Analyzing Flow-Cytometry Count Data with Regression Mixtures

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Joint work with
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Hutchinson Cancer Research Center

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Outline

1. **Introduction** to Flow-Cytometry

2. **Motivation**
   - Existing methods
   - Why a regression model?
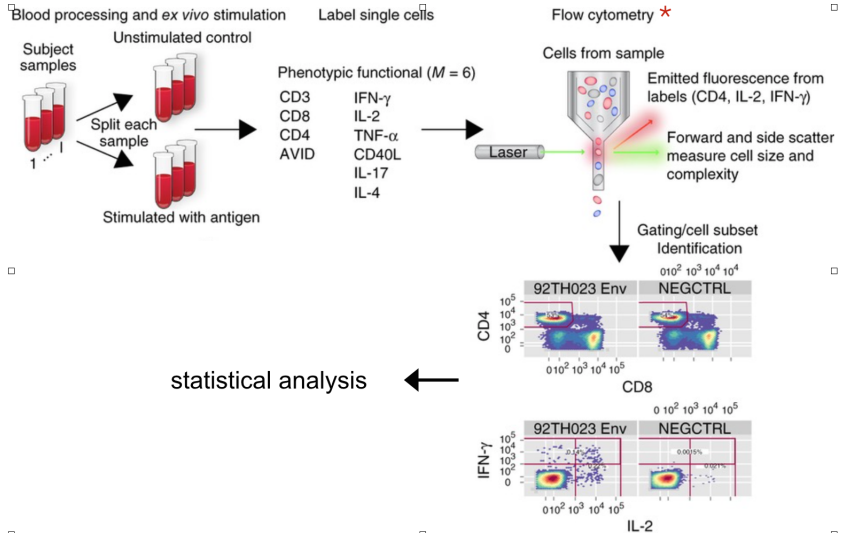
3. **Models**:
   - A Marginal Model
   - A Joint HMRF model

4. **Data analysis**
   - RV144 HIV Vaccine Trial
   - Controlled Human Malaria Infection Study.

5. **Computation** (time permitting)
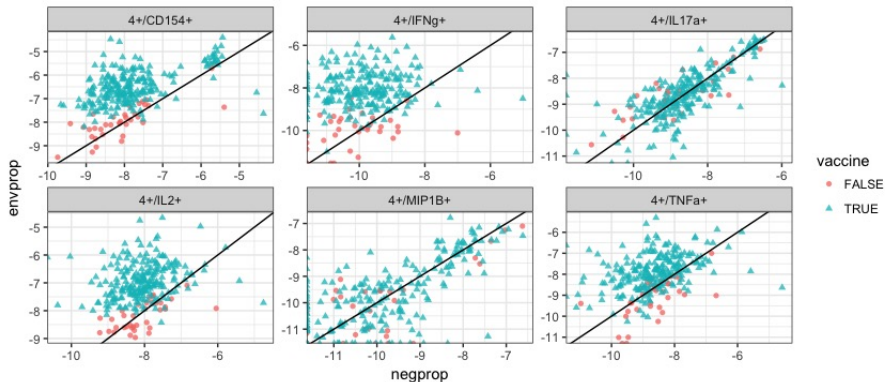
The RV144 HIV Vaccine Trial

Blood processing and *ex vivo* stimulation

Subject samples

Split each sample

1 ... I

Unstimulated control

Stimulated with antigen

Label single cells

Phenotypic functional (M = 6)

CD3   IFN-γ
CD8   IL-2
CD4   TNF-α
AVID CD40L
      IL-17
      IL-4

Flow cytometry *

Cells from sample

Laser

Emitted fluorescence from labels (CD4, IL-2, IFN-γ)

Forward and side scatter measure cell size and complexity

Gating/cell subset Identification

0 10² 10³ 10⁴

CD4 | 92TH023 Env | NEGCTRL

0 10² 10³ 10⁴ 10⁵

CD8

0 10² 10³ 10⁴ 10⁵

IFN-γ | 92TH023 Env | NEGCTRL

0.005%

0.01%

0 10² 10³ 10⁴ 10⁵

IL-2

statistical analysis

The RV144 HIV Vaccine Trial

PTID	Subset	stim	count	parentcount
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The RV144 HIV Vaccine Trial

