Mar 19, 2019

Creation and maintenance of Ashworth outcrossed DGRP population

Fergal Waldron[1], katy monteith[1], Pedro Vale[1]

[1]University of Edinburgh

dx.doi.org/10.17504/protocols.io.zbuf2nw

Vale Lab

Pedro Vale
University of Edinburgh

ABSTRACT

Infomration about the creation and maintenance of Ashworth outcrossed DGRP population

DOCX

DGRP outcrossed population foundation and maintance methods.docx

Document body

Information on creation and maintenance of Ashworth outcrossed DGRP population

Fergal M Waldron, Katy M Monteith, Pedro F Vale

The Ashworth outcrossed DGRP population was founded by FMW on 15/10/14, and is derived from 113 inbred DGRP lines sampled from a wild population in Raleigh, NC, USA [1].

The DGRP outcrossed population was founded with genetic contributions from the following 113 DGRP lines; RAL-28, RAL-31, RAL-48, RAL-49, RAL-57, RAL-59, RAL-69, RAL-75, RAL-83, RAL-91, RAL-93, RAL-101, RAL-129, RAL-138, RAL-149, RAL-153, RAL-158, RAL-189, RAL-195, RAL-208, RAL-217, RAL-228, RAL-237, RAL-239, RAL-280, RAL-287, RAL-288, RAL-301, RAL-303, RAL-304, RAL-306, RAL-309, RAL-310, RAL-317, RAL-321, RAL-324, RAL-348, RAL-350, RAL-352, RAL-354, RAL-358, RAL-360, RAL-361, RAL-365, RAL-366, RAL-373, RAL-375, RAL-377, RAL-379, RAL-380, RAL-381, RAL-382, RAL-386, RAL-390, RAL-392, RAL-395, RAL-397, RAL-399, RAL-405, RAL-406, RAL-409, RAL-426, RAL-427, RAL-437, RAL-439, RAL-443, RAL-486, RAL-491, RAL-492, RAL-502, RAL-508, RAL-509, RAL-517, RAL-528, RAL-530, RAL-535, RAL-555, RAL-559, RAL-563, RAL-566, RAL-575, RAL-584, RAL-589, RAL-627, RAL-630, RAL-634, RAL-703, RAL-712, RAL-716, RAL-732, RAL-765, RAL-774, RAL-776, RAL-786, RAL-796, RAL-805, RAL-808, RAL-818, RAL-820, RAL-821, RAL-822, RAL-832, RAL-852, RAL-853, RAL-855, RAL-859, RAL-879, RAL-882, RAL-884, RAL-897, RAL-907, RAL-908, RAL-913.

To maximise the genetic contribution of each of the founder lines to the final outcrossed population, pairwise crosses between population founder lines were carried out to produce offspring which could then be pooled into a population cage for the 1stgeneration of outcrossing. Whilst a minimum of 57 pairwise crosses would encompass inclusion of all 113 founder lines, 100 pairwise crosses were carried out as a precautionary measure against a number of crosses failing to produce offspring (an upper limit of 100 was dictated by feasibility). Pairwise crosses were set up using 2 virgin females crossed to 2 males. All virgin females and males were age-matched controlled (1-6 days old when crosses were set up). Pairwise crosses were set up in standard Lewis medium containing vials and placed at 25°C for 5 days after which adults were removed.

For pairwise crosses, DGRP outcrossed population founder lines were randomly selected to contribute females or males for the following 100 crosses (scheme is “2 virgin females from line” x “2 males from line”): RAL-390 x RAL-381, RAL-280 x RAL-301, RAL-913 x RAL-365, RAL-796 x RAL-712, RAL-589 x RAL-49, RAL-350 x RAL-382, RAL-853 x RAL-158, RAL-288 x RAL-855, RAL-49 x RAL-366, RAL-303 x RAL-908, RAL-101 x RAL-303, RAL-712 x RAL-426, RAL-321 x RAL-732, RAL-377 x RAL-101, RAL-380 x RAL-879, RAL-820 x RAL-324, RAL-882 x RAL-535, RAL-439 x RAL-634, RAL-83 x RAL-409, RAL-28 x RAL-75, RAL-409 x RAL-832, RAL-879 x RAL-237, RAL-237 x RAL-239, RAL-443 x RAL-776, RAL-908 x RAL-627, RAL-59 x RAL-584, RAL-365 x RAL-796, RAL-634 x RAL-405, RAL-392 x RAL-852, RAL-129 x RAL-350, RAL-317 x RAL-306, RAL-427 x RAL-528, RAL-373 x RAL-502, RAL-386 x RAL-28, RAL-304 x RAL-392, RAL-774 x RAL-555, RAL-306 x RAL-386, RAL-310 x RAL-309, RAL-832 x RAL-287, RAL-405 x RAL-280, RAL-57 x RAL-774, RAL-627 x RAL-228, RAL-397 x RAL-821, RAL-348 x RAL-492, RAL-437 x RAL-443, RAL-91 x RAL-31, RAL-352 x RAL-575, RAL-301 x RAL-390, RAL-48 x RAL-897, RAL-575 x RAL-808, RAL-426 x RAL-373, RAL-375 x RAL-195, RAL-31 x RAL-59, RAL-897 x RAL-310, RAL-239 x RAL-486, RAL-287 x RAL-805, RAL-584 x RAL-765, RAL-381 x RAL-149, RAL-93 x RAL-703, RAL-379 x RAL-517, RAL-821 x RAL-630, RAL-189 x RAL-853, RAL-399 x RAL-360, RAL-907 x RAL-217, RAL-535 x RAL-786, RAL-195

