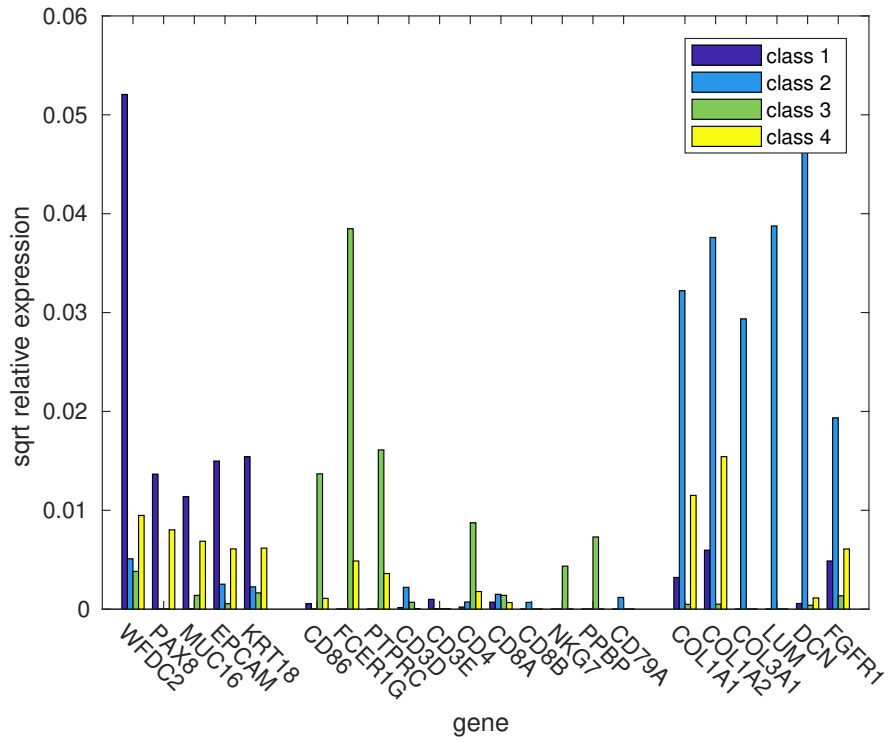
$\mathbf{X}$ are the responses, can be bulk or single-cell data
$\bar{\mathbf{U}}$ are the normalized expression profiles
$\mathbf{D}_U$ contains the expression level of each cell type (e.g. if cancer has double expression level in all genes)
$\bar{\mathbf{W}}$ is the normalized mixing matrix
$\mathbf{D}_W$ is the batch gain of the j sample (e.g. different amounts of RNA sequenced, amplification differences)

estimate $\bar{\mathbf{U}}\,\mathbf{D}_U$ and $\bar{\mathbf{W}}\,\mathbf{D}_W$ simulataneously
EM-algorithm with $Z_{i,j,k} \sim \mathcal{P}(U_{i,j}\,[\mathbf{D}_U]_{j,j}\,W_{j,k}\,[\mathbf{D}_W]_{k,k})$.

substitute $\mathcal{NB}$ for $\mathcal{P}$ for heterogeneity: mixture of $\mathcal{P}$

