# Your quick start guide to p-rep designs and how to generate them in CycDesigN

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# What is p-rep design?

A *single-location* partially replicated, or p-rep, design is a special type of unequally replicated block or row-column design in which some treatments are replicated twice, whilst the other treatments are unreplicated.

Below is an example of a single-location p-rep **block** design in which six of the 12 treatments occur in two blocks (i.e., are replicated twice), and the other six treatments occur in only one block (i.e., are unreplicated).



But wait ... doesn't partial replication run counter to the fundamental experimental design principle of replication?

Replication is a crucial feature of sound experimental design. It is essential for estimating the underlying variability (i.e., the residual variance), without which there is no basis for valid statistical inference. The key property of a p-rep design is that there is adequate replication on a subset of treatments to enable reliable estimation of the residual variance, and thus valid statistical inference. For example, in the design above, the six replicated treatments (1,4,6,7,11,12) serve to provide an estimate of the residual variance, and to allow for block effect adjustments.

Similarly, here's an example of a single-location p-rep **row-column** design, again with 12 treatments but this time with 8 treatments duplicated within the 5 row by 4 column grid. The design is constructed such that the duplicated treatments can be used to estimate the residual variance, the row effects and the column effects.

	Column 1	Column 2	Column 3	Column 4
Row 1	3	8	1	7
Row 2	10	11	6	12
Row 3	1	4	7	5
Row 4	6	12	8	9
Row 5	5	3	2	11

The p-rep design concept can also be extended across multiple locations. For a *multi-location* p-rep design, at each location some treatments occur twice, some occur only once, and others may not occur at all. Across the entire design, treatments are replicated either r or r+1 times. However, in

some situations, equally replicated designs are possible – that is, all treatments are replicated *r* times.

Here's an example of a two-location p-rep block design. Notice that in this example each treatment is equally replicated, with the unreplicated treatments at location 1 (2,3,5,7,9,10) replicated twice at location 2 (and vice-versa for those treatments duplicated at location 1).





And here's another example of a multi-location p-rep block design with equal replication. However, this time the 12 treatments are arranged across three locations but with only two blocks of size 4 per location. Thus, not all treatments can occur at all locations. And although each treatment is replicated twice over the whole design, some are replicated across different locations (e.g., treatment 5 occurs at location 1 and location 3) whereas others are duplicated within locations (e.g., treatment 7 occurs twice within location 1). This highlights a key challenge when generating multi-location p-rep designs: To even-out the partial replication within and between locations in such a way that the overall design has good efficiency.







In this final example of a multi-location p-rep block design, notice that some treatments are replicated r=2 times across the whole design, whilst the others are replicated r+1=3 times. And in particular, observe that the treatments replicated 3 times are those duplicated within a location (i.e., 1,5,7,9,11,12).







# When are p-rep designs used?

p-rep designs are useful when practical constraints limit the amount of replication possible. For example, physical constraints may limit the block size or the row-column dimensions. Alternatively, other practical considerations, such as availability of experimental material (e.g., seeds in a plant-breeding trial) or cost may limit the number of times a treatment can feasibly be replicated.

In addition, p-rep designs offer an effective way of screening a large number of treatments (e.g., different genotypes in a plant-breeding programme).

Today, p-rep designs are commonly used in early generation plant-breeding trials. They offer a new and efficient alternative to the more traditional augmented design, with its replicated check entries (i.e., standards) and many unreplicated test entries (i.e., treatments).

## How do I generate a p-rep design in CycDesigN?

We'll begin by walking through a simple example, before looking at a couple of more complex examples, including p-rep designs with standards and spatial p-rep designs.

#### Example 1: A single-location p-rep block design

Let's generate a single-location p-rep block design, with 12 treatments and three blocks of size 6 (*cf* the single-location example above).



On the main menu of CycDesigN, under Design select Partially replicated to open the window dialog for generating p-rep designs.

The top button in this dialog toggles between a Block design and a Row-column design. We're going to generate a p-rep block design.

Clicking on the next button, Parameters [], opens another window dialog where we set the parameters of our design.

