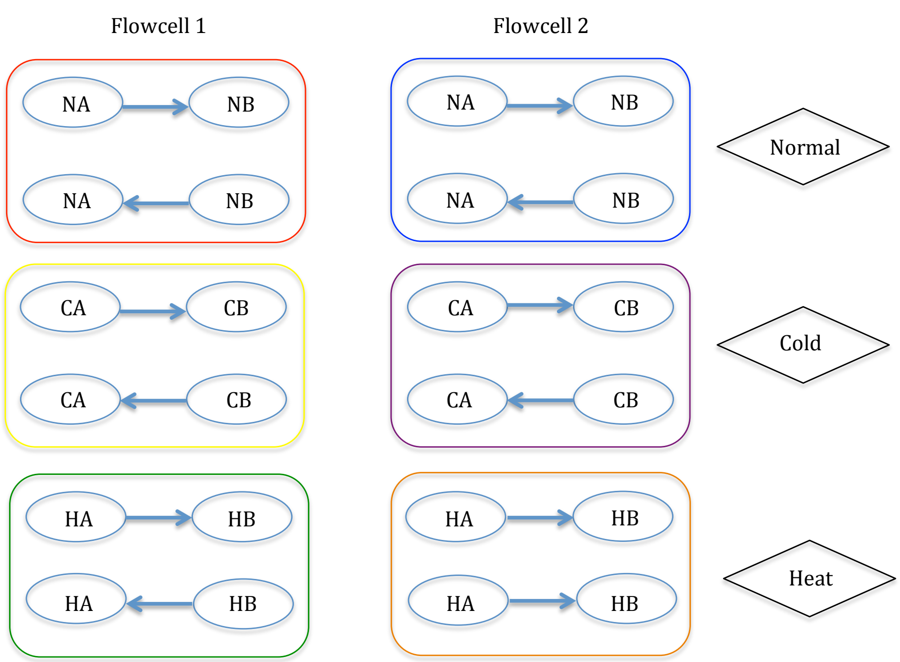
1. (a) There are two treatment factors in this experiment, temperature condition and breed.
   (b) The levels of temperature condition are normal, cold and heat; the levels of breed are breed A and breed B.
   (c) There are two types of experimental units in this experiment. Houses are the experimental units with respect to the treatment factor temperature condition because temperature conditions were randomly assigned and independently applied to houses; while chickens are the experimental units with respect to the treatment factor breed since individual chickens are the units that may be from different breeds. The number of experimental units is 6 for houses, and 24 for chickens.
   (d) The observational units should be chicken spleens obtained from each chicken. There is a one-to-one correspondence between chickens and observational units.
   (e) Houses can be considered as a pseudo-blocking factor for the treatment factor breed. Each level of breed is randomly assigned to a chicken within each house.
   (f) Based on part (c), this experiment is best described as a split-plot design. The whole-plot factor is temperature condition, the whole-plot experimental units are houses; the split-plot factor is breed, and the split-plot experimental units are chickens.

2. The rectangles indicate houses. Within each flowcell, we randomly assign 6 pairs of chickens to 6 lanes, the lane effect is partially confounded with house. Our design helps answer the primary interesting question that to find genes differentially expressed between the two breeds under each temperature condition.



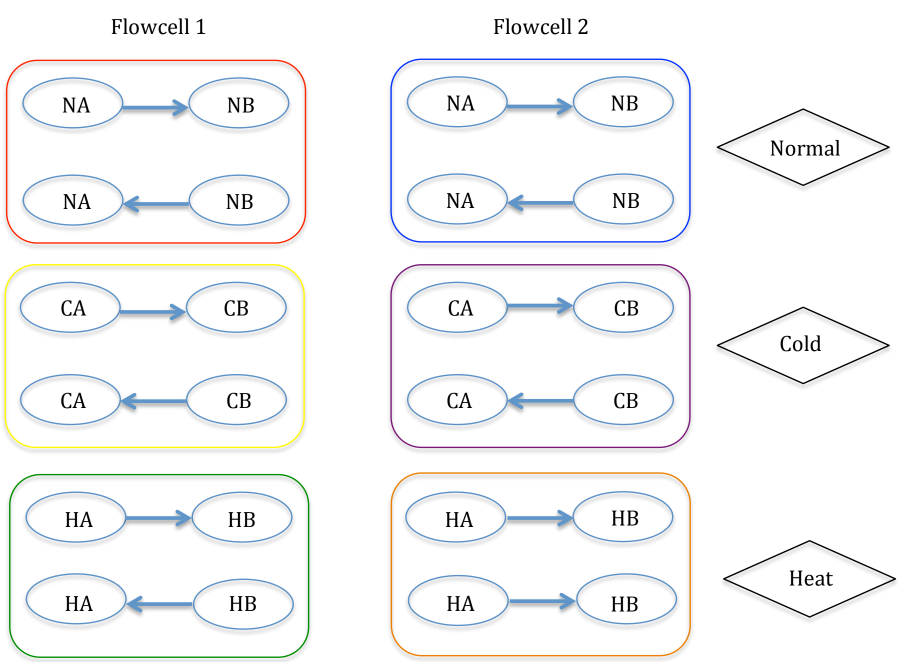
Our design is shown as above, where NA indicates the group of chickens from breed A receiving normal temperature conditon, NB indicates the group of chickens from breed B receiving normal temperature conditon; CA indicates the group of chickens from breed A receiving cold temperature conditon, CB indicates the group of chickens from breed B receiving cold temperature conditon; HA indicates the group of chickens from breed A receiving heat temperature conditon, HB indicates the group of chickens from breed B receiving heat temperature conditon.