- **262 Subjects**
  - 226 Cases
  - 36 Controls

- **2 Types of stimulus**
  - HIV antigen
  - Negative control

- **6 types of cytokines.**

Marginal Counts for RV144

A N A L Y S I S

_computational
BIOLOGY

COMPASS identifies T-cell subsets correlated with clinical outcomes

Lin Lin[1], Greg Finak[1], Kevin Ushey[1], Chetan Seshadri[2], Thomas R Hawn[2], Nicole Frahm[1], Thomas J Scriba[3], Hassan Mahomed[3], Willem Hanekom[3], Pierre-Alexandre Bart[4], Giuseppe Pantaleo[4], Georgia D Tomaras[5], Supachai Rerks-Ngarm[6], Jaranit Kaewkungwal[7], Sorachai Nitayaphan[8], Punnee Pitisuttithum[9], Nelson L Michael[10], Jerome H Kim[10], Merlin L Robb[11], Robert J O'Connell[12], Nicos Karasavvas[12], Peter Gilbert[1], Stephen C De Rosa[1,13], M Juliana McElrath[1,2,13] & Raphael Gottardo[1]

Or in general, with immune response.

Motivation: COMPASS

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Or in general, with immune response.

How do current methods work? (Approximately)

Current models are baseline/stimulation models.

- Unstimulated blood sample are compared stimulated ones.

For the unstimulated sample of the ith subject out of n, we sample a count proportion:

$$p_{i0} \sim \text{Dirichlet}(\alpha_0, \beta_0),$$

$$y_{i0} \sim \text{Multinomial}(N_{i0}, p_{i0}).$$

Let $k_i \in \{0, 1\}^p$ indicate in which subsets i responds:
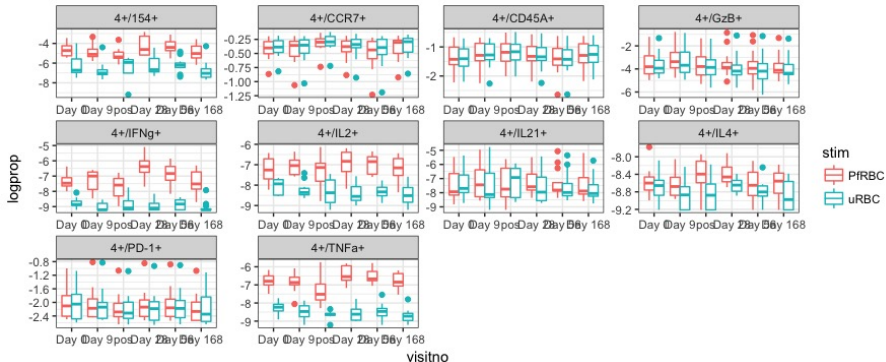
$$k_{ij} \sim \text{Ber}(w_j),$$

$$p_{i1, \tau=0} \sim \delta(p_{i0, \tau=0}), \quad p_{i1, \tau=1} | p_{i0, \tau=0} \propto \text{Dirichlet}(\alpha_1, \beta_1)$$

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Controlled Human Malaria Infection Study

- 9 subjects were infected with Malaria.
  - +3 controls.

- Blood samples were collected at 6 time points.
  - Day 0, day 9, blood parasitemia, Day 28, Day 56, Day 168.

- Two types of stimulation:
  - Infected/uninfected blood-cells.

- 53 cell subsets.
  - (10 types of cytokines in 8 cell-types)

Controlled Human Malaria Infection Study

Motivation - A Regression Model

- We want to be able to include covariates:
    - Batch effects.
    - Other covariates such as age, gender...

- Longitudal data.

- More than one stimulation.

- Explicit dependence model:
    - For the observed proportions.
    - For response/non-response.

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Motivation - Unique Challanges

- **Dependence**
  - Within sample between cell subsets.
  - Within subject / across time.

- **Heterogenous treatment effect**

- **Over-dispersed Binomial counts**

A Marginal Model - Single Subset

Indexing: **i**-subject, **t**- stimulation/time-point.

Overdispersion $\Rightarrow$ Beta Binomial Model.

$$\text{logit}(\mu_{it}) = X_{it}\beta,$$

$$p_{it} \sim \text{Beta}(M\mu, M(1-\mu)),$$

$$y_{it} \sim \text{Binom}(N_{it}, p_{it})$$

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Dependence $\Rightarrow$ 'random' subject baseline:

$$\nu_i \sim N(0, \sigma^2)$$

$$\text{logit}(\mu_{it}) = X_{it}\beta + \nu_i$$

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Non-response $\Rightarrow$ Mixture-Model:

$$k \sim Ber(\theta),$$

$$\text{logit}(\mu_{itk}) = X_{it}\beta + T_{it}\tau_k + \nu_i,$$

- T a matrix of covariates related to the treatment.
- τ_k equals 0 if $k = 0$ or $\tau \neq 0$ if $k = 1$.

