INTRODUCTION TO EXPERIMENTAL DESIGN AT CRUK-CI

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21 JANUARY 2021

tinyurl.com/cruk-edesign

Agenda

WHY PERFORM EXPERIMENTS?

WHAT MAKES FOR A WELL DESIGNED EXPERIMENT?

KEY ASPECTS OF EXPERIMENTAL DESIGN

- Experimental variables
- Power: variance and replicates
- Bias: confounding factors, randomisation, and controls

DESIGN PARAMETERS

EXPERIMENTAL DESIGN PROCESS AT CRUK-CI

BREAKOUT SESSIONS: PRACTICALS

Why Perform Experiments?

BECAUSE MY SUPERVISOR TOLD ME TO

BECAUSE THEY DID IT IN THIS OTHER PAPER

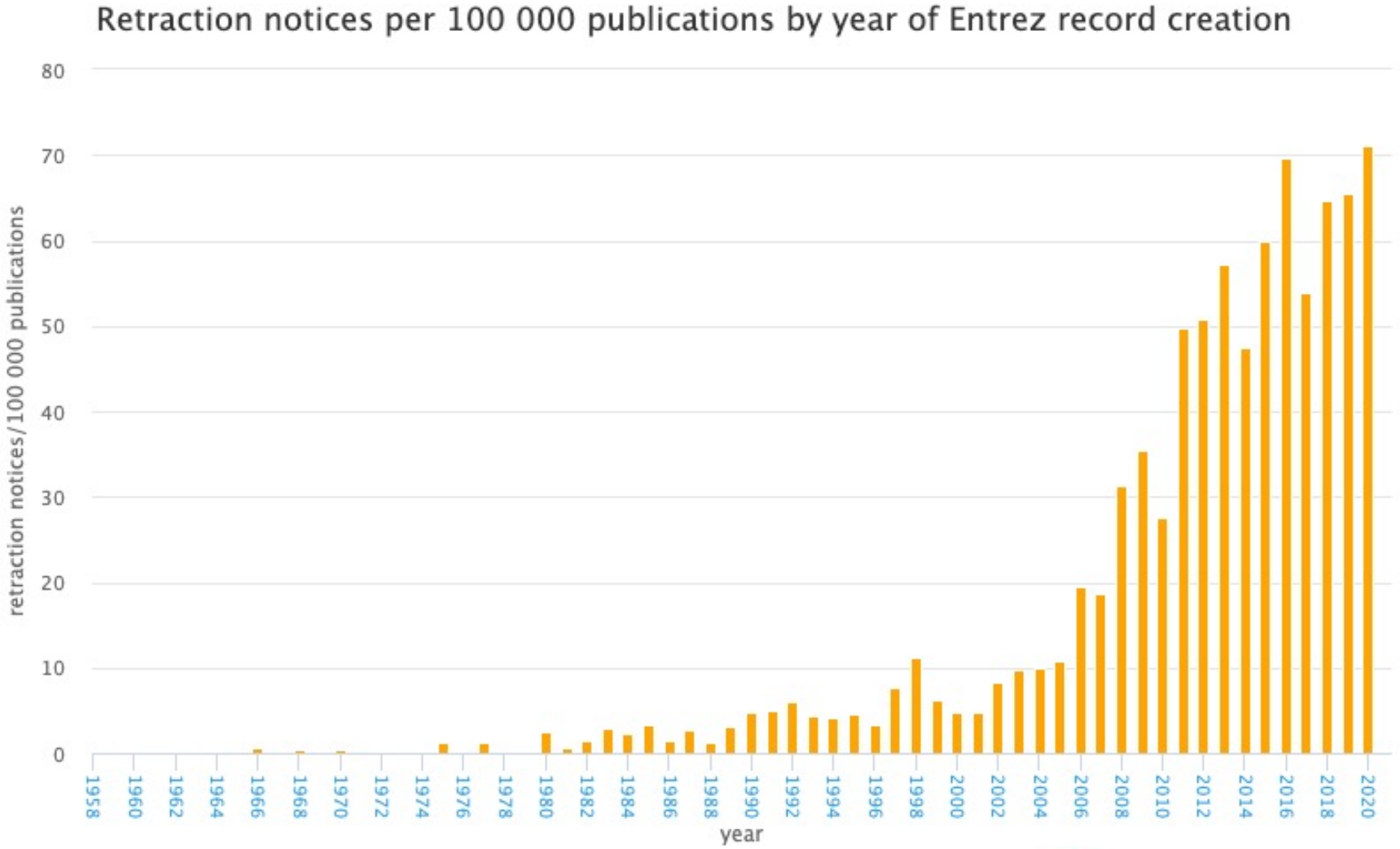
BECAUSE WE GOT A COOL NEW PIECE OF TECH AND I WANT TO TRY IT OUT

BECAUSE I DON'T KNOW WHAT ELSE TO DO

TO GET EVIDENCE (HOPEFULLY) SUPPORTING A HYPOTHESIS

Reproducible Research

Crisis in Reproducible Research



http://neilfws.github.io/PubMed/pmretract/pmretract.html

47 of 53 high-profile cancer studies were not reproducible!

