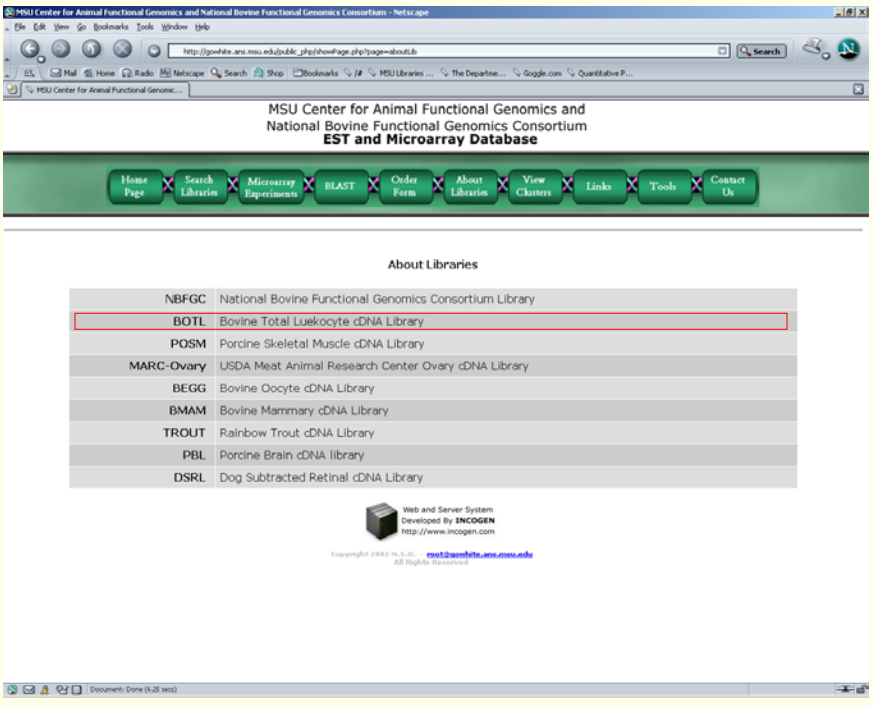
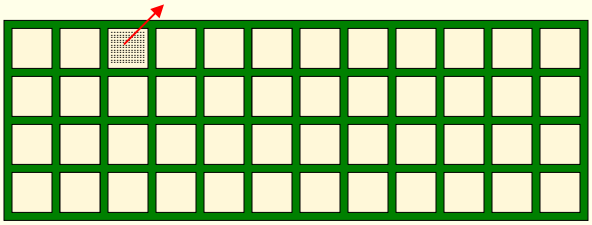


Center for Animal Functional Genomics
Michigan State University

- cDNA microarray BOTL-3
- 709 bovine EST clone inserts
- 345 amplicons (known genes)

Slides: 4 x 12 patches
(9 x 9 spots /each)



Controls

β actin
RPL19
GAPDH
Lambda Q
DMSO
Blank
etc.

Different types of "replication" in microarray experiments

(Churchill, 2002)

1. Assessing measurement error
   - ⇨ Multiple spots per gene on an array
   - ⇨ Multiple arrays to study the same sample

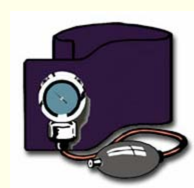
   * Useful for quality control …

   But not too useful for inference on treatment effects!

2. Assessing biological variability
   - ⇨ True replication

But… Should use appropriate error terms for testing treatments !

Example 1



⇨ Effect of diet on blood pressure

① Two individuals (one vegetarian; another non-vegetarian)

② Same gender, same age, nonsmoking, etc.

③ The diastolic pressure is measured 3 times

(consecutive days, for example)

Vegan	Meat
80	80
95	100
110	90

Can we use a t-test to compare both groups ?

Do I have biological replication here ?

?

