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Optimizing Animal Randomization for In Vivo Experiments

Forked from a private protocol



DOI

<https://dx.doi.org/10.17504/protocols.io.261ge19bov47/v1>

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External link: <https://randmice.com>

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Protocol status: Working

We use this protocol and it's working

Created: February 09, 2026

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Keywords: randomization, mice, animal, animal randomization with randmice, animal randomization, randomization protocol, randmice, animals between the group, number of animal, animal, experiment, randomization, optimizing animal randomization for in vivo experiment, optimizing animal randomization, animals per group, combinations of animal assignment, experimental group, animal assignment, random assignment, laboratory animal, vivo experiment, mice, online tool randmice, testing combination, scale experiment, group, lowest covariate imbalance, distribution with the lowest covariate imbalance

Abstract

This protocol describes a standardized procedure to distribute laboratory animals (mice, rats) into experimental groups using the online tool Randmice (<https://randmice.com>). The algorithm minimizes inter-group heterogeneity by iteratively testing combinations of animal assignments and selecting the distribution with the lowest covariate imbalance. This approach reduces operator bias, improves reproducibility, and supports compliance with the 3Rs principle (Replacement, Reduction, Refinement) as required by European Directive 2010/63/EU. The tool is optimized for small-scale experiments ($N < 12$ animals per group), where random assignment leads to high inter-group heterogeneity (Bertsimas et al., 2015).



Before randomization

- 1 Identify all animals with a unique tag
- 2 Measure the covariates to be balanced across groups. Example of common covariates are: body weight, tumor volume (left and/or right flank), blood pressure, any other quantitative variable relevant to the experiment

Run randomization

- 3 Open <https://randmice.com> via a web browser



LABTOOL

I can help to

Reducing heterogeneity for *in vivo* experiments

Randomize

FREE



[See use cases](#)

Intro page at randmice.com

- 4 Login if you have an account. Otherwise, enter your email address to access the form.
- 5 You have now access to the form to enter your data



Enter your data

Email*

sylvain.carlioz@gmail.com

Number of groups*

2

Number of iterations*

10e6 -- Very fast (<2min) & good results



Own reference

☒ Compare with original balancing

Data

+ Add column

	Tag	Covariate1	Covariate2	Covariate3
1	Tag			
2				
3				
4				
5				
6				
7				
8				

▼ See instructions

Randomize

6 Enter the **Number of groups**



- 7 Choose the **Number of iterations** for the randomization. 10^6 is standard and run very fast (less than 2min) and generates good results. 10^7 is a bit longer and gives excellent results.
- 8 Fill your **Own reference** if needed
- 9 Check/uncheck **Compare with original balancing** if you want to compare your own randomization with the one from randmice.
- 10 Enter your data. The 1st fill is a header that describes each column. It can be any text. Tag must be unique identifier. See instructions link for more information.
- 11 Click **Randomize** when everything is ready.

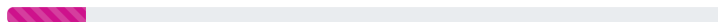
The randomization will proceed. You can close the window if you want, the report will be sent in your mailbox when it is ready.



Menu ▾

🔄 Your randomization is now running...

You will **receive an email** with the PDF report when the balancing is complete.
This window is for monitoring the progression and can be safely closed.



DON'T FORGET TO SIGN IN !

You can [create an account](#) to save all your work in one place and easily access to your previous reports and more whenever you need.



CITE US !

As per our Terms of Use for the free mode, we kindly ask you to cite our tool in the published paper, preferentially in the "Materials and Methods" section of your publication. This will help to continue our work and keep a free version. Thank you for your support!



SHARE !

Help Us Spread the Word! If our tool helped you better run your experiment, we'd love to hear about it! Share your experience on social media and help other researchers to discover a smarter way to improve their study design. Tag us and use the hashtag **#RunBetterExperiments** – we can't wait to see your work!