nature International weekly journal of science

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Archive > Volume 483 > Issue 7391 > Comment > Article

NATURE | COMMENT

Drug development: Raise standards for preclinical cancer research

C. Glenn Begley & Lee M. Ellis

Affiliations | Corresponding author

Nature **483**, 531–533 (29 March 2012) | doi:10.1038/483531a

Published online 28 March 2012

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Need for Good Design

Consequences of Poor Experimental Design…

- **Cost** of experimentation. We have a responsibility to CRUK donors!
- **Limited & Precious** material, esp. clinical samples.
- **Immortalization** of data sets in public databases and methods in the literature. Our bad science begets more bad science.
- **Ethical concerns** of experimentation: animals and clinical samples.

A Well-Designed Experiment:

Should have

- CLEAR OBJECTIVES
- FOCUS AND SIMPLICITY
- SUFFICIENT POWER
- RANDOMISED COMPARISONS

And be

- PRECISE
- UNBIASED
- AMENABLE TO STATISTICAL ANALYSIS
- REPRODUCIBLE

Ronald A. Fisher(1890-1962)

"TO CONSULT THE STATISTICIAN AFTER AN EXPERIMENT IS FINISHED IS OFTEN MERELY TO ASK HIM TO CONDUCT A POST MORTEM EXAMINATION. HE CAN PERHAPS SAY WHAT THE EXPERIMENT DIED OF."

"… VERY OFTEN, … THE MOST ELABORATE STATISTICAL REFINEMENTS POSSIBLE COULD INCREASE THE PRECISION BY ONLY A FEW PERCENT, YET A DIFFERENT DESIGN INVOLVING LITTLE OR NO ADDITIONAL EXPERIMENTAL LABOUR MIGHT INCREASE THE PRECISION TWO-FOLD, OR FIVE-FOLD OR EVEN MORE."

Aspects of Experimental Design

- **EXPERIMENTAL FACTORS**
- **POWER**
  - Sources of Variance
  - Replicates
- **BIAS**
  - Confounding factors
  - Randomisation wherever a decision is to be made
    - Controls for both measured and unmeasured factors
  - Controls

Experimental Factors

Experimental Factors

FACTORS: ASPECTS OF EXPERIMENT THAT CHANGE AND INFLUENCE THE OUTCOME OF THE EXPERIMENT

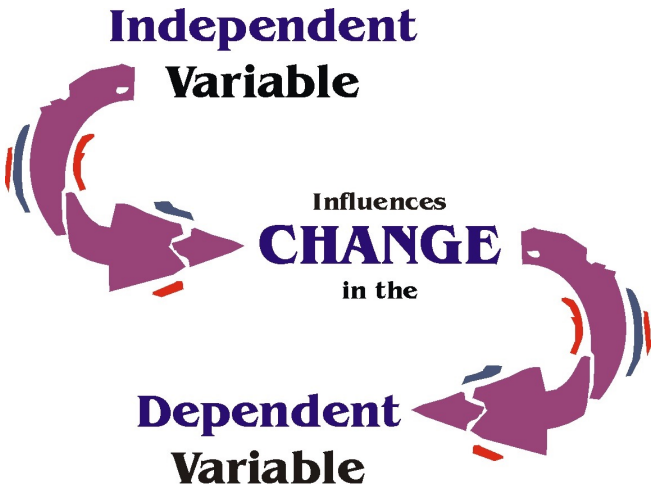
- e.g. time, weight, drug, gender, ethnicity, country, plate, cage etc.

VARIABLE TYPE DEPENDS ON TYPE OF MEASUREMENT:

- Categorical (**nominal**) , e.g. gender
- Categorical with ordering (**ordinal**), e.g. tumour grade
- **Discrete**, e.g. shoe size, number of cells
- **Continuous**, e.g. body weight in kg, height in cm

INDEPENDENT AND DEPENDENT VARIABLES

- Independent variable (IV): what you change
- Dependent variable (DV): what changes due to IV
- "**If** (**independent** variable), **then** (**dependent** variable)"



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Capturing Variance

Sources of Variation

BIOLOGICAL "NOISE"

- Biological processes are inherently stochastic
- Single cells, cell populations, individuals, organs, species….
- Timepoints, cell cycle, synchronized vs. unsynchronized