RNA-seq: results

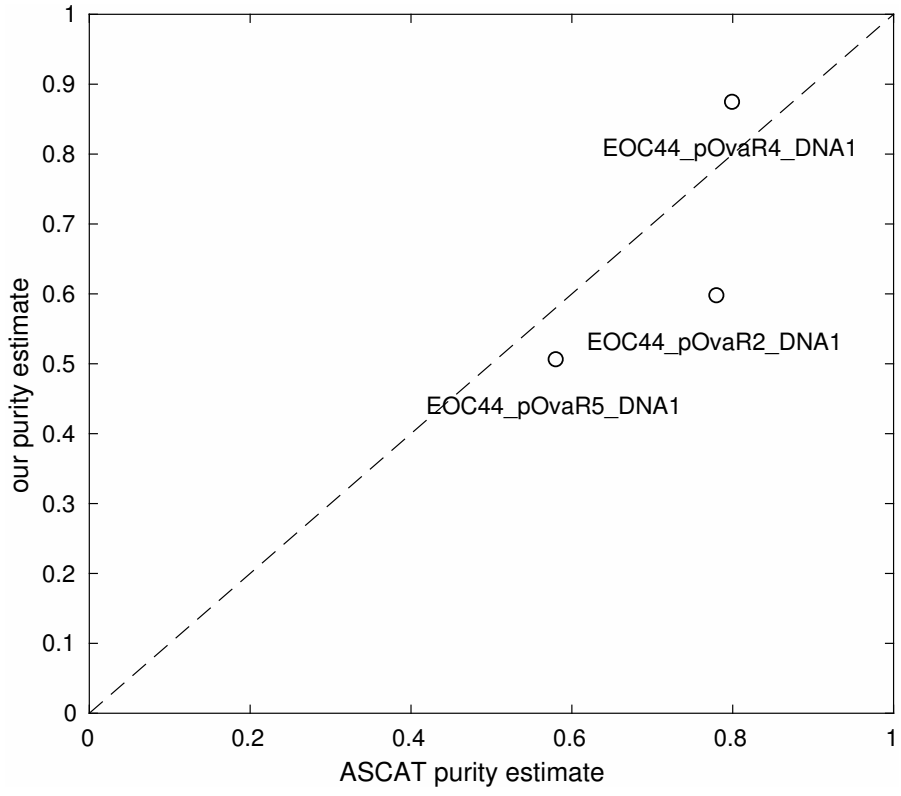


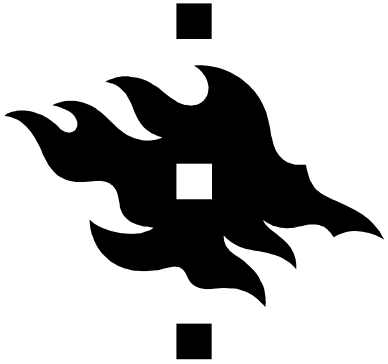


	p_EOC	p_fibro	p_immune	p_unknown	i_EOC	i_fibro	i_immune
class 1	0.0605	0	0.0029	0	0.0346	0	0
class 2	0.0058	0	0	0	0	0.2277	0.0058
class 3	0.0029	0	0.1095	0.1066	0.0058	0	0.0951
class 4	0.2767	0.0029	0.0346	0	0.0144	0.0115	0.0029

$\sim$85.3% accuracy of discovering hand-labeled cell types in 347 single-cell profiles

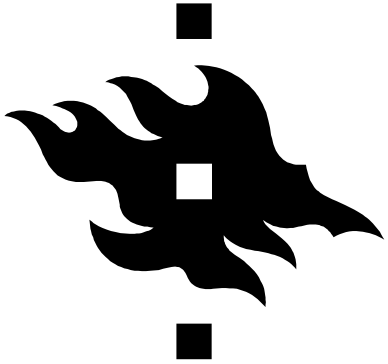
RNA-seq: results





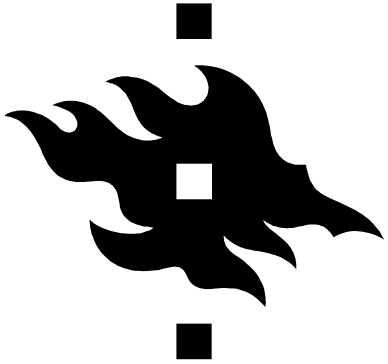
RNA-seq: results

- Our decomposition allows:
  - Blind clustering of single-cell data to identify candidate cell types
  - Decomposition of bulk samples into their components
    - Using single-cell + 1 unknown for 1 bulk sample
    - "Clean" a bulk sample using a bulk normal
- Correct batch effects etc. in the process
- The development & evaluation are still ongoing



Conclusion

- Remove biases that result from sample impurities
- Adapt to patient-specific profiles
- Adapt to missing or suspect controls
- All parameters estimated from the data–allow more flexible experimental settings
- Better accuracy–less prominent differences can be detected
- Accurate and unbiased quantification is required for integrative analyses to identify malfunctioning the biological processes
- Allows NGS of patient-derived samples to be used as personalized diagnostic and prognostic biomarkers and aids discovering therapeutic targets



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