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## CycDesigN 8.0

For our example, we set the Number of treatments to 12, the Number of standard types to 0, and the Number of locations to 1, then click Next to set further parameters.

Note: Standards (e.g., check entries) can be incorporated into p-rep design. These are replicated more frequently than the treatments. In an *augmented p-rep design*, a design for a single-location, all treatments are unreplicated except for the standard types.

Partial replication parameters	?	×
Number of treatments	12	▲ ▼
Number of standard types Edit standards	0	•
Number of locations	1	▲ ▼
< Back	Next	>

In the next window we set the minimum Number of replications of the treatments across the whole design. In p-rep designs, treatments are replicated either *r* or *r*+1 times across the whole design. Thus, this design parameter is set to the value given by *r*. For a single-location p-rep, this will be 1: recall that only a subset of treatments in a single-location p-rep are duplicated, with the remainder being unreplicated.

Next, we set the Number of units per block, which in our example is 6.

Partially replicated parameters	? ×
Number of replications Number of units per block	
< Back	Next >

Click Next to open the final window for parameter setting. Set the Number of blocks per location to 3. Notice that the Number of duplicates has automatically changed to 6. This is because our design of three blocks of size 6 provides a total of 18 experimental units to which our 12 treatments are randomly assigned. This enables six of the 12 treatments to be duplicated (18-12=6).

Clicking Next closes the final parameter setting window.

The numbers in the square brackets on Parameters [] button in the Partially replicated design window correspond to the values of the design parameters we just have set.

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Block design	
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Seed: 1126	<ul> <li>Minimum number of treatment replications across the whole design (i.e., r)</li> <li>Number of locations</li> <li>Number of standards</li> <li>Number of treatments</li> </ul>

The Seed button allows us to set the seed used by CycDesigN's algorithm to generate the design. By default, the seed is taken from the computer clock.

Clicking Next on the Partially replicated design window starts the algorithm for generating the design.



When the updating process has stabilised, that is when there's no improvement to the average efficiency factor after a minute or two, we click Next to randomize the design and generate the output.

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In the Randomization/Output window, we tick Randomize to randomize the design, and set the Number of randomizations to 1 to produce one randomization of our design. The Seed button allows us to set the seed of the random number generator used to randomize the design. If we use the same seed more than once, we will get the same random numbers, and hence the same randomization.

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Block design	Randomize 🗹
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Single factor	Blocks in columns 5
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Seed: 1126	Design file: CycDesigN.txt
	CycAnalysis [No]
Next >	<back next=""></back>
Decin V VI	Design A Design A

This button toggles between the blocks of the design being printed in columns or in rows in the .txt Design file.

For your design, CycDesigN generates a:

- Log file containing all the necessary information to reproduce the exact same design at a later date, and a,
- **Design file** giving details on the design type, parameters chosen and the final design layout.

We can use the Log file and Design file buttons to specify the names of these files and where they are saved. Default names are automatically generated, and unless specified using these buttons, the files are saved in the working directory.

(Note: the working directory can changed be via Setup on the main menu bar.)

Two other files with the same name as the log file will also be generated:

- a .csv file containing the design in long format, and
- a .aux file for use by the CycAnalysis module (see below)

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This button toggles between opening (yes) and not opening (no) the CycAnalysis window with the design just generated pre-loaded.

Note: In the CycAnalysis window we can generate template code for the analysis of our design using Genstat or SAS, save test data in a .xlsx file, and also make changes to the treatment and blocking factors, such as renaming them. The CycAnalysis window can also be accessed by clicking on Analysis in the main menu bar.

And finally, clicking Next on the Randomization/Output window will generate our design and the accompanying output.



Note: you can use View on the main menu bar to open your log and design file in CycDesigN.

#### Example 2: A multi-location p-rep block design

In this example, assume there are four locations, three blocks of size 5 per location and 16 treatments, each of which is to be replicated at least three times across the whole design. Therefore, in the design parameter windows, set:

- Number of treatments to 16
- Number of standard types to 0
- Number of locations to 4
- Number of replications to 3
- Number of units per block to 5
- Number of blocks per location to 3

For the final parameter, Number of duplicates per location, we have a choice of settings: 0 to 4. Let's duplicate two treatments per location.