TECHNICAL NOISE

- Reagents, antibodies, temperatures, pollution
- Platforms, runs, operators

CONSIDER IN ADVANCE AND CONTROL

REPLICATION REQUIRED TO CAPTURE VARIANCE

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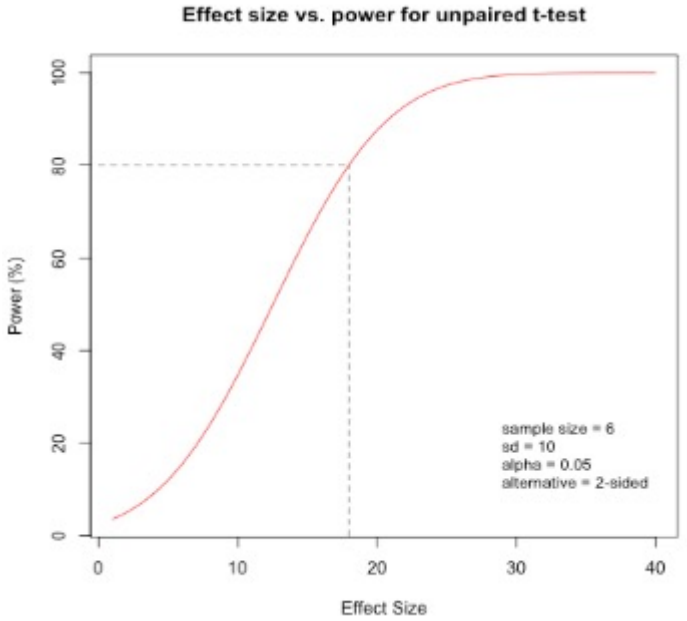
Sample size and experimental power

WHY DO YOU NEED REPLICATES?

CALCULATING APPROPRIATE SAMPLE SIZES

- Power calculations
- Planning for precision
- Resource equation



EXPERIMENTAL POWER

- **Power**: the **probability** of detecting an **effect** of a specified size if present.
  - Identify and control the **sources of variability**
  - Power calculations estimate sample size required to detect an effect *if degree of variability is known*
  - Using **appropriate numbers** of samples (sample size/replicates)
  - If adding samples increases variability, that alone won't add power!

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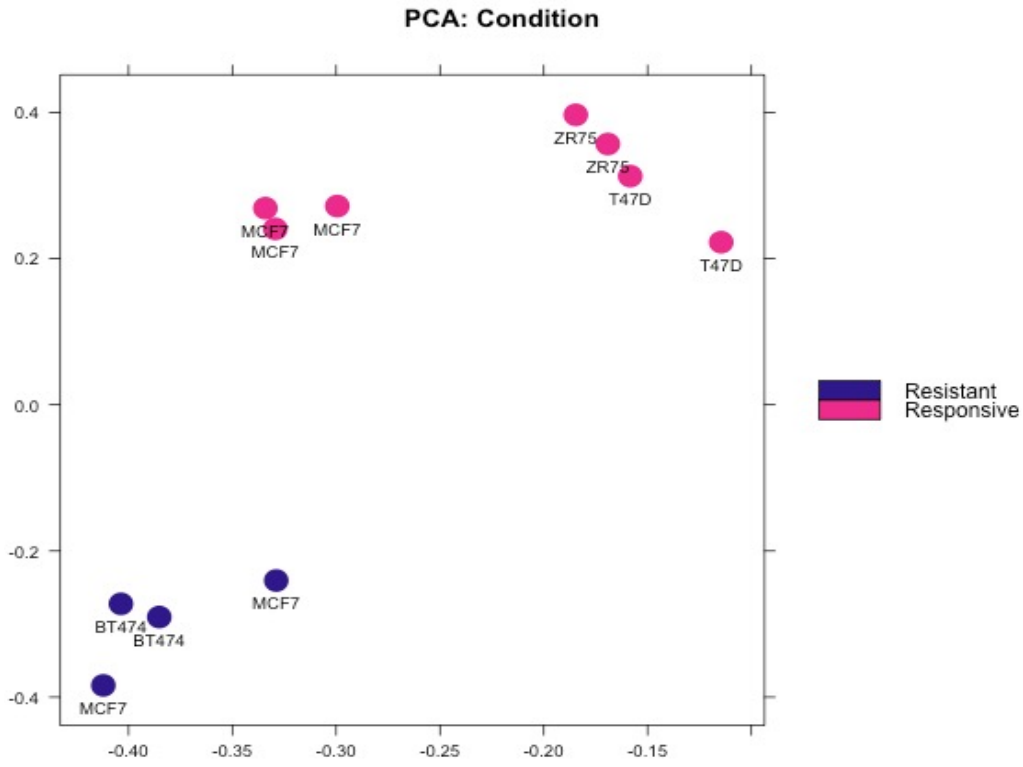
Types of Replication

