

Tutorial of the Breeding Planner (BP) for Marker Assisted Backcrossing (MABC)

BP system consists of three tools relevant to molecular breeding.

- MARS: Marker Assisted Recurrent Selection
- MABC: Marker Assisted Backcrossing
- MAS: Marker Assisted Selection for pyramiding multiple genes
- This tutorial is designed for MABC

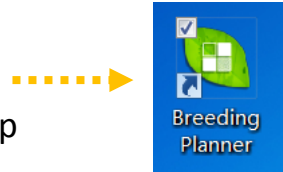
November 2013



Please make sure you have installed Java jdk 1.6 or 1.7 in your computer!

Open the Breeder Planner (BP) software

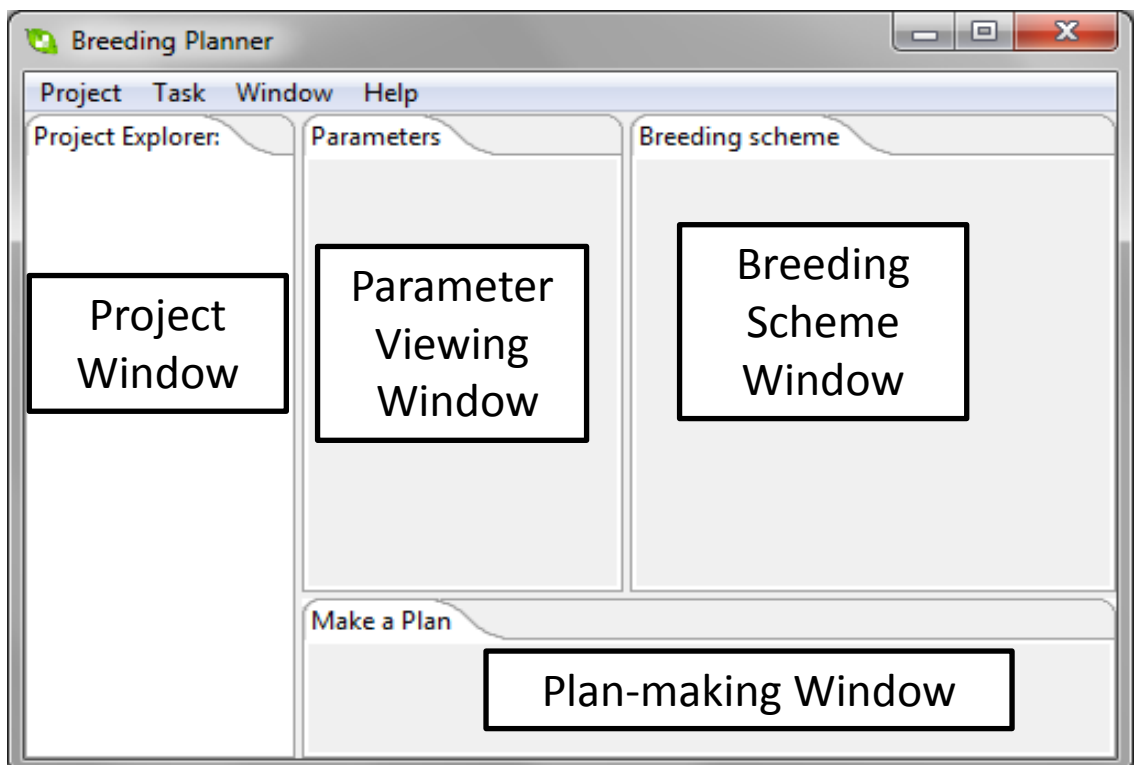
1. **Open the software:** The BP software can be opened by double click the **software icon** in your computer desktop



2. **Overview of the software.**

The screen is split into four windows.

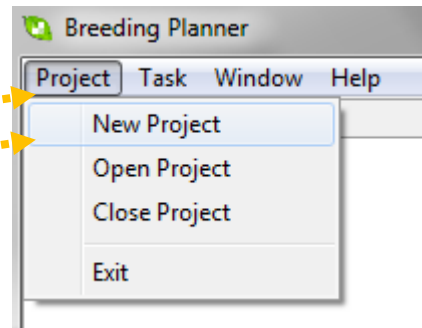
- **Project Window:** List all molecular breeding programs you have planned. Three distinct breeding programs can be considered: MARS, MABC and MAS.
- **Parameter Viewing Window:** You can view your breeding parameters in this window.
- **Breeding Scheme Window:** Once the breeding parameters are specified, a breeding flowchart will be demonstrated in this window.
- **Plan-making Window:** You can select the current stage/generation of your breeding programs in this window. A detailed plan for the near future will be made by the BP system.