3. **Phase 2:**
In order to not break up the whole-plot, we confound the houses with sessions so that four chickens within the same houses are processed in the same session. The first letter of each house N, C, H denote three temperature conditions (normal, cold and heat) respectively, while the second number represents the first or second house receiving the same temperature condition. The houses are randomly ordered to process. Within a given session, we confound the processing order with replicates. The design we have with this is as below:

Processing Order/ House (Session)	1	2	3	4
N1	A	B	A	B
C2	B	A	B	A
H1	A	B	A	B
H2	B	A	B	A
C1	A	B	A	B
N2	B	A	B	A

Phase 3:
Since the session effects for obtaining chicken spleens and the session effects for RNA extraction are not of scientific interest, they are intentionally confounded with each other. Furthermore, the order that RNA is extracted from samples is chosen to match the chicken spleens obtaining order. We sequence two pairs of samples for each house in two different lanes in same flowcell. The session factor can be partially confounded with lane so that the libraries from any one session are sequenced across two lanes from one flowcell. The order factor is intentionally confounded with lane (two lanes for each house) and barcode combination so that first A represents first of the two lanes and barcode 1, first B represents first of the two lanes and barcode 2, second A denotes second of the two lanes and barcode 2, while second B denotes second of the two lanes and barcode 1. The RNA-seq experimental design is as follows, where the term X_{ijkl} represents the experimental unit that received breed level X, temperature condition i, house blocking effect j, first or second of the two lanes k within each house and barcode l.

Processing Order/ House (Session)	1	2	3	4
N1	A_{N111}	B_{N112}	A_{N122}	B_{N121}
C2	B_{C211}	A_{C212}	B_{C222}	A_{C221}
H1	A_{H111}	B_{H112}	A_{H122}	B_{H121}
H2	B_{H211}	A_{H212}	B_{H222}	A_{H221}
C1	A_{C111}	B_{C112}	A_{C122}	B_{C121}
N2	B_{N211}	A_{N212}	B_{N222}	A_{N221}



4. (a) i. $E(2Y_1 + 3) = 2\mu_1 + 3$

   ii. $E(Y_1 + Y_2 + Y_3) = \mu_1 + \mu_2 + \mu_3$

   (b) i. $E(c) = c$

   ii. $Var(c) = 0$

   (c) i. $Var(Y_1 + Y_2) = 2\sigma^2$

   ii. $Var\{(Y_1 + Y_2)/2\} = \frac{1}{4}(\sigma^2 + \sigma^2) = \frac{\sigma^2}{2}$

   (d) i. $Var(\sum_{i=1}^{n} Y_i) = n\sigma^2$

   ii. $Var(\bar{Y}) = Var(\frac{1}{n}\sum_{i=1}^{n} Y_i) = \frac{1}{n^2}Var(\sum_{i=1}^{n} Yi) = \frac{\sigma^2}{n}$

   (e) i. For design 1,

$$
\begin{aligned}
Var(\bar{Y}_{1\cdots} - \bar{Y}_{2\cdots}) &= Var\left((\mu + \tau_1 + \bar{\delta}. + \bar{s}. + \bar{b}_{1}. + \bar{e}_{1\cdots}) - (\mu + \tau_2 + \bar{\delta}. + \bar{s}. + \bar{b}_{2}. + \bar{e}_{2\cdots})\right) \\
&= Var\left((\tau_1 - \tau_2) + (\bar{b}_{1}. - \bar{b}_{2}.) + (\bar{e}_{1\cdots} - \bar{e}_{2\cdots})\right) \\
&= Var\left((\bar{b}_{1}. - \bar{b}_{2}.) + (\bar{e}_{1\cdots} - \bar{e}_{2\cdots})\right) && \text{Fixed effects have no contribution to variance} \\
&= 2Var(\bar{b}_{1}.) + 2Var(\bar{e}_{1\cdots}) && \text{Due to independence and equal variance} \\
&= \frac{2\sigma_b^2}{8} + \frac{2\sigma_e^2}{8} \\
&= \frac{\sigma_b^2 + \sigma_e^2}{4}.
\end{aligned}
$$

   ii. For design 2,

$$
\begin{aligned}
Var(\bar{Y}_{1\cdots} - \bar{Y}_{2\cdots}) &= Var\left((\mu + \tau_1 + \bar{\delta}. + \bar{s}. + \bar{b}_{1}. + \bar{e}_{1\cdots}) - (\mu + \tau_2 + \bar{\delta}. + \bar{s}. + \bar{b}_{2}. + \bar{e}_{2\cdots})\right) \\
&= Var\left((\tau_1 - \tau_2) + (\bar{b}_{1}. - \bar{b}_{2}.) + (\bar{e}_{1\cdots} - \bar{e}_{2\cdots})\right) \\
&= 2Var(\bar{b}_{1}.) + 2Var(\bar{e}_{1\cdots}) \\
&= \frac{2\sigma_b^2}{4} + \frac{2\sigma_e^2}{8} \\
&= \frac{2\sigma_b^2 + \sigma_e^2}{4}.
\end{aligned}
$$