A Marginal Model - Recap

Indexing: **i**-subject, **t**- stimulation, **k**- cluster.

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Model can be estimated via an EM algorithm

Wellness of Fit Evaluation

How do we evaluate the model?

- We fit the model without information regarding the true treatment allocation.

- The model should be able to discriminate between vaccinees and placebos.

- We use three type of figures:
  - Scatter plots w/classification information.
  - Receiver-Operator Curves.
  - False Detection Rates.

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Marginal Model - Results
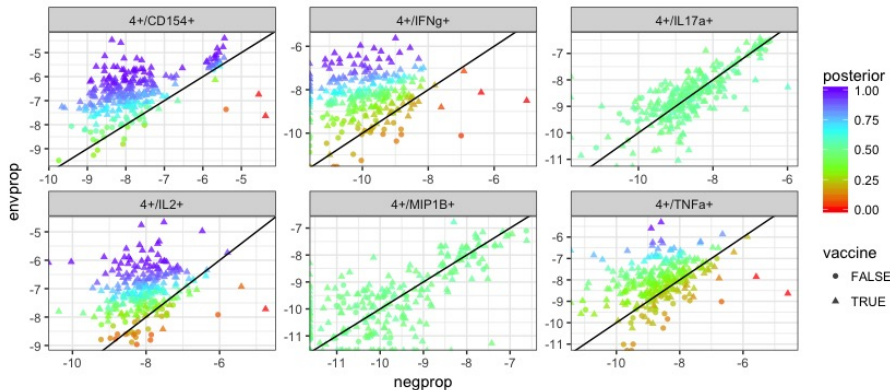
Figure: Posterior Probabilities for RV144 dataset - Independence Model

Comparison w/ MIMOSA - Finak et al. (2013)
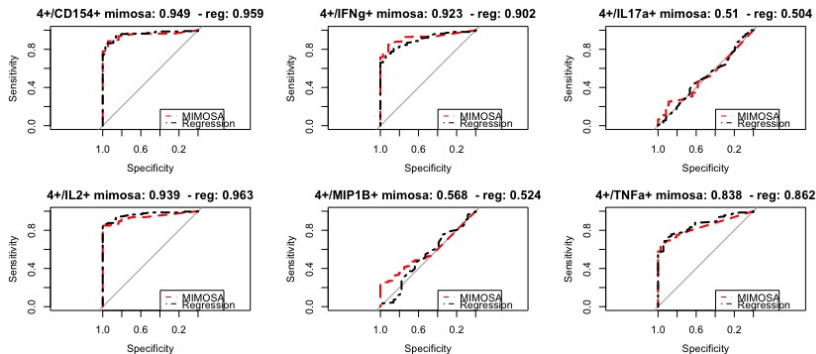
Figure: Comparison with MIMOSA (univariate COMPASS)

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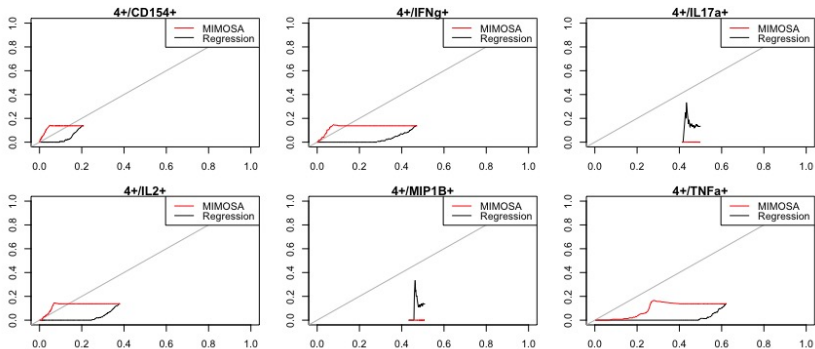
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Subject-Response Model

Performing analysis for each cell-subset at a time doesn't use all of the information available.

- Random Effects are correlated, can be estimated better simultaneously.

- Correlation structure might be of interest in itself.

- Response is probably not independent across cell-subsets.

- We might be able to improve classification of response by looking at several cell-subsets at once.

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A Hidden Markov Random Field Model

Indexing: **i**-subject, **t**- stimulation, **j**- subset, **k**- cluster.

Denote cluster (Response) by a $k \in \{0, 1\}^p$ vector with 1 indicating a responsive subset.

We assume an Ising model for the dependence structure between subsets:

$$P(k) \propto \sum_{j=1}^{p} k_j \theta_j + \sum_{s \neq t} k_t k_s \theta_{st},$$

$$P(k_j = 1 | k_{-j}) = \theta_j + \sum_{t \neq j} k_t \theta_{tj}.$$

We can induce sparsity through an ℓ_1 penalty.