#### Note on the number treatment replications across the whole design, r

In a multi-location p-rep design, individual treatments are replicated either r or r+1 times. The range of possible r values depends on the number of locations (which we'll denote by c).

For *c* locations, *r* must be at least *c*-2, and no larger than 2c-1 (i.e.,  $c-2 \le r \le 2c-1$ ).

In our example, with c = 4 locations, r can thus range from 2 to 7.

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#### Example 3: A multi-location p-rep block design with standard types

Now let's modify the design above to include two standard types (denoted by S1 and S2), each of which is to occur three times per location. We do this by setting the Number of standard types to 2, then clicking in the Edit standards button and entering the required number of standards per location into the field for each standard type.



To accommodate these standards in our design, we either must change the dimensions of the block design (i.e., increase the number blocks and/or increase the number of units per block) OR decrease the number of times our treatments are replicated across the design. Reducing replication is never ideal, so, if possible, you should aim to increase the dimensions of the block design, preferably by increasing the units per block. Let's assume we can increase the units per block from 5 to 7 – giving 6 more plots per location. This will enable us to accommodate the 6 standards per location (2 standard types by 3 reps) without reducing the amount of replication possible for the treatments.



When generating a p-rep block design with standards, CycDesigN will set out the standards at each location such that they occur as evenly as possible within the blocks. In this particular example, each standard type thus appears exactly once in every block.

#### Example 4: A multi-location p-rep row-column design with standards

Until now we've only considered p-rep designs laid out in blocks. Let's look at one laid out in rows and columns at each location.

As above, let's assume there are four locations, 16 treatments (each of which is to be replicated at least three times across the whole design), and two standard types (each of which is to be replicated three times per location), but this time, within each location the design is to be arranged in a five row by four column grid.

Using the top button in the Partially replicated design menu dialog, toggle to Row-column design. In the design parameter windows, set:

- Number of treatments to 16
- Number of standard types to 2
- Edit standards to 3 for each standard type



- Number of locations to 4
- Number of replications to 3
- Number of rows to 5
- Number of columns to 4

For the final parameter, Number of duplicates per location, we have a choice of settings: 0 to 3. Let's duplicate three treatments per location.

Notice that the 5<sup>th</sup> button in the Partially replicated design menu dialog is active. This button toggles between spatial and non-spatial designs. A common problem with classical row-column designs is that replications of the individual treatments may be clustered together. A *spatial* row-column p-rep design takes the distribution and separation of the treatments across the rows and columns into account, generating a design more robust to spatial trends and environmental heterogeneity across the row-column grid. Let's generate a spatial design.

To learn more about spatial design in CycDesigN, please check out the tutorial video: <u>https://youtu.be/aZRzIBUNM14</u> and the reference paper: <u>https://onlinelibrary.wiley.com/doi/epdf/10.111</u> <u>1/jac.12463</u>



Click Next on the Partially replicated design menu dialog and CycDesigN will run through its iterative procedure to optimize the standards, ensuring they are evenly spread across the rows and columns. When this process has stabilized click Spatial. CycDesigN will now run through its algorithm to optimize the spatial criteria of the design.

When the updating process has stabilised, click Next, set your desired options in the Randomization/Output menu, and finally, click Next to generate your design and save the output.

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The "Treatment span for duplicates" table summaries how close the duplicated treatments within a location are. For example, a span of 3 for a treatment in the row direction means that its duplicates range over 3 rows. CycDesigN's spatial algorithm tries to maximize the overall span of the treatment replications. In our design, at each location, the duplicated treatments span at least 2 columns and at least 3 rows.