Example 2

⇨ Multiple individuals per group

Vegan				Meat			
V_1	V_2	...	V_n	M_1	M_2	...	M_n
80	88	...	73	110	93	...	115

$$y_{ij} = \mu + G_i + \varepsilon_{ij}$$

$$\begin{cases} i = 1, 2 \\ j = 1, 2, \ldots, n \end{cases}$$

ANOVA TABLE

SV	DF	E[MS]
Group	1	$\sigma_R^2 + n\phi_G$
Residual	2(n - 1)	σ_R^2
Total	2n - 1	

Example 3

⇨ Repeated measurements in each individual

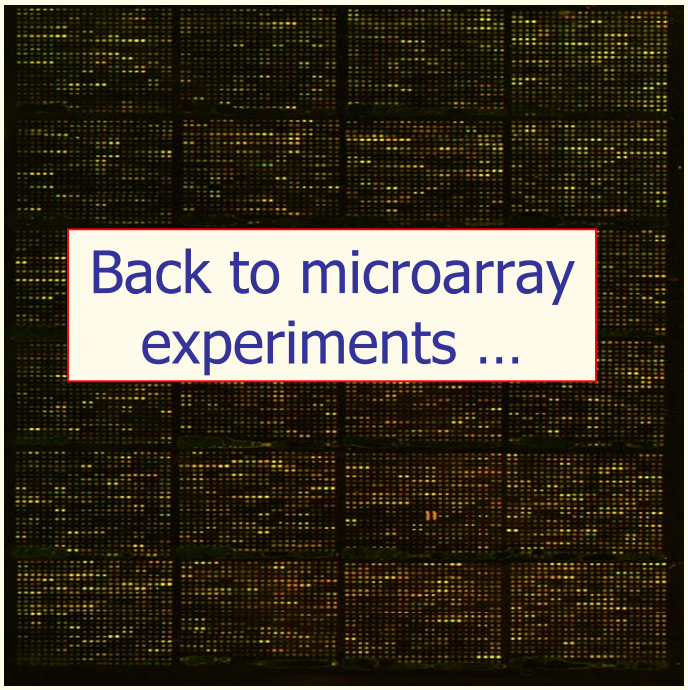
Vegan				Meat			
V_1	V_2	...	V_n	M_1	M_2	...	M_n
80	88	...	73	110	93	...	115
105	89	...	79	106	105	...	90
90	100	...	75	105	89	...	97

$$y_{ij} = \mu + G_i + I_{ij} + \varepsilon_{ijk}$$

$$\begin{cases} i = 1, 2 \\ j = 1, 2, \ldots, n \\ k = 1, 2, \ldots, r \end{cases}$$

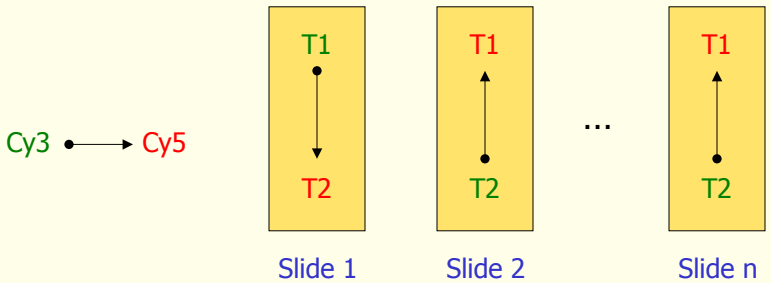
ANOVA TABLE

SV	DF	E[MS]
Group	1	$\sigma_S^2 + n\sigma_I^2 + nr\phi_G$
Indiv.|Group	2(n - 1)	$\sigma_S^2 + r\sigma_I^2$
Residual	2n(r - 1)	σ_S^2
Total	2nr - 1	



TWO-SAMPLE EXPERIMENTS

- Slides as blocks
  - Two treatments (different experimental conditions)
  - J 'biological' replications per treatment
  - J slides total



TWO-SAMPLE EXPERIMENTS

- One slide per experimental unity (individual or pooled mRNA)
  - Model:

$$\log(y_{gijkm}) = \mu_g + T_{gi} + B_{gj} + D_{gk} + TB_{gij} + \text{interactions} + S \mid B_{g(j)m} + \varepsilon_{ijkm}$$

  - ANOVA:

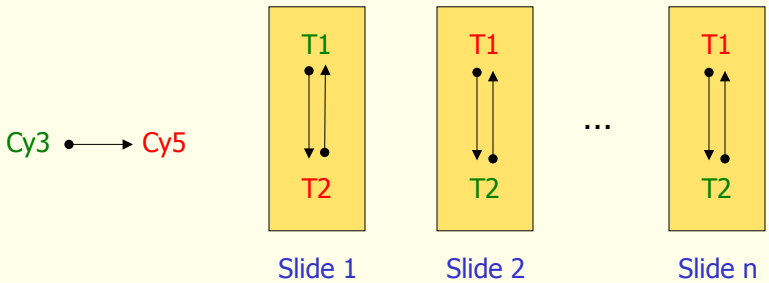
Biological replication

SV	DF
Trt (T)	1
Animal (B)	J - 1
Dye (D)	1
T*B	J - 1
Others	ϕ_1
Spot (S|B)	J(s – 1)
Residual	ϕ_2
Total	2sJ - 1

$$F_T = \frac{MS(T)}{MS(T*B)}$$

TWO-SAMPLE EXPERIMENTS

- Slides as blocks
  - Two treatments (different experimental conditions)
  - J 'biological' replications per treatment
  - 2J slides total (dye swap)



TWO-SAMPLE EXPERIMENTS

- Two slides (dye swap) per experimental unity
  - Model:

$$\log(y_{gijklm}) = \mu_g + T_{gi} + B_{gj} + D_{gk} + TB_{gij} + A \mid B_{g(j)l} + \text{interactions} + S \mid A_{g(l)m} + \varepsilon_{ijklm}$$

- ANOVA:

Biological replication

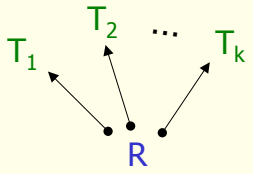
SV	DF
Trt (T)	1
Animal (B)	J - 1
Dye (D)	1
T*B	J - 1
Array (A|B)	J
Others	ϕ_1
Spot (S|B)	2J(s – 1)
Residual	ϕ_2
Total	4sJ - 1

$$F_T = \frac{MS(T)}{MS(T*B)}$$

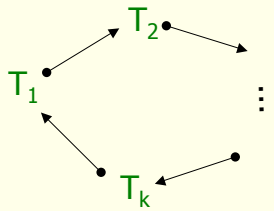
MULTIPLE SAMPLES (TREATMENTS)

- Incomplete block structure
  - k treatments (different experimental conditions)
  - n 'biological' replications per treatment
  - k × n slides total

Reference Design



Loop Design



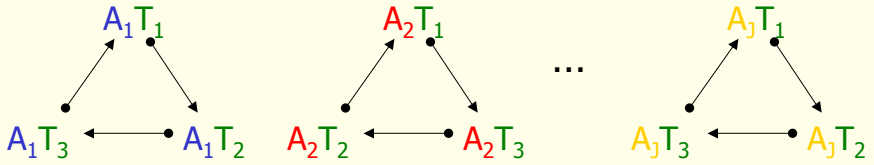
Cy3 → Cy5

LOOP EXPERIMENTS

- Each animal under all trts (within a loop)

I treatments
J animals (or pools)
I × J slides total (J loops of I slides)

2 (I × J) if dye swap



Cy3 ⟶ Cy5

LOOP EXPERIMENTS

- Animal ⇔ Loop
- Model:

$$\log(y_{gijklm}) = \mu_g + T_{gi} + B_{gj} + D_{gk} + TB_{gij} + A \mid B_{g(j)l} + \text{interactions} + S \mid A_{g(l)m} + \varepsilon_{ijklm}$$

- ANOVA:

SV	DF
Trt (T)	I - 1
Animal (B)	J - 1
Dye (D)	1
T*B	(I - 1)(J - 1)
Array (A|B)	J(I – 1)
Others	ϕ_1
Spot (S|B)	IJ(s – 1)
Residual	ϕ_2
Total	2sIJ - 1

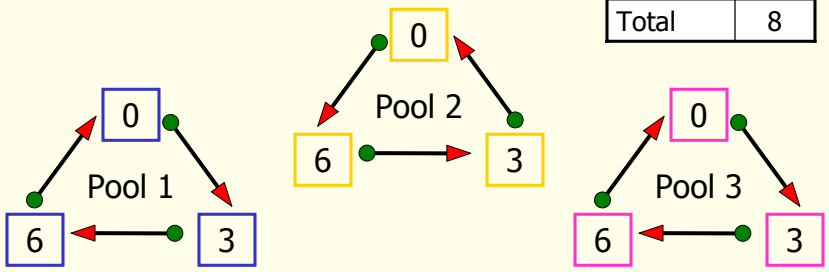
$$F_T = \frac{MS(T)}{MS(T*B)}$$

Cartilage Impact

Pooi-See Chang and Dr. Mike Orth

- Time after impact (0, 3 and 6 h)
- Three pools of three animals each
- Multiple Loop Design