x RAL-395, RAL-852 x RAL-913, RAL-502 x RAL-818, RAL-361 x RAL-375, RAL-138 x RAL-491, RAL-808 x RAL-93, RAL-517 x RAL-208, RAL-153 x RAL-189, RAL-149 x RAL-352, RAL-732 x RAL-509, RAL-818 x RAL-563, RAL-630 x RAL-57, RAL-395 x RAL-380, RAL-358 x RAL-822, RAL-765 x RAL-406, RAL-703 x RAL-859, RAL-406 x RAL-153, RAL-508 x RAL-379, RAL-716 x RAL-427, RAL-509 x RAL-358, RAL-555 x RAL-48, RAL-360 x RAL-321, RAL-786 x RAL-69, RAL-855 x RAL-354, RAL-559 x RAL-437, RAL-563 x RAL-361, RAL-158 x RAL-559, RAL-805 x RAL-884, RAL-208 x RAL-566, RAL-492 x RAL-397, RAL-382 x RAL-399, RAL-75 x RAL-508, RAL-884 x RAL-138, RAL-530 x RAL-83, RAL-69 x RAL-348. Offspring from pairwise crosses were collected 28 days after parents were removed and pooled into a large *Drosophila* population cage, for the 1st generation of outcrossing and subsequent embryo collection.
For this, and each subsequent generation of outcrossing, the outcrossed DGRP population was maintained employing a method used to maintain constant larval densities (223 ± 14.3 (95% CI)) in stock bottles [2]. Briefly this method involves populating a large *Drosophila* cage with thousands of flies on the day 1, providing these with fruit juice (grape/apple) agar plates for embryo laying. After a 24 hr habituation period, agar plates are replaced (day 2). On the day 3, agar plates are recovered and embryos are collected from the surface. Using PBS and a brush, concentrated egg/PBS solutions are prepared, and these are squirted on the surface of Lewis media in bottles. This process is typically carried out every 20-25 days. The outcrossed DGRP populations is maintained at a density of 20-25 bottles (20 bottles maintains the population at >4000 individuals).

Below is a table outlining details of the outcrossed DGRP population foundation and maintenance.

Date	Action	Temp	No. Bottles	Worker
18/10/2014	Tipped DGRP onto new food, and placed at 18C for virgin collection in mid-Oct	18C	NA	FMW/WHP
07/10/2014	Tipped out adult DGRPs from vials for subsequent virgin collection	25C	NA	FMW
8/102014 - 14/10/2014	Virgins and males collected	25C	NA	FMW
15/10/2014	Pairwise crosses (n=100)set up (virgin male and female flies from 113 lines)	25C	NA	FMW
20/10/2014	Pairwise crosses parents removed from vials	25C	NA	FMW
19/11/2014	Egg squirts from offspring of pairwise crosses: 1st generation of complete outcrossing	25C	30	FMW
18/12/2014	Tipped flies from old bottles into fresh bottles	25C	30	FMW
30/01/2015	Egg squirts: 2nd generation of complete outcrossing	25C	30	FMW
06/03/2015	Tipped flies from old bottles into fresh bottles	25C	30	FMW
09/04/2015	Egg squirts: 3rd generation of complete outcrossing	25C	25	FMW
19/05/2015	Egg squirts: 4th generation of complete outcrossing	25C	21	FMW
19/06/2015	Egg squirts: 5th generation of complete outcrossing	25C	25	FMW
30/07/2015	Egg squirts: 6th generation of complete outcrossing	25C	25	FMW
01/10/2015	Egg squirts: 7th generation of complete outcrossing	25C	25	FMW
29/10/2015	Egg squirts: 8th generation of complete outcrossing	25C	20	KMM
02/12/2015	Tipped flies from old bottles into fresh bottles	25C	20	KMM
06/01/2016	Egg squirts: 9th generation of complete outcrossing	25C	20	KMM
10/02/2016	Egg squirts: 10th generation of complete outcrossing	25C	20	KMM
22/02/2016	Tipped flies from old bottles into fresh bottles	25C	20	KMM
25/03/2016	Egg squirts: 11th generation of complete outcrossing	25C	24	KMM
26/04/2016	Egg squirts: 12th generation of complete outcrossing	25C	22	KMM
18/05/2016	Egg squirts: 13th generation of complete outcrossing	25C	20	KMM
14/06/2016	Egg squirts: 14th generation of complete outcrossing	25C	22	KMM
14/07/2016	Egg squirts: 15th generation of complete outcrossing	25C	22	KMM
10/08/2016	Egg squirts: 16th generation of complete outcrossing	25C	25	KMM

07/09/2016	Egg squirts: 17th generation of complete outcrossing	25C	25	KMM
04/10/2016	Egg squirts: 18th generation of complete outcrossing	25C	25	KMM
02/11/2016	Egg squirts: 19th generation of complete outcrossing	25C	25	KMM
30/11/2016	Egg squirts: 20th generation of complete outcrossing	25C	25	KMM
05/01/2017	Egg squirts: 21st generation of complete outcrossing	25C	25	KMM
09/02/2017	Egg squirts: 22nd generation of complete outcrossing	25C	25	KMM
01/03/2017	Egg squirts: 23rd generation of complete outcrossing	25C	25	KMM
05/04/2017	Egg squirts: 24th generation of complete outcrossing	25C	25	KMM

References

1. Mackay, T. F. C. et al. The Drosophila melanogaster Genetic Reference Panel. *Nature* **482**, 173–178 (2012).
2. Clancy, D. J. & Kennington, J. W. A simple method to achieve consistent larval density in bottle cultures. *Drosoph. Inf. Serv.* **84**, 168–169 (2001).