BIOLOGICAL REPLICATION:

- *In vivo:*
  - Patients
  - Mice
- *In vitro:*
  - Different cell lines
  - Re-growing cells (passages)



TECHNICAL REPLICATION:

- Experimental protocol
- Measurement platform (i.e. sequencer)

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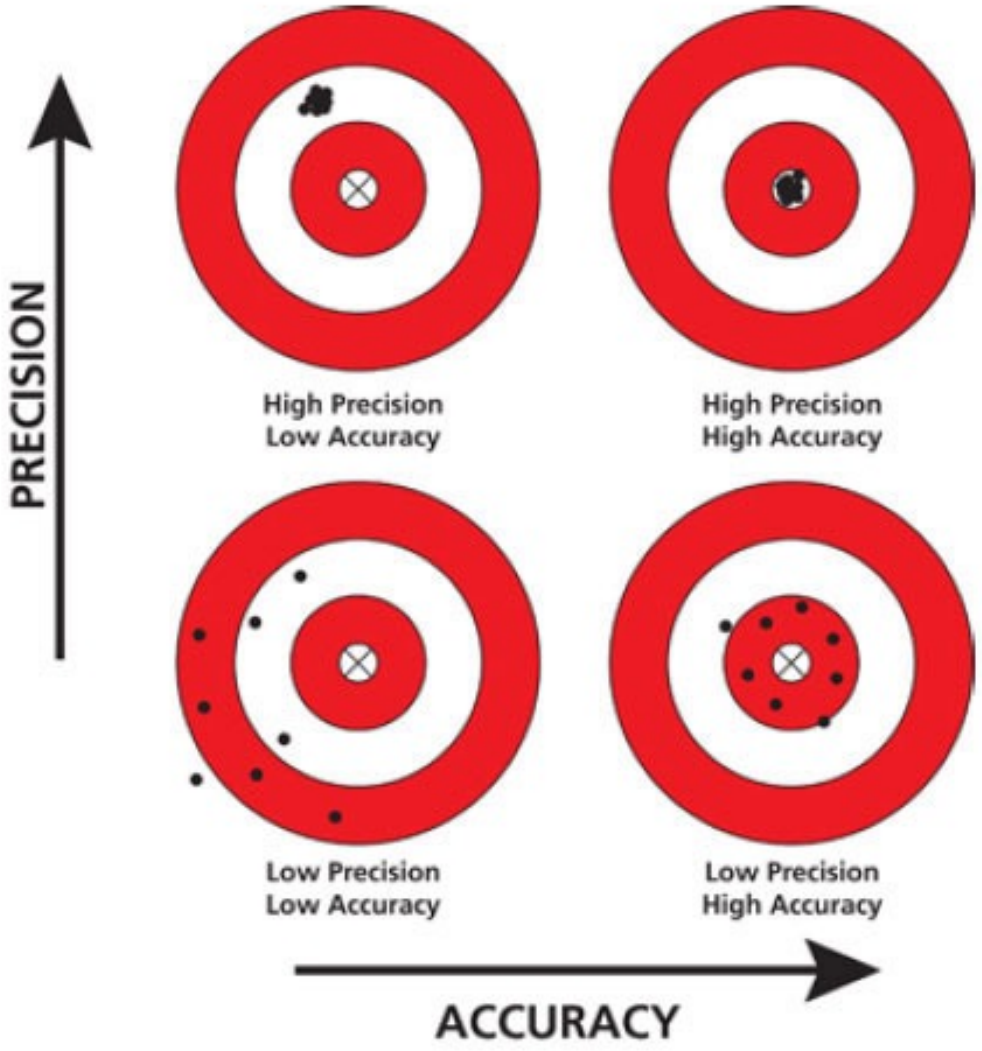
Precision, Accuracy, Confounders, and Bias