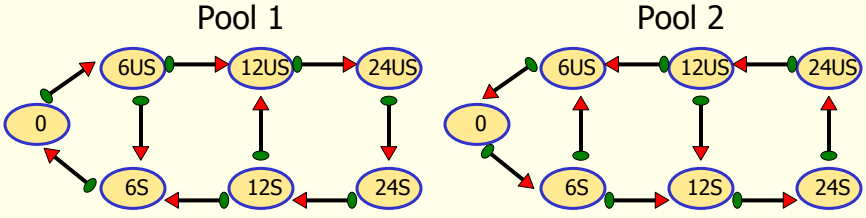
S.V.	d.f.
Pool	2
Time	2
Residual	4
Total	8



In Vitro Bovine Immune Response to *Trypanossoma brucei* Infection

Dr. Emmeline Hill and Dr. Paul Coussens

- Time course (0, 6, 12 and 24 h)
- Stimulated (S) and Non Stimulated (NS)
- Two pools of two animals



Error Term for testing Trt: Pool*Treatment (6 d.f.)

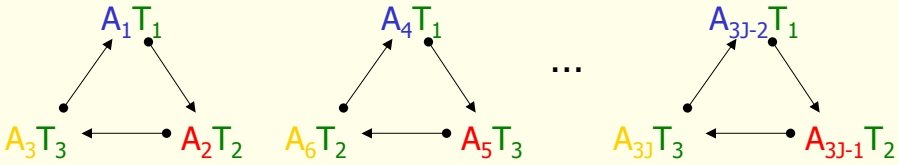
LOOP EXPERIMENTS

- Each animal under one trt

I treatments

J animals per treatment (I × J animals in total)

I × J slides total (J loops of I slides)



Cy3 ⟶ Cy5

LOOP EXPERIMENTS

- Model:

$$y_{gijklmn} = \mu_g + T_{gi} + B \mid T_{g(i)j} + D_{gk} + L_l + A \mid L_{g(l)m} + \text{interactions} + S \mid A_{g(m)n} + \varepsilon_{ijklmn}$$

- ANOVA:

SV	DF
Trt (T)	I - 1
Dye (D)	1
Animal (B|T)	I(J - 1)
Loop (L)	J - 1
Array (A|L)	J(I - 1)
Others	ϕ_1
Spot (S|B)	IJ(s – 1)
Residual	ϕ_2
Total	2sIJ - 1

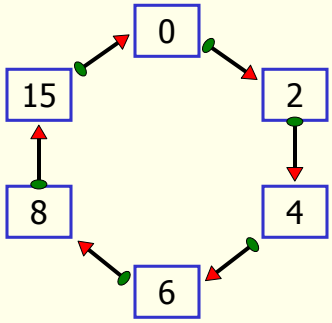
$$F_T = \frac{MS(T)}{MS(B \mid T)}$$

mRNA Profiles Post Infection

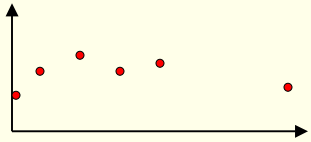
Dr. Line Morgills and Dr. Jeanne Burton

- Time after infection (0, 2, 4, 6, 8, 15 days)
- A different animal per time point
- Loop Design

Cy3 → Cy5



S.V.	d.f.
Linear	1
Quadratic	1
Lack of Fit	3
Total	5



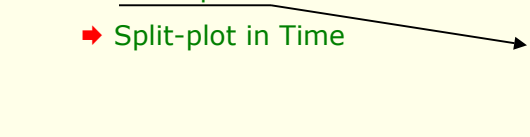
OTHER EXPERIMENTAL DESIGNS

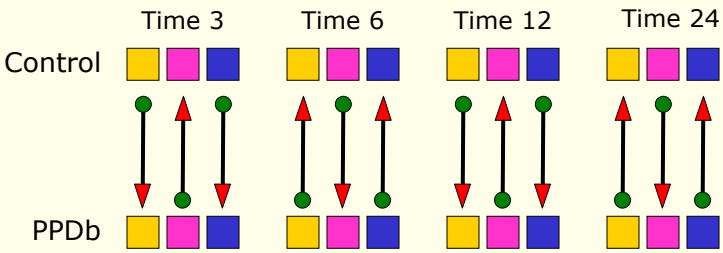
- SPLIT-PLOT
  - Within a CRD or RBD (on plots)
  - Split-Plot on Time

