Experimental Design & Ethics

S1.W1.1. Introduction to experimental design

- Elements to consider in experimental design

S1.W1.2. Experimental design I: Elements of experimental design

- Replicates
  - **techinical replicate**: 同一测试方法多测几次
  - **biological replicate**: 多个个体/样本
  - **method replicate**: 不同实验方法重新验证同个实验目的
- Randomisation and Stratification方法
  - If you can (and want to), fix a variable
  - If you do not fix a variable, stratify it
  - If you can neither fix nor stratify a variable, randomise it.

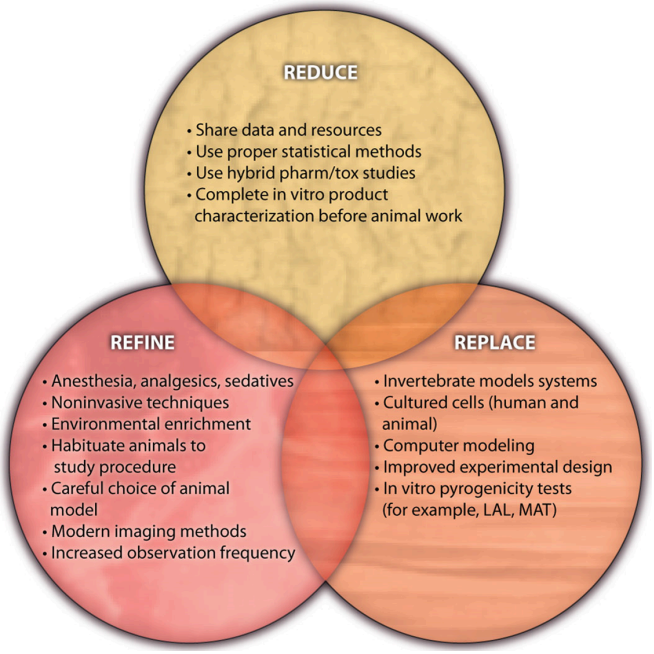
S1.W1.3. Bioethics I: Scientific integrity

- 5 principles:
  - 1.Truthfulness (Trustworthiness/Honesty)
  - 2.Diligence (You did what you claim to have done + did it properly!)
  - 3.Objectivity (No conflict of interest - Political/Economic/ Commercial/Bias)
  - 4.Circumspection (Avoid over-optimism and exaggeration, “We think” vs “we hope” the data shows x.)
  - 5.Collegiality (Equal treatment of data regardless of status of authors + all who contributed are credited).
- Hawthorne Effect: 指人们在被观察或研究时，其行为会发生改变

S1.W2.1. Bioethics II: Animal Experimentation

- Mouse model limitations
  - Rats lack gall bladders

    - Vitamin C is vital for only a few vertebrate species (notably humans and guinea pigs).
    - Of the 19 non-inhalation carcinogens that cause cancer in humans – only 7 do so in mice or rats.
- Developmental Areas:
    - Fundamental Biomedical Research, Applied Research, Toxicity Testing
- **The 3 R's Principle**



- Types of animal protectionist

Category (Examples of Organizations)	Beliefs about Animals	Major Goals	Primary strategies
Pro-research with animals *(Research Defense Society; AMRIC)*	•Animal experiments are essential for medical and scientific advances.	•Humane use of animals in experiments	•Encourage self-regulation by scientists within legal constraints.
Welfarists *(RSPCA, ASPA)*	•Animals as objects of Compassion deserving protection •Recognizing clear boundaries between species	•Avoidance of cruelty	•Protective legislation •Humane education •Shelters
Pragmatists *(FRAME; UFAW, Dr Hawden Trust)*	•Animals deserving of moral consideration. • Balance between human and animal interests.	•Eliminate unnecessary suffering •Reduce, replace and refine use of animals	•Public protests •Pragmatism (cooperation, negotiation and short-term compromises).
Fundamentalists *(NAVS;BUAV, PETA, Animal Aid; Advocates for animals)*	•Animals have absolute moral right to live without human interference. •Equal rights across species	•Total and immediate elimination of all animal experimentation	•Moralist Rhetoric and condemnation •Direct action and civil disobedience, •Animal sanctuaries.

S1.W2.2. Experimental design II

- **Accuracy**: 测得真实、正确数据的能力, 对应Systematic error, (TP+TN) / total
- **Precision**: 良好的可重复性 (Reproducibility), 对应Random error, TP / (TP+FP)
- **Sensitivity (recall)**: TP / (TP + FN)