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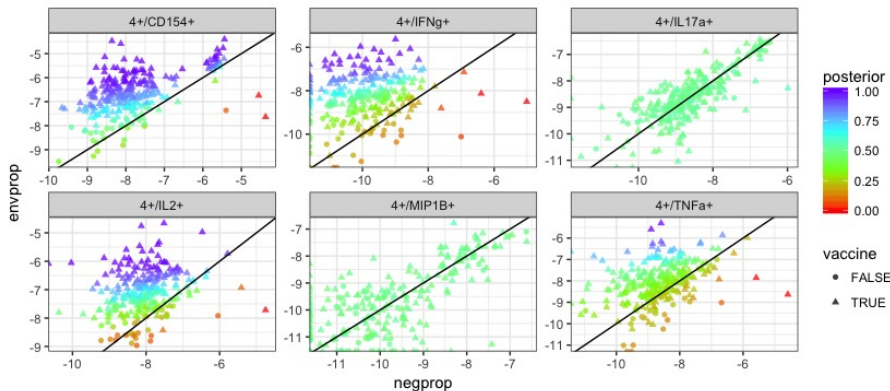
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HMRF Model - Results
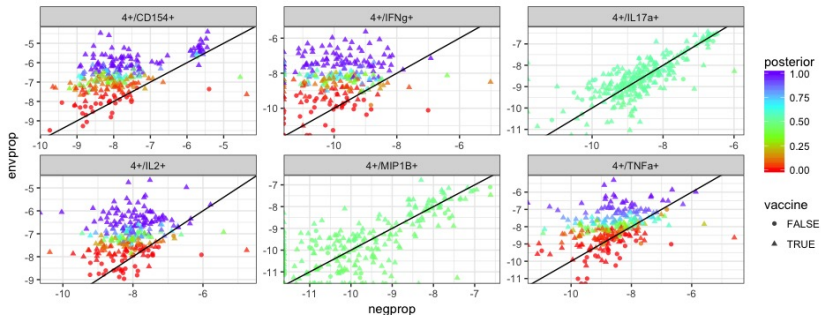
Figure: Scatter Plot for HMRF Modle Model

Subset-Response Model - Results
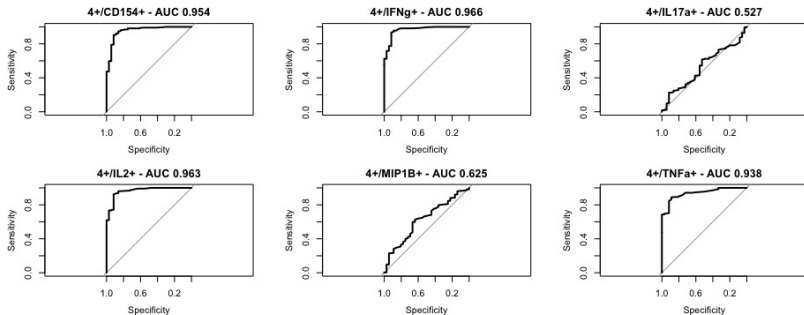
Figure: ROC for HMRF Modle

# Subset-Response Model - Results

Figure: FDR for HMRF Modle

Simulated Data Experiment

- Posterior probabilities are not well calibrated.
    - Might be due to true non-response.

- Is the optimization algorithm estimating the model properly?

- Does the model fit the data well?

- To find out:
    - We generate data according to the estimated model.
    - Fit should be perfect.
   - Is the artificial data similar to the real data?

