

Step 3: Specify the design

PERMANOVA requires a **design file** to run.

You can see the Factors associated with the holdfast data matrix (or its resemblance matrix) by clicking on **Edit > Factors....** These factors will be 'visible' to the PERMANOVA dialog that we will use to create our design file.

For this study, we want to create a design file that has all of the information that PERMANOVA will need to construct the correct partitioning, the correct pseudo-F ratios and the correct permutation algorithms to test every term in the model that is implied by the design. For this example, we have a fully hierarchical (nested) study design with three random factors: Locations, Sites (within Locations) and Areas (within Sites).

1. From the resemblance matrix ('Jaccard'), click **PERMANOVA+ > Create PERMANOVA Design....**

The screenshot shows the PRIMER 7 interface. The 'PERMANOVA+' menu is open, highlighting 'Create PERMANOVA Design...'. The background displays a 'Samples' table with distance values for various samples.

	Berghan11c	Berghan11d	Berghan11e	Berghan14a	Berghan14b
Berghan11d	50	33.333	40		
Berghan11e	44.828	33.333	53.125	53.333	
Berghan14a	26.667	23.077	36.364	27.273	31.25
Berghan14b	30.769	27.273	28.125	31.034	30.769
Berghan14c	14.286	5.8824	10.714	12	14.286
Berghan14d	30.769	21.739	32.258	35.714	26.667
Berghan14e	31.707	23.077	38.636	41.463	41.463
Berghan33a	20	14.286	27.586	21.429	21.429
Berghan33b	8.3333	25	13.793	15.385	11.111
Berghan33c	31.818	27.778	33.333	22.222	26.923
Berghan33d	20.833	27.778	28.571	17.857	22.222

2. In the 'PERMANOVA design properties' dialog, pick a title for your design file, and indicate the number of factors. For the holdfast example, we will choose (Title: **Three-way nested design**)&(Number of factors: **3**), then click **OK**.

PERMANOVA design properties

Title:

Number of factors:

OK Cancel Help

- You will see an empty design file with three rows, one for each factor. Each row will correspond to a factor in your design. You will need to specify, in turn, the name and properties of each factor for the analysis in its own row.

Design1

Three-way nested design

Factor	Nested in	Fixed/random	Contrasts
		Fixed	
		Fixed	
		Fixed	

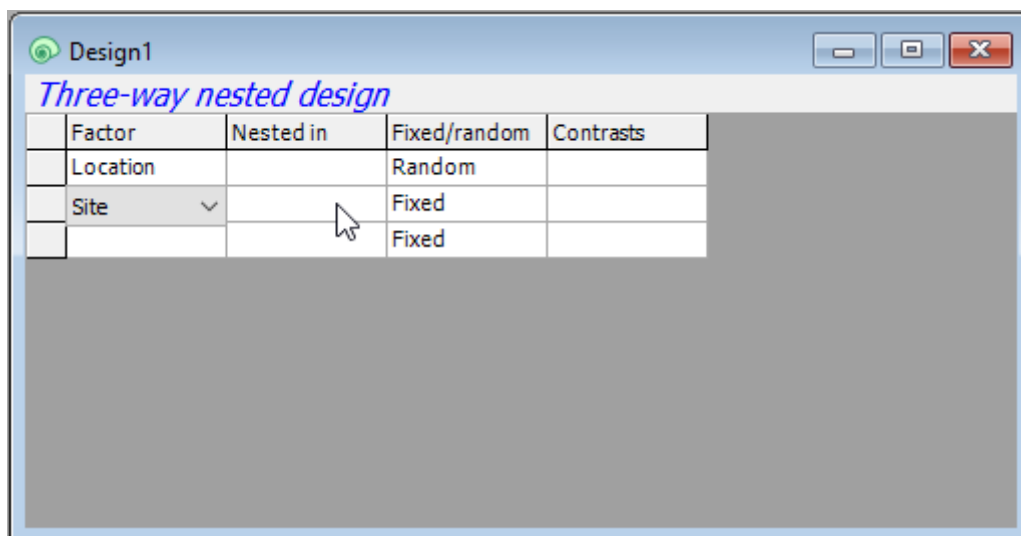
- Location:** First, in row 1, click in the blank cell under the word 'Factor', and you will see a drop-down menu listing all of the factors associated with the resemblance matrix from which this design file was created. Choose 'Location' to fill this cell. Location is not nested in anything, so we leave the second cell in row 1 blank. In the third cell of row 1, we have to specify that Location is a **random** factor, so click on the word 'Fixed' and change it to 'Random'.