- **Specificity**: TN / (TN + FP)
- **Power** of a hypothesis test: the probability that the test correctly rejects the null hypothesis (H0)
- **Type I error**: "false positive", 指当原假设（H0）实际上是正确的，但我们却错误地拒绝了; 犯第一类错误的概率即为α (自己设的significance level)
- **Type II error**: "false negative", 指原假设（H0）实际上是错误的，但我们却没有拒绝, 犯第二类错误的概率即为β, 1-β就是Power
- **Effect size**: a number measuring the strength of the relationship between two variables in a population, or a sample-based estimate of that quantity. Its significance depends of the sample size
- **Internal Standard**（内标）是一种分析化学技术，常用于定量分析。它是指在样品中加入一个已知浓度的化合物（内标物），这个化合物与目标分析物在化学性质上相似，但在样品中并不存在，也可以与目标分析物分离开来。通过比较目标分析物和内标物的峰面积或高度，并结合内标物质的已知浓度，就可以计算出被分析物的含量，这可以提高分析结果的精确性和准确性，特别是在样品的处理和测量过程中可能导致的损失和变化。
- **Significant Figures**（有效数字）是指一个数值中能表示其精确性的数字位数。有效数字包括所有已知的数字及最后一个不确定的数字。它关系到测量结果的精确性和不确定性。例如，在数值 0.00456中，有效数字为3（4、5和6），而在数值 100.0中，有效数字为4（1、0、0和小数点后的一位0）。

S1.W3.1. Experimental design III: Experimental bias

- Bias: 所有造成Systematic error的因素
  - Survivorship Bias (e.g.)
  - Sampling/Ascertainment/Selection bias：选取样本的方式不恰当
  - Experimenter bias：实验者受自身期望、偏好等主观因素影响
  - Reporting Bias：选择性报告或不客观描述解释
  - Biased Model Systems：模型自身局限、不合理假设或数据输入偏差等
  - Publication Bias：学术期刊倾向于发表显著结果研究，导致已发表研究不能全面反映领域真实情况而产生的偏差。
- 如何设计unbiased的实验?
  - Define the population from which you want to draw a conclusion
  - Define your inclusion and exclusion criteria
  - Split treatment and control group after exclusion has been done
  - Use a blinded approach
  - Run a pilot study to discover potential problems

S1.W5.1. Experimental design IV: Types

- Completely randomised design: 将实验对象完全随机地分配到不同处理组
- Randomised complete block design: 将实验对象按性质相同或相近分为若干区组，再将每个区组内的对象随机分配到不同处理组
- Split-plot design: 将实验单元分为主区和副区，分别安排不同处理因素
- Randomised Control Trial: 将研究对象随机分配到干预组和对照组，以评估干预措施效果
  - Placebo: 一种中立的控制干预，但可以与积极效果（安慰剂效应）相关联。
  - Nocebo: 一种负面的控制干预，受试者会经历负面影响
  - Hawthorne effect: 实验对象的行为会随着被观察而改变
  - Demand characteristics: 由受试者猜测研究目的并采取相应行动而产生的效应
  - 'Screw-you' effect: 实验对象的行为可能会破坏实验者的目的
- Cohort Studies
  - Retrospective (回顾性): 通过回顾过去的暴露和疾病发生情况，分析暴露因素与疾病关系
  - Prospective (前瞻性): 从现在开始追踪观察不同暴露组的研究对象，以确定疾病发生情况及与暴露因素关系
- Cross-Over design: 让研究对象在不同时期先后接受不同处理，以比较不同处理效果
- Cross-sectional: 在某一特定时间点对研究对象的特征和疾病状况进行调查
- Longitudinal: 对同一组研究对象在较长时间内进行跟踪，观察其变化
- Meta-analyses: 综合多个同类研究结果，进行定量分析以得出更具普遍性结论
- Multi-batch analyses: 对多个批次的数据进行分析，以考虑批次效应并获得更可靠结果

S1.W7.1. Experimental design V: interpolation/extrapolation and mixed effects models

- Interpolation: Constructing new data points within the range of known data
- Extrapolation: Applying your model to calculate values outside of the range of observed data sample
- Mixed effects models: 统计模型，同时包含固定效应和随机效应，用于分析具有层次结构或聚类结构的数据，能在考虑个体差异等随机因素的同时，研究固定因素对响应变量的影响
- Random effects model: 将个体或群体的效应视为随机变量，强调从总体中抽取的样本所具有的随机性，更适合于研究个体或群体间的差异及变化，以及总体特征。
- Nested design: 一种实验设计方法，指的是一个因子（如细胞）被包含或嵌套在另一个因子（如胰腺）中，且不同组的嵌套对象彼此独立，不能跨组比较。主要作用：用来区分不同来源的变异，比如区分基因型的差异、胰腺之间的差异、以及细胞之间的差异。

S1.W10.1. Introduction to experimental design framework ***

- **Experimental purpose**: BG, Question, Hypothesis, Pre
- **Model system**: Objects, A/D, (Ethics)
- **Measurement approach**: Methods, A/D
- **Group Setting**: Controls (N, P, Internal), Replicates, Variables (Independent, Nuisance, Dependent (Response)), Settings (Standardization, Randomization, Blocking)
- **Experimental output**: Data format (Img, Numeric, Omics), Data type (Quantitative, Qualitative (Categorical)), Data sharing
- **Analysis**: Data processing (filtration, normalization (e.g. β-actin/GAPDH, housekeeping pro)), Statistics, Visualization
- **Critical thinking**: Bias/Nuisance, Ethics, Reproducibility