12 In your mailbox, you will find the report.

Below is an example:

Randomization Report

Powered by:



Reference

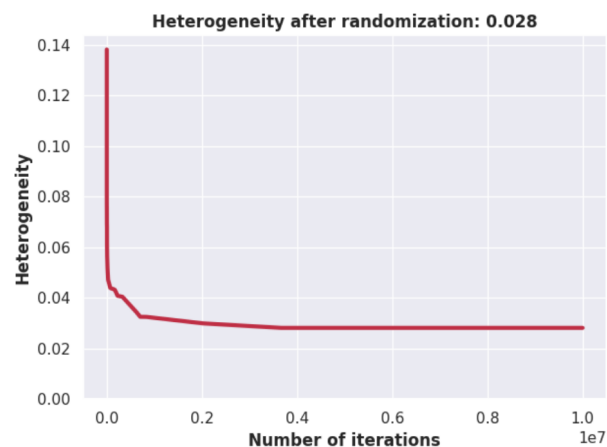
Date 23/05/2025 12:54
 Randmice reference 20250523_1244_dece2d2d
 Own reference

Parameters of the randomization

Number of covariates 2
 Number of animals 48
 Number of groups 6
 Number of iterations 1.0E+07
 Execution time 617.0227s
 Comparison with existing True
 Algorithm Bruteforce. Minimize heterogeneity.
 Price FREE

Analysis

Convergence of the algorithm

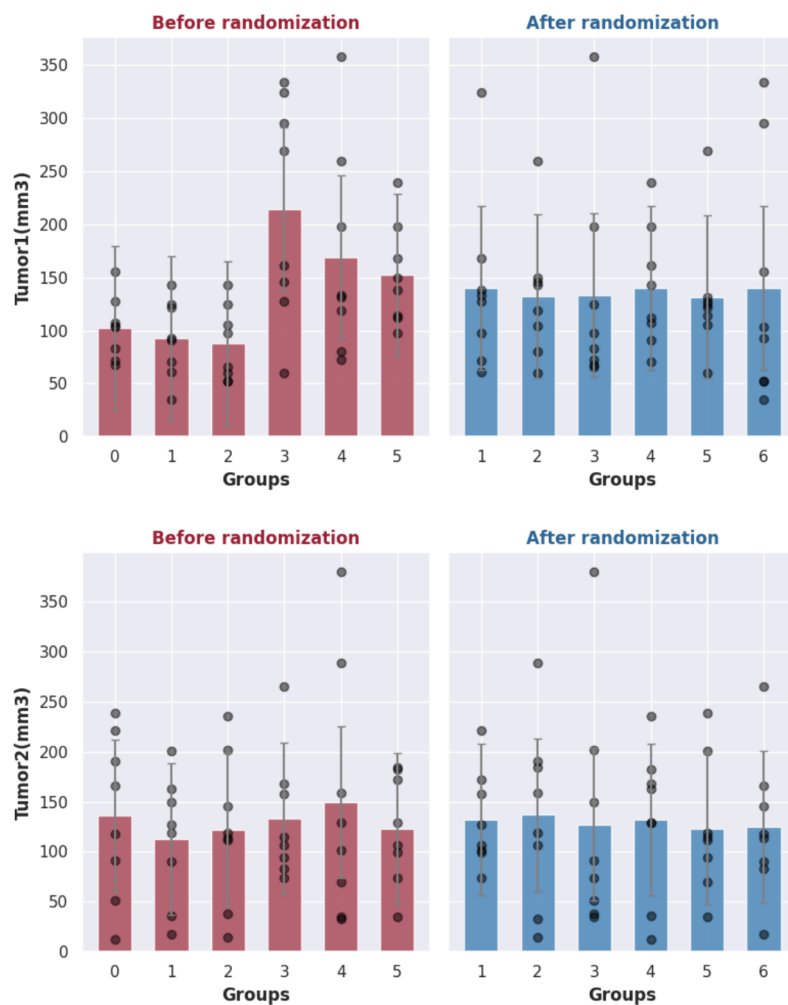


For the definition of heterogeneity, please see at the end of the report.

Randomization Report

Randomization

Individual covariates



Randomization Report

Groups

Group #1

Tag	Tumor1(mm3)	Tumor2(mm3)
145	71.241000	221.675000
150	60.783000	126.683000
153	127.070000	157.969000
197	323.870000	74.359000
164	133.275000	101.743000
142	97.687000	106.331000
185	167.752000	171.788000
152	138.570000	99.764000
mean	140.031000	132.539000
std	77.125159	45.084718

Group #2

Tag	Tumor1(mm3)	Tumor2(mm3)
176	104.02100	190.393000
172	143.27800	118.588000
178	59.56500	14.132000
175	145.68800	107.103000
193	259.33200	289.193000
168	80.05200	158.372000
184	119.25800	32.801000