Design1

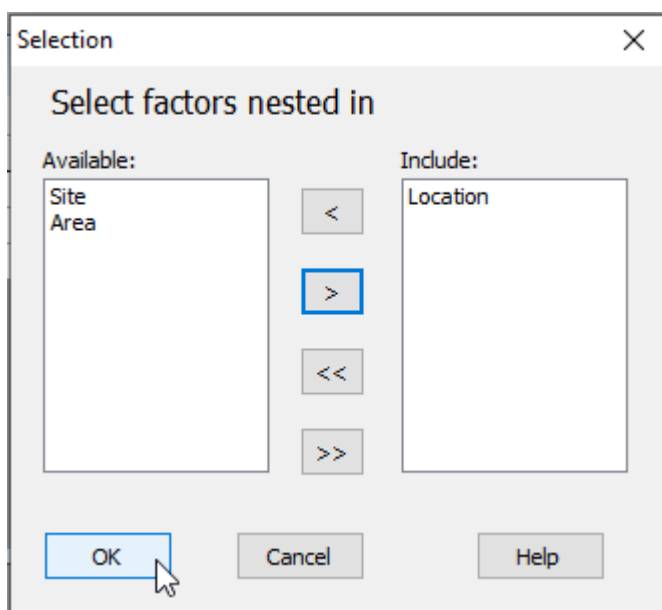
Three-way nested design

Factor	Nested in	Fixed/random	Contrasts
Location		Fixed	
		Fixed	
		Random	

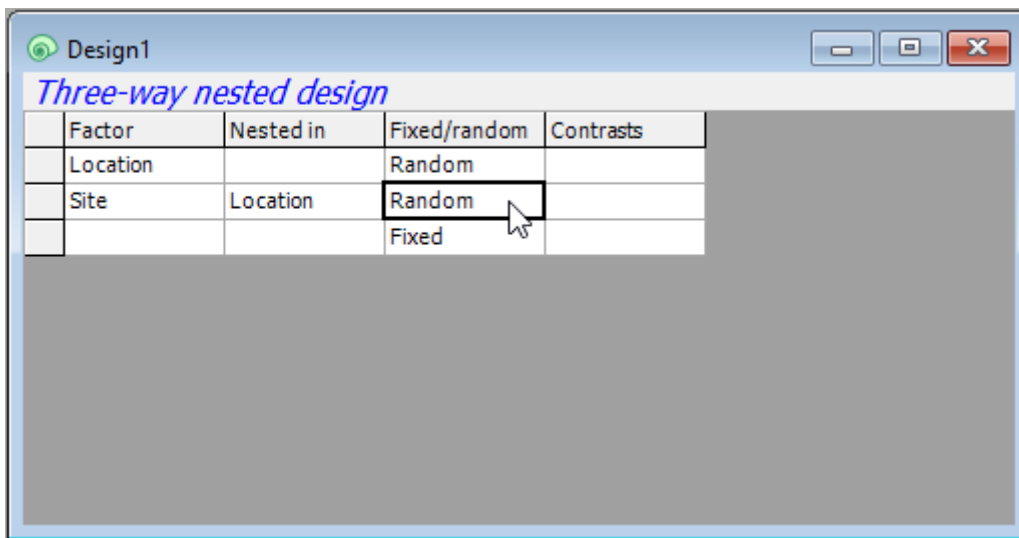
4. **Site:** Next, we need to specify the second factor in the design in row 2 of the design file. Click on the cell in row 2 of the 'Factor' column (column 1) and choose 'Site'.



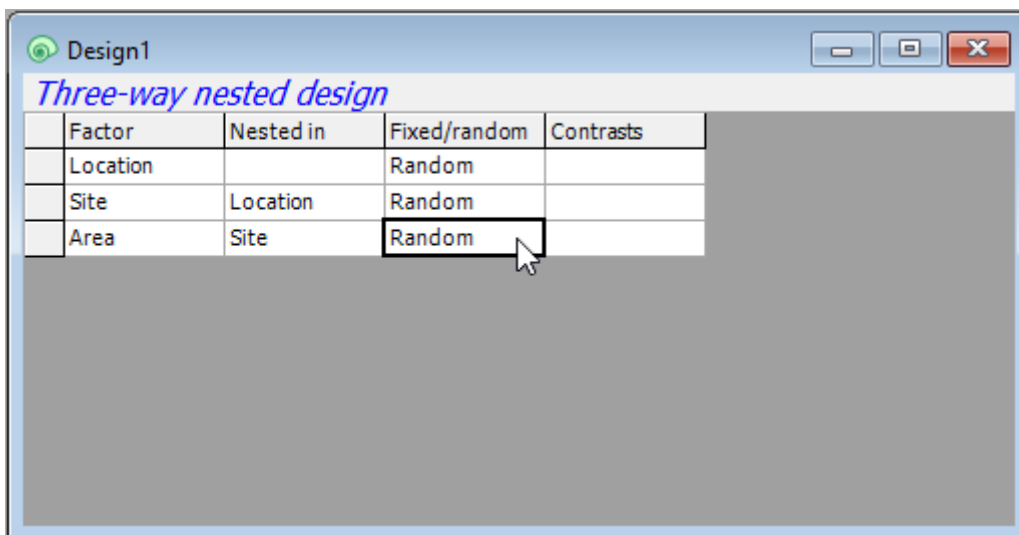
5. Sites are **nested in Locations**, so we have to specify that in column two ('Nested in') accordingly. Click on the cell in row 2 in column 2 and a 'Selection' dialog will pop up to allow you to choose the factors within which 'Site' is nested. You will need to click on the word 'Location' (in the 'Available:' box on the left), then on the single-right-arrow button () to move it over into the 'Include:' box (on the right), then click **OK**, like so:



6. Make sure that the factor 'Site' is also specified in column 3 as 'Random'. (This happens automatically after specifying a nested term in column 2, because nested terms are, almost always, random factors.) Your design file should now look like this:



7. **Area:** Finally, we need to specify the third factor (Areas) correctly in row 3 of the design file. Under 'Factor' choose 'Area'. Under 'Nested in', specify 'Site', and make sure that column three has the word 'Random'. The final three-way nested design file should look like this:



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