Precision and Accuracy



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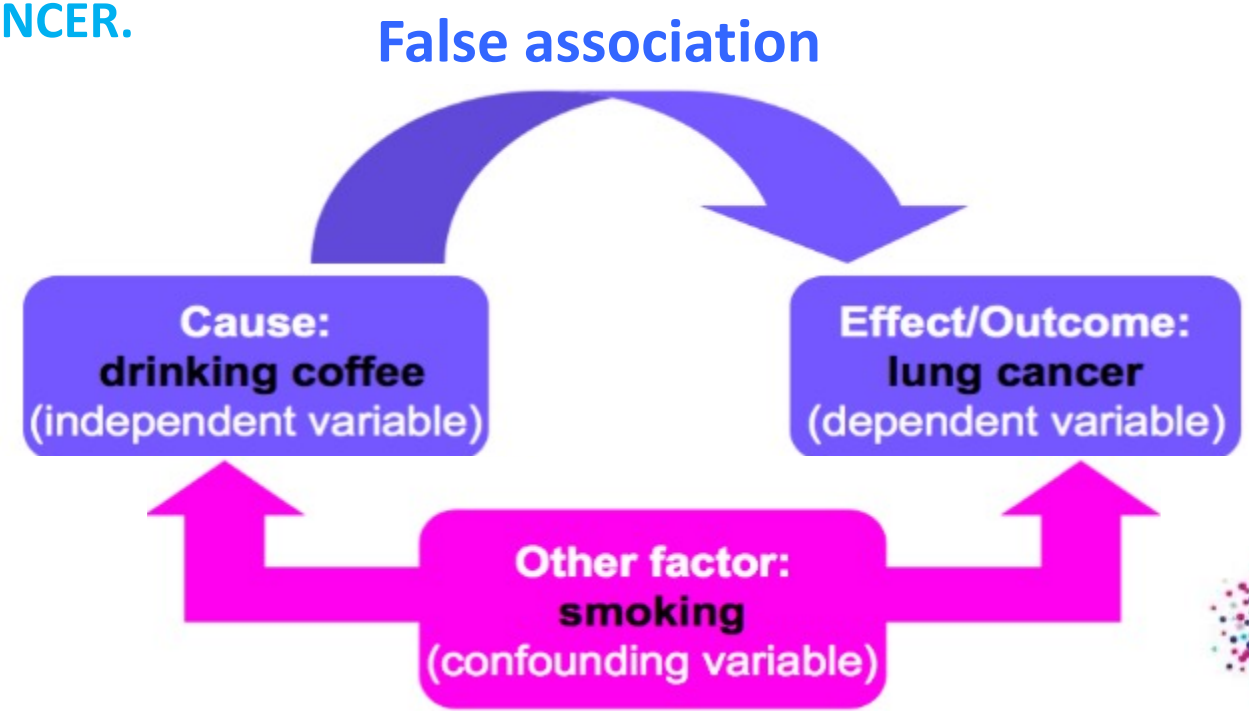
Confounding Factors

ALSO KNOWN AS EXTRANEOUS, HIDDEN, LURKING OR MASKING FACTORS, OR THE THIRD VARIABLE OR MEDIATOR VARIABLE.

MAY MASK AN ACTUAL ASSOCIATION OR FALSELY DEMONSTRATE AN APPARENT ASSOCIATION BETWEEN THE INDEPENDENT & DEPENDENT VARIABLES.

HYPOTHETICAL EXAMPLE WOULD BE A STUDY OF COFFEE DRINKING AND LUNG CANCER.



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Confounding Factors

OTHER EXAMPLES:

- Democrats were less satisfied with their sex lives than Republicans. (ABC poll report).
- Overweight (not obese) people have longer life expectancy than thin people
  (US Centre for Disease Control).

TECHNICAL CONFOUNDING FACTORS

AN EXPRESSION OF CONCERN HAS BEEN PUBLISHED ON THIS PAPER; SEE LAST PAGE

Sciencexpress Report

Genetic Signatures of Exceptional Longevity in Humans

Paola Sebastiani,[1]* Nadia Solovieff,[1] Annibale Puca,[2] Stephen W. Hartley,[1] Efthymia Melista,[3] Stacy Andersen,[4] Daniel A. Dworkis,[3] Jemma B. Wilk,[5] Richard H. Myers,[5] Martin H. Steinberg,[6] Monty Montano,[3] Clinton T. Baldwin,[6,7] Thomas T. Perls[4]*

[1]Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA. [2]IRCCS Multimedica, Milano, Italy; Istituto di Tecnologie Biomediche, Consiglio Nazionale delle Ricerche, Segrate, 20122, Italy. [3]Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA. [4]Section of Geriatrics, Department of Medicine, Boston University School of Medicine and Boston Medical Center, Boston, MA 02118, USA. [5]Department of Neurology, Boston University School of Medicine, Boston, MA 02118, USA. [6]Departments of Medicine and Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA 02118, USA. [7]Center for Human Genetics, Boston University School of Medicine, Boston, MA 02118, USA.