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How close are we to the distribution of the data?
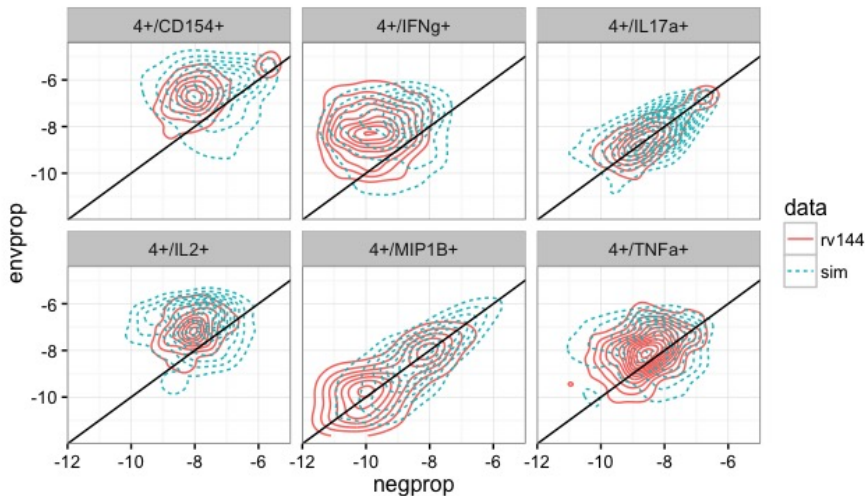
Results for Simulated Data
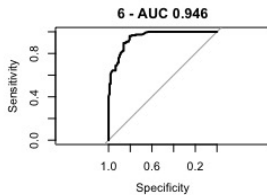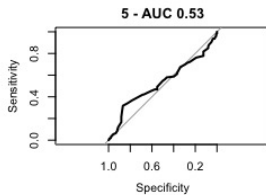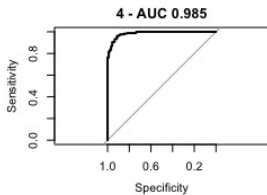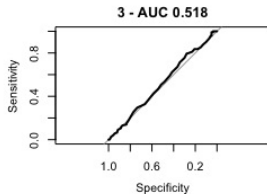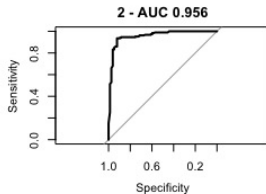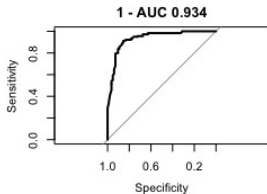
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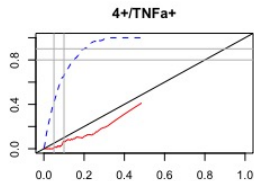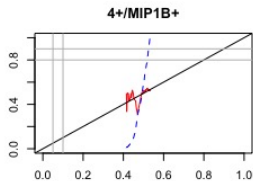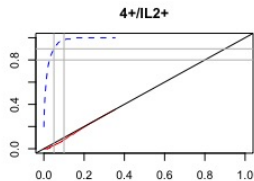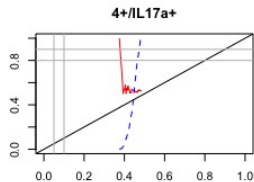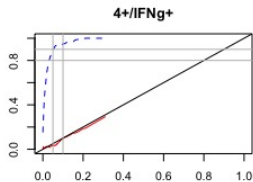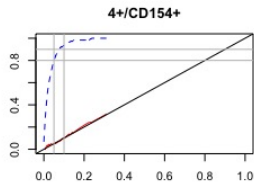
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RV144 Booleans Dataset

- So far we worked with marginal counts - can be obtained from bulk assays.

- single-cell measurements enable a more comprehensive understanding of cellular functions.

- **The degree of functionality** (numbered of expressed cytokines) of responsive cell-subsets has been correlated with favorable outcomes in vaccine studies.
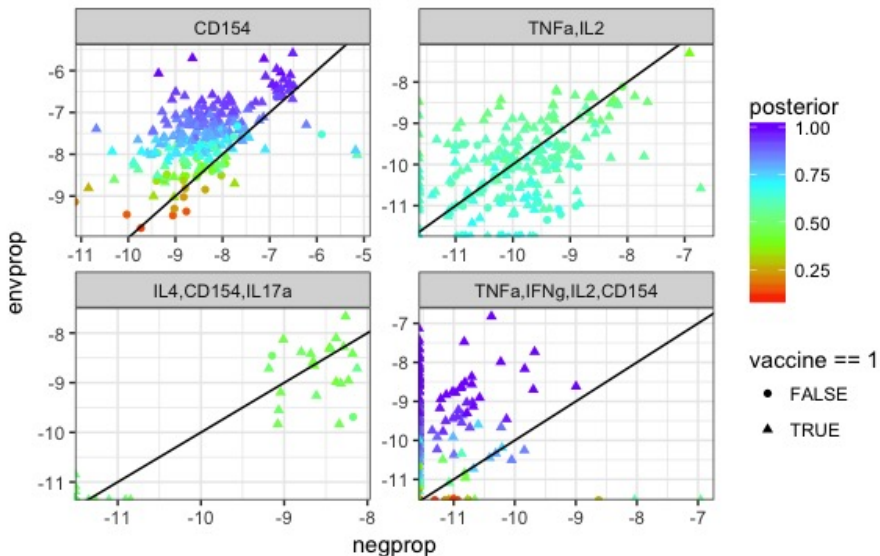
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  - 226 Cases
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- **2 Types of stimulus**
  - HIV antigen
  - Negative control