Bovine TB Experiment

Kieran Meade and Dr. Paul Coussens

- PPDb stimulation over time (3, 6 12, and 24 h)
- Three pools with two infected animals each
- Split-plot in Time





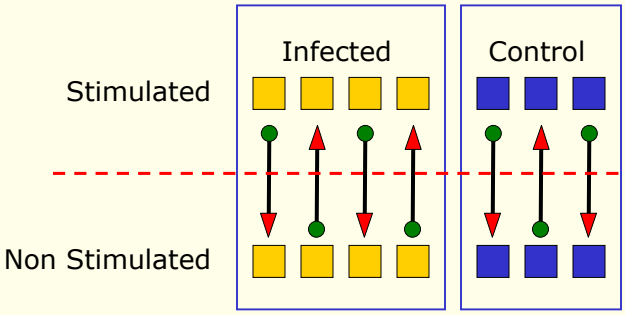
S.V.	d.f.
Pool	2
Trt	1
Res(a)	2
Plot	5
Time	3
Trt*Time	3
Res(b)	12
Total	23

Peripheral Blood Mononuclear Cells from MPTb Infected Cows

Dr. Paul Coussens

- Infected (n_1 = 4) and Control (n_2 = 3) Cows
- Two treatments: Stimulation or not
- Split-plot Design

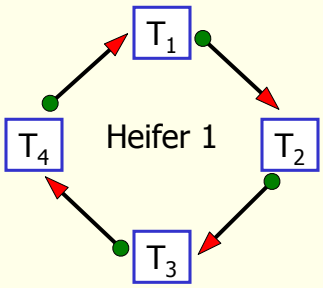
S.V.	d.f.
Group	1
Res(a)	5
Plot	6
Trt	1
Group*Trt	1
Res(b)	5
Total	13



Leptnin and IGF-I Intramammary Infusion

Brett Etchebarne and Dr. Mike VandeHaar

- Four (2 × 2) treatments, one in each quarter
- Six Heifers, Loop Design
- Treatments:



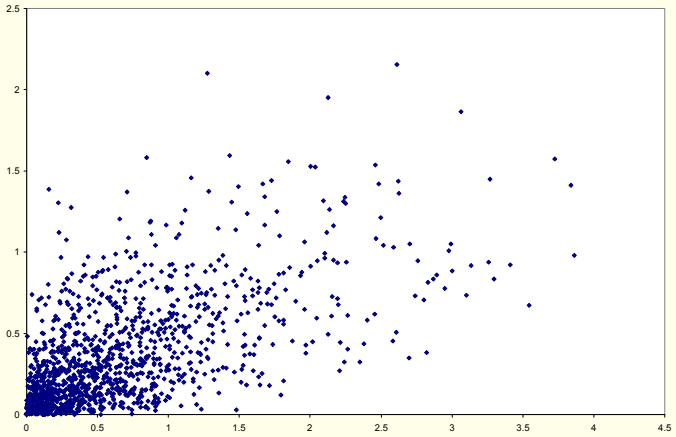
Trt	IGF	Lept
1	N	N
2	N	Y
3	Y	N
4	Y	Y

S.V.	d.f.
Dye	1
Trt	3
Heifer	5
Trt*Heifer	15
Array|Heifer	18
Spot|Array	48
Residual	53
Total	133

Leptnin and IGF-I Intramammary Infusion

Brett Etchebarne and Dr. Mike VandeHaar

- P-values (log scale) from fixed and mixed effects models



FINAL REMARKS

- ⇨ Experimental Design
- ⇨ Technical Replication:
  - Measurement error assessment
  - Future allocation of sampling efforts
- ⇨ Biological replication & Error variance
- ⇨ Borrowing information across genes (variance)

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