- GWAS STUDY: 800 CENTENARIANS VS. CONTROLS
- FOUND 150 SNPS PREDICTING CENTENARIANS WITH 77 % ACCURACY
- PROBLEM: THEY USED DIFFERENT SNP CHIPS FOR CENTENARIANS AND CONTROLS
- RETRACTED IN 2011 FOLLOWING INDEPENDENT REVIEW AND QC OF DATA

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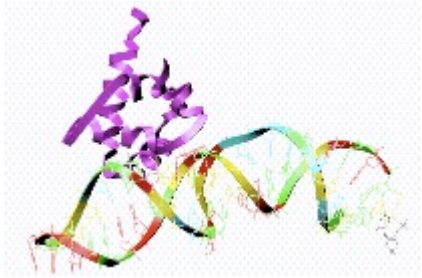
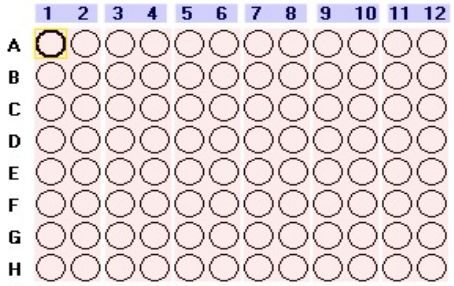
http://www.the-scientist.com/blog/display/57558/

Technical Confounding Factors: Batch Effects

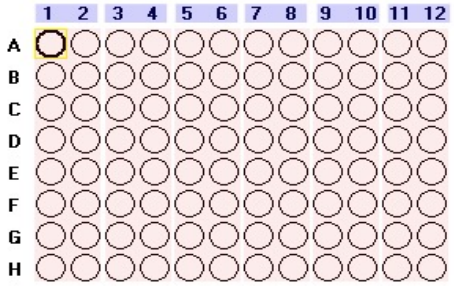


RNA Extraction

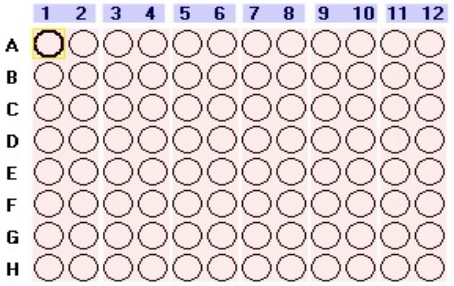
Day1, Plate 1



Day2, Plate 2



Day3, Plate 3



Control

Treatment 1

Treatment 2

The difference between Control, Treatment 1 and Treatment 2 is confounded by **day** and **plate**.

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Addressing confounding batch effects

RANDOMISATION

- Statistical analyses assume randomised comparisons
- May not see issues caused by non-randomised comparisons
- Make every decision *random* not *arbitrary*
- Caveat: over-randomization can increase error

BLINDING

- Especially important where subjective measurements are taken
- Potentially multiple degrees of blinding (*eg*. double-blinding)

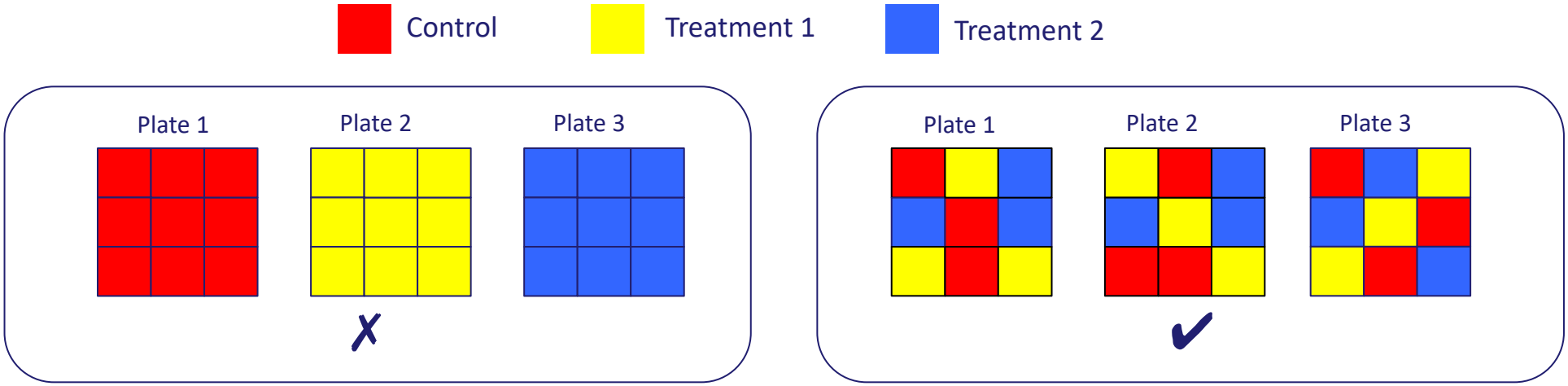
DETECTION VS CORRECTION

Addressing confounding batch effects

Rand

Randomised Block Design

Blocking is the arranging of *experimental units* in groups (blocks) that are similar to one another.



RBD across plates so that each plate contains spatially randomised **equal proportions** of:

- Control
- Treatment 1
- Treatment 2

controlling plate effects.