- **23 types of cells with non-negligible counts.**
  - (At least two instances of *count* $\geq$ 5)
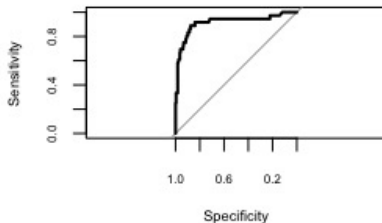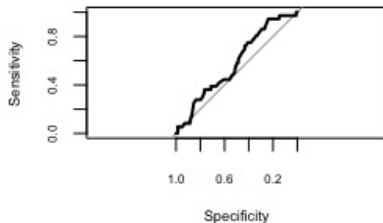
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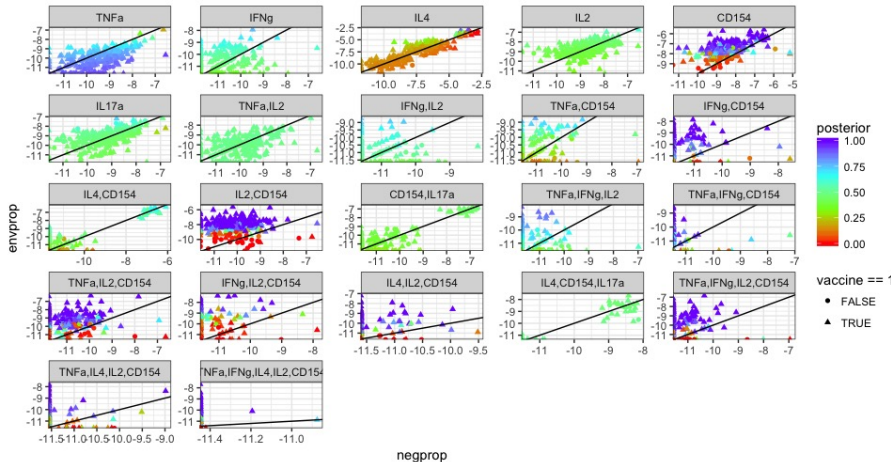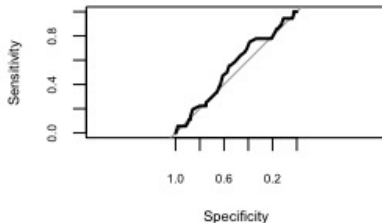
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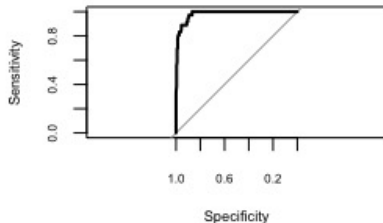AUC - Overall 0.989
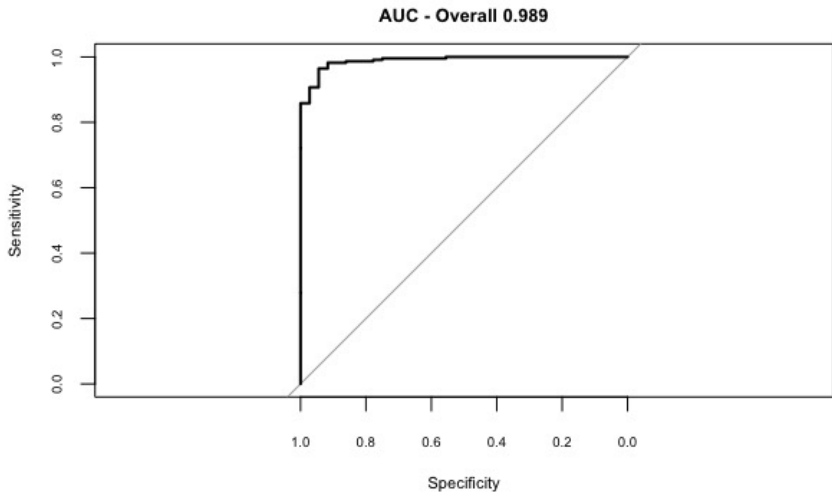
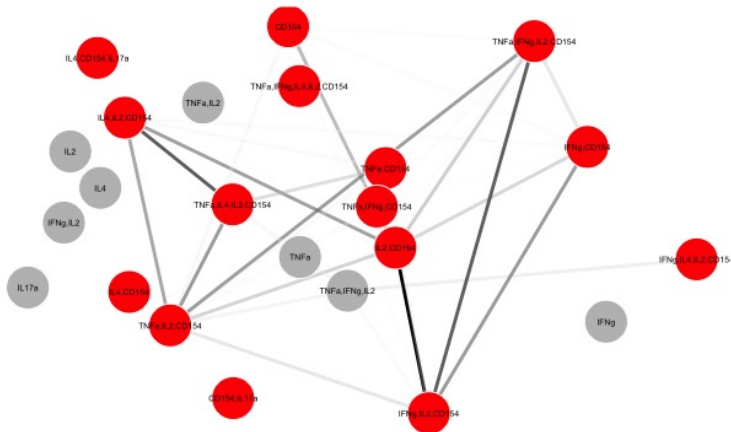# RV144 - Booleans Dataset