#### Real-world examples in plant-breeding

Today, p-rep designs are commonly used in early generation plant-breeding trials. Typically, such trials are laid out in a rectangular grid of experimental units (i.e., in rows and columns), rather than in blocks. A spatial design will be more robust to spatial trends and environmental heterogeneity across the row-column grid than the non-spatial variant, enabling more reliable estimates of the treatment effects. <u>Piepho et al.'s 2020 paper</u> in the Journal of Agronomy and Crop Science, entitled *"Generating row–column field experimental designs with good neighbour balance and even distribution of treatment replications"*, provides three real-world examples of p-rep row-column designs, which they generate to be spatially robust. Let's also generate these three spatial p-rep row-column designs in CycDesigN.

Recall that in CycDesigN, *spatial* row-column p-rep designs are generated by toggling the 5<sup>th</sup> button in the Partially replicated design menu to Spatial. A spatial design will be more robust to spatial trends and environmental heterogeneity across the row-column grid than the non-spatial variant, enabling more precise estimates of the treatment comparisons.



# Example 3 in Piepho et al. (2020) : Single-location spatial p-rep row-column design augmented with a standard type

In this single-location trial, there are 252 treatment lines, of which 36 are duplicated. In addition, there is a single standard variety, replicated 12 times. The trial is laid out over 25 rows and 12 columns.

The Log file from generating a design for this trial in CycDesigN is below. Notice the settings of the design properties and parameters at the top of the Log file.

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Example 4 in Piepho et al. (2020) : Multi-location spatial p-rep row-column design

In this multi-location p-rep trial, 450 breeding lines of triticale (a hybrid of wheat and rye) are tested at 10 locations. Each line is replicated 12 times across the entire trial. At each location, the layout has 18 rows and 30 columns, and 90 duplicated lines.

Below is the top section of the Log file (which includes the design properties and parameters) from generating a multi-location spatial p-rep row-column design for such a trial.

Log file	: C:\Program	Data\DATA	\CycDesigN	.html														×
File Edit	-																	
Design r	roperties																	
Par	tially re	plicate	d															^
Row	-column d	esign																
spa	tial																	
Design p	arameters																	
Num	ber of tr	eatment	3		=	450												
Num	ber of st	andard	types			0												
Num	wher of re	plicati	ons			10												
Num	ber of ro	ws				18												
Num	ber of co	lumns			=	30												
Num	wher of du	plicate	3			90												
Treatmen	it spans f	or dupl	icates															
Minim	um treatm	ent span	ns															
locati	on colu	mns r	ows															
1	15	8																
2	15	8																
4	15	ă																
5	15	8																
6	15	8																
7	15	8																
9	15	ă.																
10	15	8																
Random n	umber see	d for d	esian aen	neration	= 1104													
Average	efficienc	y facto	rs (Upper	r bounds	)													
ROW	-corumn	0.9084	51 (N/A)	)														
Random n	umber see	d for d	esign rai	ndomizat	ion = 95	9												
Randomiz	ed design	1																
+																		
column	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	1
row +	230	326	92	425	407	314	271	356	25.8	31.0	150	35	145	55	291	124	284	31
2	280	380	245	18	153	343	52	62	350	105	130	231	446	148	272	229	3 60	24
3	382	43	426	57	299	239	282	363	10	332	435	166	355	298	362	220	75	34
4 1	179	99	428	213	138	22	429	5	329	266	450	324	129	202	430	177	398	21
5	102	228	327	200	12	224	20	401	210	223	27	114	212	313	369	348	225	31
7	417	433	65	95	204	70	151	30	390	98	3	208	160	371	370	165	219	3(
8 1	59	169	385	149	263	89	248	68	359	71	216	115	364	203	238	434	181	1
9	182	116	400	354	189	76	222	351	40	232	252	240	342	315	142	424	365	
10	319	436	173	48	26	83	214	325	330	418	352	156	340	158	123	63	373	1
12	346	348	260	387	439	322	409	259	276	330	287	306	265	448	392	93	97	4:
13	422	147	378	118	312	421	1	211	308	302	29	24	384	427	4.4	345	82	( )
14	94	227	357	447	250	235	416	247	32	337	122	234	195	127	193	159	432	
15	388	414	225	294	413	386	383	303	269	170	226	255	309	180	336	175	274	11
17	358	438	21	320	104	90	110	270	331	241	119	108	191	420	389	199	415	1!
18	412	267	423	163	289	42	91	67	112	333	411	278	376	50	188	201	102	2(
	1.50	305	264	41.4	300	1 2 2	364	24.2	100	399	1 20	112	11.6	22.2	0.65	400	275	21
2		300	204	414	390	132	304	243	100	332	130	113	110	223	200	100	215	4.
	328	48	443	208	74	420	413	9	171	85	418	333	211	283	369	95	2	2:
3	328	48 437	443 103	208 192	74 247	420 438	413 289	9 244	171 212	85 168	418 75	333 433	211	283 138	369	95 119	2 387	2:
3 4	328 38 309	48 437 137	443 103 431	208 192 13	74 247 298	420 438 285	413 289 317	9 244 324	171 212 365	85 168 395	418 75 59	333 433 401	211 8 122	283 138 67	369 384 140	95 119 51	2 387 46	2:
3 4 5	328 38 309 106	48 437 137 270	443 103 431 133	208 192 13 47	74 247 298 305	420 438 285 376	413 289 317 277	9 244 324 302	171 212 365 230	85 168 395 69	418 75 59 383	333 433 401 160	8 122 378	283 138 67 11	369 384 140 64	95 119 51 82	2 387 46 377	2: 2:

# *Example 5 in Piepho et al. (2020) : Multi-location spatial p-rep row-column design augmented with a standard type*

In this trial, 330 lines of winter barley are tested across 5 locations. Each line is replicated 6 times across the entire trial. At each location, the design has 15 rows and 28 columns, and 66 duplicated lines. In addition, at each location, a standard variety is replicated 24 times.

Below is the top section of the Log file (including the design properties and parameters) from generating a design for this spatial p-rep trial.

<pre>ret c un Pesion proporties Pesion proporties Pesion proporties Pesion proporties Peritally replicated Rev-colum design Pesitally replicated Rev-colum design Pesital Pesion proporties Partially replicated Perital to rever = 33 Partially replicated Partially replicated Perital to rever = 4 Partially replicated Partially</pre>	Log file:	: C:\Program[	Data\DATA\C	CycDesigN.ht	tml													×
Design properties Treatestry replications Bunker of remained types Humber of ionations Humber of remained types Humber of diplicates Humber of displicates Humber of displicates Humber of design generation = 149 Aversage efficiency factors (Dyper bounds) Humber of design remomination = 4 Random number seed for design remomination = 4 Random number seed for design remomination = 4 Random list of the factors (Dyper bounds) Humber of 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 1 1 2 3 4 5 6 7 8 9 10 13 220 79 13 303 260 120 126 126 126 126 126 126 126 126 126 126	File Edit																	_
Representation design generation = 130           Number of treatments           Number of treatments           Number of treatments           Number of treatment spans           Number of columns           14           14           14           14           14           14           14           14           14           Number of duplicates	Design p Par	properties rtially re	s eplicate	d														^
Design parameters         Number of treatands (ypes)       - 10         Number of treatands (ypes)       - 10         Number of treatands (ypes)       - 10         Number of treatands (ypes)       - 20         Number of treatands (ypes)         Number of treatands (ypes)         1       14       7         2       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       1       13       14       15         4       14       14       14       14       14       14	Rov	-column (	design															
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Humber of isotations       -       1       124           Humber of isotations       -       5         Humber of isotations       -       6         Humber of isotations       -       6         Humber of diplicates       -       66         Treatment spans for duplicates       -       66         Humber of diplicates       -       66         1 / 2 / 3 / 7       -       -         3 / 24 / 7       -       -         4 / 14 / 7       -       -         5 / 24 / 7       -       -         4 / 14 / 7       -       -         5 / 24 / 7       -       -         4 / 14 / 7       -       -         7 / 2 / 3 / 4 / 7       -       -         8 / 24 / 7       -       -         4 / 14 / 14 / 7       -       -         8 / 24 / 7       -       -       -       -         8 / 24 / 7       -       -       -       -       -       -         8 / 24 / 8100       107       210       11 / 12 / 13 / 14 / 15 / 14 / 15       14 / 15       14 / 15         1 / 36 / 137 / 153 / 120 / 133 / 135 / 250 / 277 / 251 / 16 / 13 / 13 / 25 / 25 / 133 / 330 / 100 / 128 / 15 / 13	Design p Num	p <b>arameter</b> mber of t:	s reatment	3		-	330											
Treatment agons for duplicates	Nun	mber of st	tandard	types		=	1 (	24)										
Number of roug         =         13 Number of columns         =         23 e           Treatment spans for duplicates         =         66           Treatment spans for duplicates         =         66           Treatment spans for duplicates         =         66           1         5.6         7         9         1	Nun	wher of re	eplicati	ons		=	6											