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Randomised Block Design

Good design example: Alzheimer's study from GlaxoSmithKline

Plate effects by *plate*

Left PCA plot show *large plate effects*.
Each colour corresponds to a different plate

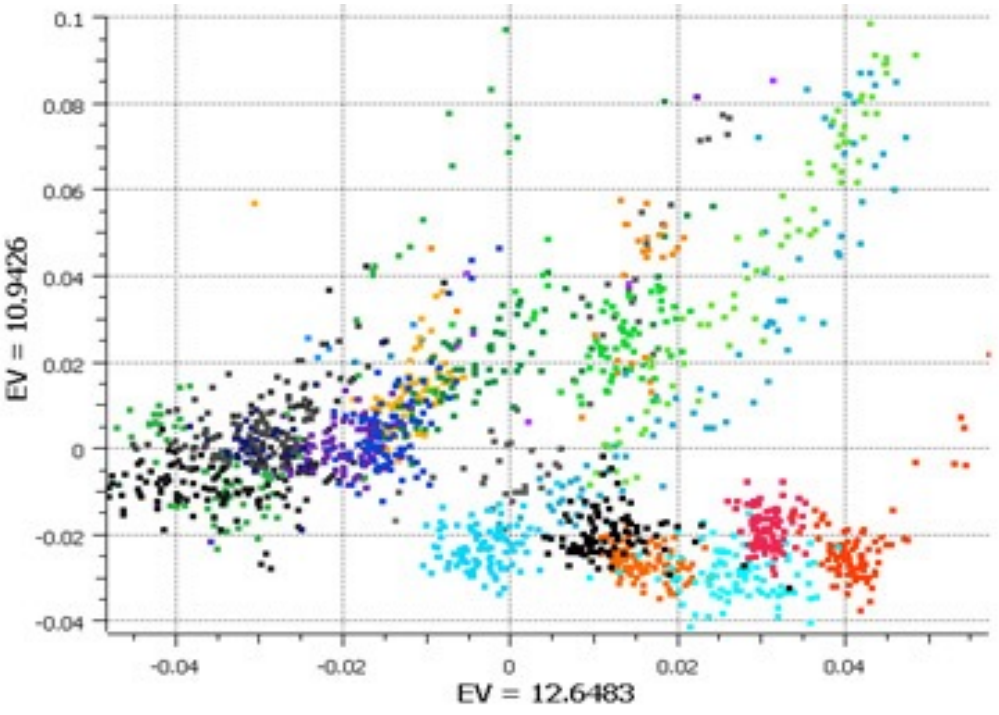
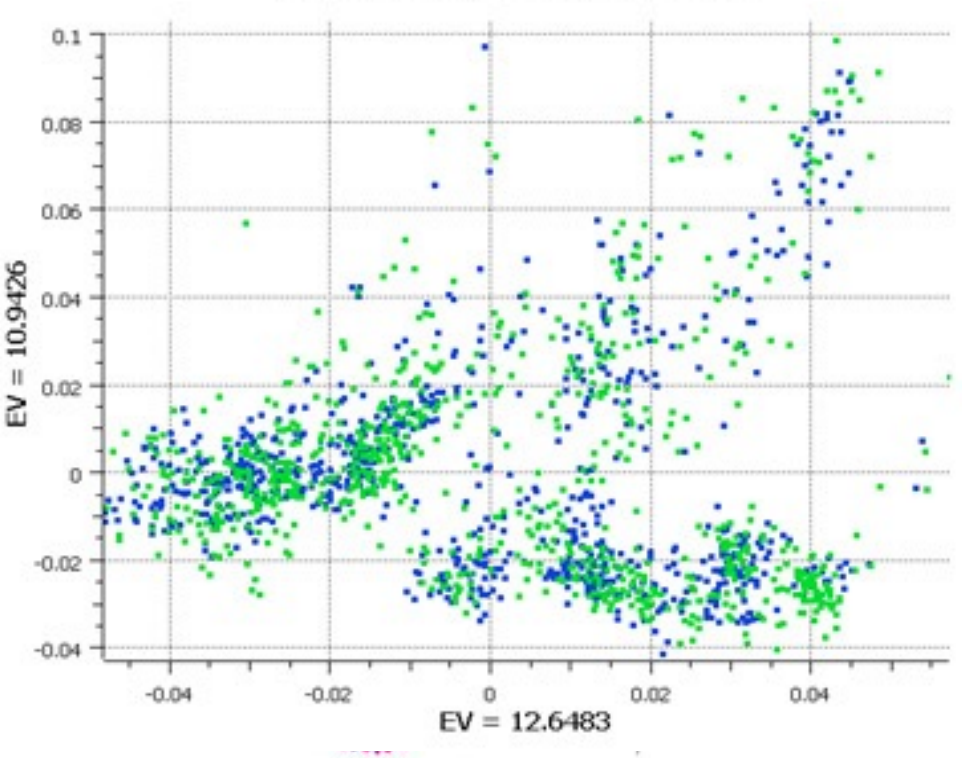