Randomization Report

159	149.78200	184.098000
mean	132.62200	136.835000
std	56.71387	83.572646

Group #3

Tag	Tumor1(mm3)	Tumor2(mm3)
191	67.403000	90.791000
146	83.222000	51.581000
183	124.437000	149.117000
129	97.728000	38.022000
131	66.057000	201.678000
163	358.042000	379.814000
166	72.153000	34.606000
195	197.723000	74.350000
mean	133.345625	127.494875
std	94.282813	109.660727

Group #4

Tag	Tumor1(mm3)	Tumor2(mm3)
158	107.279000	12.111000
194	90.961000	35.823000
133	70.334000	163.191000
177	142.504000	235.451000
167	161.079000	167.860000

Randomization Report

140	197.967000	128.944000
151	239.128000	182.265000
200	112.041000	129.517000
mean	140.161625	131.895250
std	53.323849	69.934167

Group #5

Tag	Tumor1(mm3)	Tumor2(mm3)
181	127.291000	238.556000
180	121.916000	200.236000
173	124.263000	112.227000
154	105.722000	118.632000
137	60.306000	114.396000
187	269.400000	94.729000
155	131.393000	70.000000
169	114.317000	34.979000
mean	131.826000	122.969375
std	56.131387	62.132613

Group #6

Tag	Tumor1(mm3)	Tumor2(mm3)
196	155.176000	117.370000
170	103.717000	165.791000
132	93.085000	17.473000

Randomization Report

139	35.077000	89.780000
136	52.562000	113.906000
149	52.497000	145.278000
130	295.646000	265.118000
199	334.280000	82.682000
mean	140.255000	124.674750
std	107.254764	67.529551



Randomization Report

Material and methods

Algorithm: **Bruteforce. Minimize heterogeneity.**

Animals were randomized using Randmice (<https://randmice.com>) on the basis of minimizing the heterogeneity between the groups. The algorithm randomly distribute all animals between the different groups and calculates the mean of each covariates and minimize the difference between the maximum and the minimum of all groups. Between 10^6 to 10^8 iterations can be performed to find the optimum.

13 You will also receive a CSV file to import if needed



	Group	Tag	Tumor1 (mm3)	Tumor2 (mm3)
1	1	145	71.241	221.675
2	1	150	60.783	126.683
3	1	153	127.07	157.969
4	1	197	323.87	74.359
5	1	164	133.275	101.743
6	1	142	97.687	106.331
7	1	185	167.752	171.788
8	1	152	138.57	99.764
9	2	176	104.021	190.393
10	2	172	143.278	118.588
11	2	178	59.565	14.132
12	2	175	145.688	107.103
13	2	193	259.332	289.193
14	2	168	80.052	158.372
15	2	184	119.258	32.801
16	2	159	149.782	184.098
17	3	191	67.403	90.791
18	3	146	83.222	51.581
19	3	183	124.437	149.117
20	3	129	97.728	38.022
21	3	131	66.057	201.678
22	3	163	358.042	379.814
23	3	166	72.153	34.606
24	3	195	197.723	74.35
25	4	158	107.279	12.111
26	4	194	90.961	35.823



27	4	133	70.334	163.191
28	4	177	142.504	235.451
29	4	167	161.079	167.86
30	4	140	197.967	128.944
31	4	151	239.128	182.265
32	4	200	112.041	129.517
33	5	181	127.291	238.556
34	5	180	121.916	200.236
35	5	173	124.263	112.227
36	5	154	105.722	118.632
37	5	137	60.306	114.396
38	5	187	269.4	94.729
39	5	155	131.393	70.0
40	5	169	114.317	34.979
41	6	196	155.176	117.37
42	6	170	103.717	165.791
43	6	132	93.085	17.473
44	6	139	35.077	89.78
45	6	136	52.562	113.906
46	6	149	52.497	145.278
47	6	130	295.646	265.118
48	6	199	334.28	82.682
49				



Protocol references

Bakhos Jneid *et al.*, Selective STING stimulation in dendritic cells primes antitumor T cell responses.*Sci. Immunol.* **8**, eabn6612(2023).DOI:[10.1126/sciimmunol.abn6612](https://doi.org/10.1126/sciimmunol.abn6612)