Figure: Estimated Ising Model - Red marks CD154

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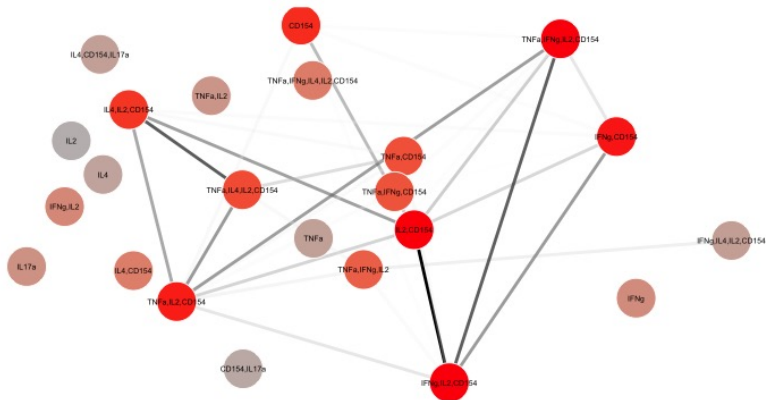
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Controlled Human Malaria Infection Study

Blood Parasitemia Day 11-19

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- Individuals who experience malaria infections develop immunity.
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  - Even at day 0!
  - What is the profile of the immune response?

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  - We might expect to see a rise in response during experiment.
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Controlled Human Malaria Infection Study
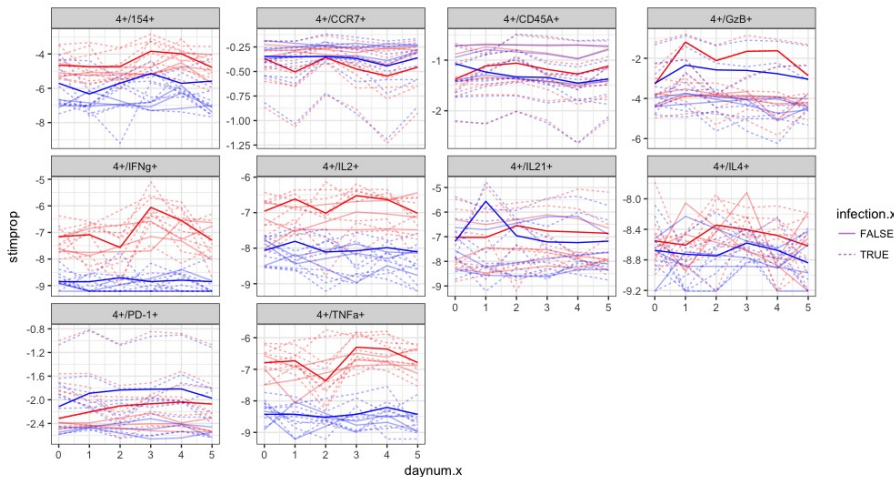
malaraFourPlusSmoothed

Figure: CD4 Helper Cells

Standard errors for significance tests computed using Jackknife.

|	4+	4+/CXCR5+	56+dim	56+hi	8+	8+/CXCR5+	NK T cells	PD-1+
154+	0.029	0.004			0.103	0.75	0.006	0.024
CCR7+	0.649	0.996			0.596	0.51		
CD45A+	0.575	0.307			0.543	0.54		
IFNg+	0.001	0.006	0.065	0.146	0.001		0.052	0.097
IL2+	0	0.005			0.119	0.56	0.321	0.052
IL21+	0.676	0.649	0.751	0.589	0.649		0.71	
IL4+	0.12	0.543		0.751	0.649		0.583	
TNFa+	0	0.001	0.261	0.309	0.276		0.053	0.09
GzB+	0.583		0.511	0.001	0.589		0.596	
PD-1+	0.751				0.596	0.83		