Plate effects by *case/control*

Right PCA plot shows each plate cluster contains *equal proportions* of cases (blue) and controls (green).



http://blog.goldenhelix.com/?p=322

Experimental Controls

Experimental Controls

CONTROLLING ERRORS

- –Type I: False Positives (reject true H0)
  - Use Negative controls: A group that should have minimal or no effect
- Type II: False Negative (fail to reject a false H0)
  - Use Positive controls: A group where known response expected

TECHNICAL CONTROLS

- Detect/correct technical biases
- Normalise measurements (quantification) e.g. spike-ins

Examples of Experimental Controls

- **WILD-TYPE ORGANISM (KNOCKOUTS)**
- **INACTIVE siRNA (SILENCING)**
- **VEHICLE (TREATMENTS)**
- **INPUT: FRAGMENTED CHROMATIN (ChIP)**
- **SPIKE-INS (QUANTIFICATION/NORMALISATION)**
- **“GOLD STANDARD” DATAPOINTS**
- **MULTI-LEVEL CONTROLS**
  - e.g. contrast Vehicle/Input vs. Treatment/Input

Design Parameters for Sequencing Experiments

Design Issues: Sequencing Experiments

PLATFORMS

LIBRARY PREPS

MULTIPLEXING AND POOLING STRATEGIES

SINGLE-END VS PAIRED END

SEQUENCING DEPTH

- Coverage
- Lanes

VALIDATION

- Knock-downs
- Pull-downs

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Experimental Design process at CRUK-CI

CRUK-CI Experimental Design Process

- Students required to take (this) Experimental Design class
- All sequencing and proteomics experiments require experimental design review meeting
  - Simple form with key aspects of experiment
  - Attended by Scientists, Genomics/Proteomics Core, Bioinformatics Core, Statistician
  - Project opened in LIMS afterwards
- Randomisation and Layouts
  - Checkpoint for experiment
  - Project cleared for sample submission
- Keys:
  - Form and meeting not onerous
  - (Currently) not chargeable
  - Scientists agree process improves experiments!

Experimental Design Meetings - Genomics

TUESDAY 30 MIN SLOTS (2:00-3:00PM) WITH BIOINFORMATICS GENOMICS/PROTEOMICS CORES

REQUIREMENTS:

- Email CRIExperimentalDesign@cruk.cam.ac.uk to request meeting
- Fill in Experimental Design Form and return 1 week prior to meeting
- **Your attendance**
- Provide **project *background*** (a few slides from you)

DISCUSSION:

- Planning, time-scale, cost, aims, scope, questions
- Choosing the correct technology
- Effect size & Sample-size calculation?
- Sample collection and processing methods
- Sample information (meta-data) collection
- Randomisation, Blocking and Replication issues
- Technical issues e.g. what sequencing depth?
- Will Bioinformatics Core help with/do analysis?
- Analysis deliverables

Experimental Design Meetings - Proteomics

TUESDAY 30 MIN SLOTS (2:00-3:00PM) WITH BIOINFORMATICS GENOMICS/PROTEOMICS CORES

REQUIREMENTS:

- Email ProteomicsProjectDesign@cruk.cam.ac.uk to request meeting
- Fill in ProteomicsMetadataTemplate.xls **Your attendance**
- Provide **project *background*** (a few slides from you)

DISCUSSION:

- Planning, time-scale, cost, aims, scope, questions
- Choosing the correct technology
- Sample collection and processing methods
- Sample information (meta-data) collection
- Randomisation, Blocking and Replication issues
- Will Bioinformatics Core help with/do analysis?
- Analysis deliverables

Experimental Design Guide

- HTTPS://SHAREPOINT.CRI.CAMRES.ORG/SITES/BIOINFORMATICS/PUBLIC/INRODUCTIONTOEXPERIMENTALDESIGN/EXPERIMENTALDESIGNMANUAL.PDF
- TINYURL.COM/CRUK-EDESIGN

Practicals

1. Genomic/Clinical: Identification of prognostic biomarkers in human prostate cancer patients (Chandu/Stéphane)
2. RNA-seq/Animal: Effects of mutant vs wildtype HHEX in liver and brain development (Abbi/Mark)
3. Quantitative Proteomics/Cultured Cells: AR interactome differences between drug responsive/resistant conditions (Rory)
4. ChIP-seq/Animal: Evolution of transcription factor binding in mouse strains (Ash/Mark)