Humber of duplicates       =       66         Winimum treatment spans for duplicates       Number of duplicates       Number of duplicates         1       14       7         2       14       7         3       14       7         3       14       7         5       14       7         5       14       7         5       14       7         8       15       7         Remote number seed for design generation = 149         Average afficiency factors (Opper bounds) Rev-Golum         Remote number seed for design randomization = 4         Randomization = 4         Randomization = 10         201       1       12       13       14       15       16       1         201       1       2       3       4       5       6       7       6       9       10       11       13       14       15       16       1         201       1       13       14       15       16       1         201       13       16       20       20       13       16       1	Nun Nun	wher of ro	ວຟສ ວໄນຫກສ				15 28											
Transment spans for duplicates         Minimus treatment spans         1       14       7         2       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         3       14       7         5       14       7         S       14       7         Average afficiency factors (Uppor hounds)       8         Row-Column       0.990100 (0/A)         Row-Column       1       2       3       4       5       6       7       8       9       10       11       12       13       14       15       16       1         1       36       137       155       242       81       51       20       21 <td>Nun</td> <td>mber of di</td> <td>uplicate</td> <td>3</td> <td></td> <td>=</td> <td>66</td> <td></td>	Nun	mber of di	uplicate	3		=	66											
Treatment spans for dupileates         Number seven to columns proves         1       14       7         2       14       7         3       14       7         3       14       7         5       14       7         5       14       7         5       14       7         S       14       7         Average efficiency factors (Opper bounds) Rev=Colum       0.88180 (IVA)         Fandom number seed for design randomization = 4       4         State 1       1       36       137       165       42       51       51       250       247       102       213       14       15       16       1         1       36       137       165       42       51       250       247       102       233       140       15       16       1																		
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1       110       249       235       161       75       7       253       17       174       194       295       90       160       S1       122       98       16         2       305       319       82       232       173       113       157       14       265       47       15       26       316       263       302       S1       S         3       154       274       130       50       171       187       107       S1       259       101       25       54       74       220       63       53       25         4       46       318       72       95       49       257       226       150       202       58       328       286       S1       303       198       240       28         5       147       306       207       268       180       239       252       33       S1       287       222       29       309       297       204       12       2         5       147       306       207       268       180       239       272       12       2       309       297       204       12       <	14 15	304 271	108 127	231 212	111 87	23 189	313 131	67 323	32 225	125 105	106 90	64 314	155 S1	276 238	279 326	194 233	63 203	22 15
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3       154       274       130       50       171       187       107       S1       259       101       25       54       74       220       63       53       25         4       46       318       72       95       49       257       226       150       202       58       328       286       S1       303       198       240       28         5       147       306       207       268       180       239       252       33       S1       287       222       29       309       297       204       12       2         5       147       306       207       268       180       239       252       33       S1       287       222       29       309       297       204       12       2	2	305	319	235	232	173	113	157	14	265	47	295	26	316	263	302	90 S1	S
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	-	147	26	207	200	197	100	202	220	10	207	000	229	509	120	204	10	20

This brings us to the end of our quick-start guide to p-rep designs in CycDesigN. To learn more about using CycDesigN, including how to generate other types of designs, please check out the tutorial videos: <u>https://cycdesign.kb.vsni.co.uk/videos/</u>