Controlled Human Malaria Infection Study
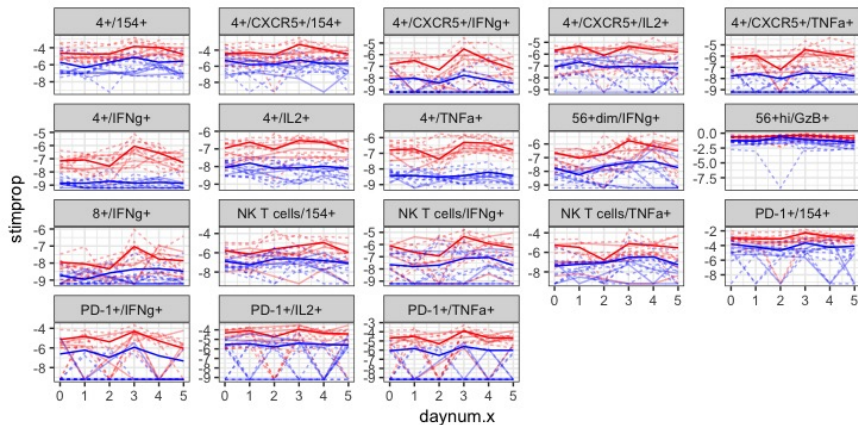
Figure: Significant Subsets

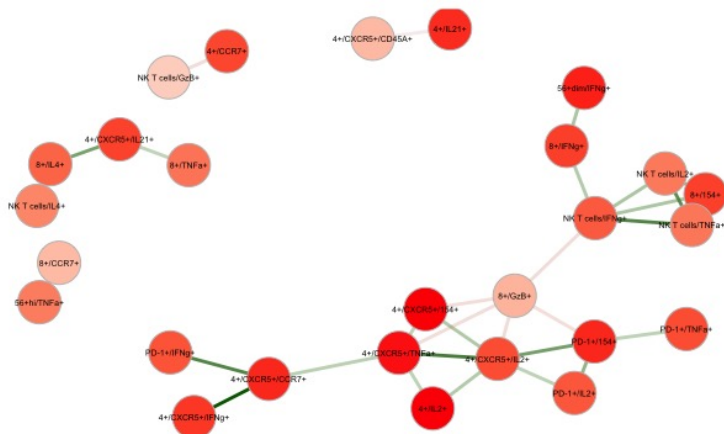# Controlled Human Malaria Infection Study

Figure: Estimated Graph - Red marks probability of response

Computation - A Difficult Likelihood

With cluster assignments and random effects known:

$$f(y_i|\nu_i, k_i) = \prod_j \prod_t f(y_{ijt}|\nu_{ij}, k_{ij})$$

The log-likelihood of the data is given by:

$$\ell(\beta, \tau, \theta, \Sigma) = \sum_{i=1}^{n} \log \left(\sum_{k \in \{0,1\}^J} P_\theta(k) \int_{\mathbb{R}^J} f_{\beta,\tau}(y_i|\nu_i, k_i)\varphi(\nu_i; 0, \Sigma)d\nu_i \right)$$

The log-likelihood is intractable for large J.

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Computation - How About EM?

The integrals are replaced with conditional expectations:

$$\sum_{i=1}^{n} \log \left(\sum_{k \in \{0,1\}^J} P_\theta(k|y_i) \int_{\mathbb{R}^J} f_{\beta,\tau}(y_i|\nu_i, k_i) f_\Sigma(\nu_i|y_i, k) d\nu_i \right)$$

We can use sampling to approximate the intractable integrals:

$$k_{i1}^*, ..., k_{iM}^* \sim P_\theta(k|y_i)$$

$$\nu_{i1}^* \sim f(\nu_i|y_i, k_{i1}), ..., \nu_{iM}^* \sim f(\nu_i|y_i, k_{iM})$$

$$(\beta, \tau, \theta, \Sigma) = \arg \max \sum_{i=1}^{n} \frac{1}{M} \sum_{m=1}^{M} \sum_j \sum_t \log f(y_{ijt}|\nu_{im}^*, k_{im}^*)$$

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Computation - Stochastic EM Algorithm

We alternate between a stochastic E-step and an M-step.

S - Step
- k^* - Gibbs sampler.
- $\nu^*|k^*$ - component-wise MH algorithm.

M - Step
- β, τ - **glm, glmnet** for sparsity or **gamlss** for BB.
- θ - Pseudo-likelihood or **isingFit** for sparsity .
- Σ - Option for sparse estimation via **PSDE** package.

Thank you!

Questions?

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