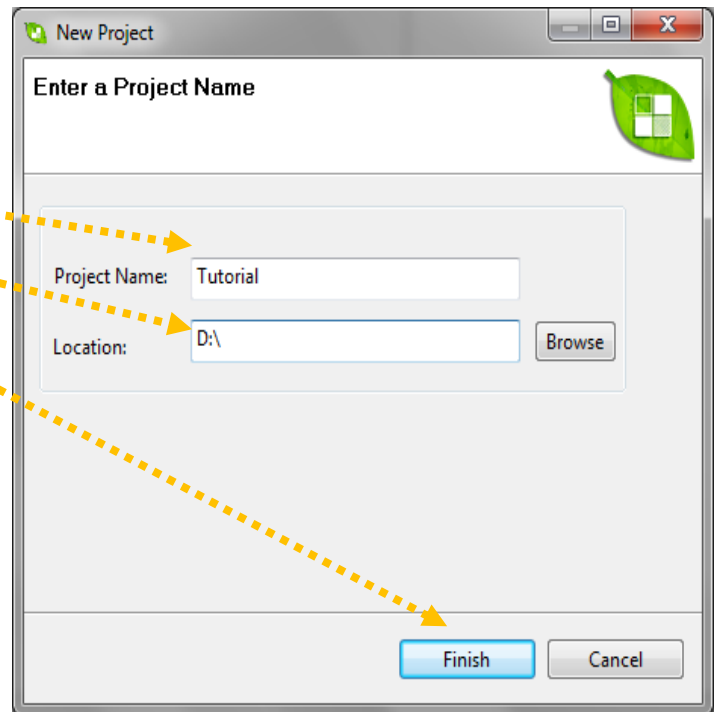
Build a new project

3. Build a new project

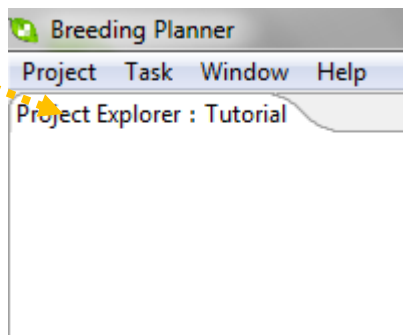
- Click **Project** menu
- Choose **New Project**



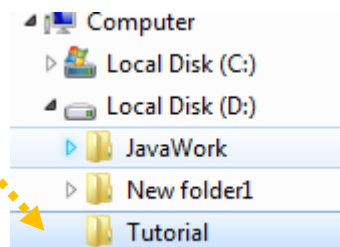
- Specify Project Name (Tutorial for example) and Project Path (D:\ for example)
- Click **Finish** to complete



New project is displayed in the **Project Window**



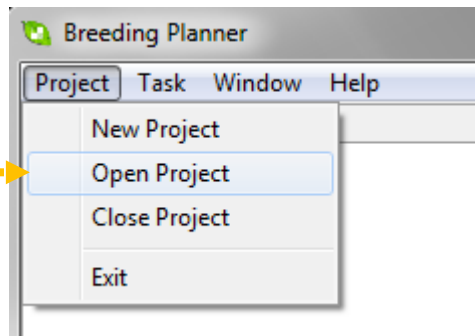
All inputs and outputs will be automatically saved in path of the project (**D:\Tutorial\...** for example)



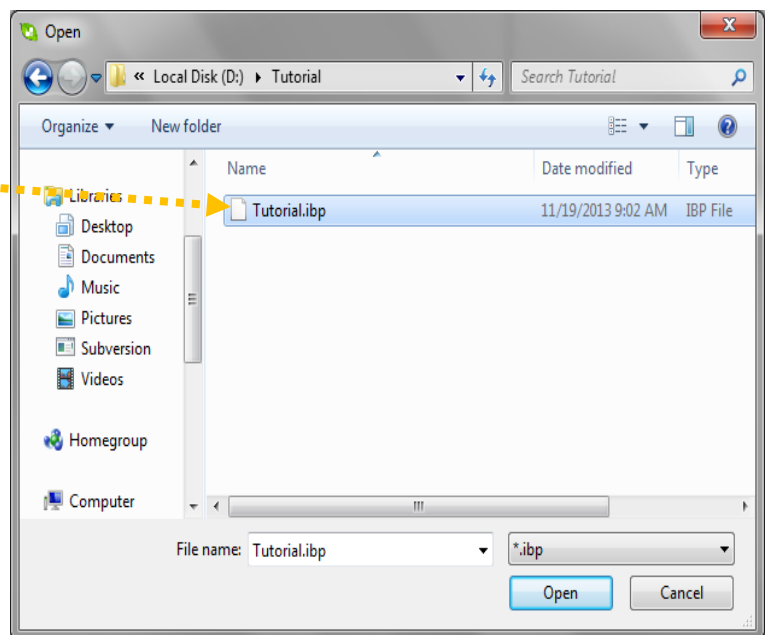
Open an existing project

4. Open an existing project

- Click **Project** menu
- Choose **Open Project**



- Choose an existing project in your computer

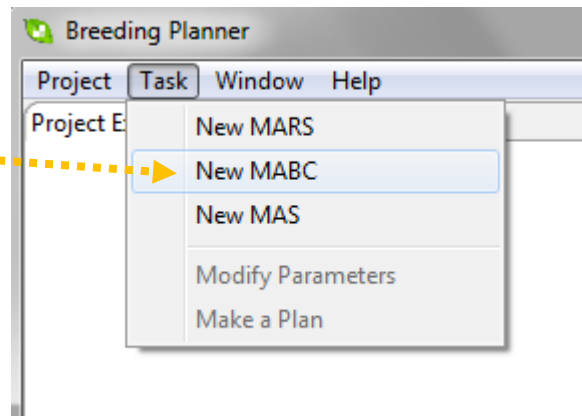


- **Please Note:** The BP system is project-based. When you first use the system, you need to build a new project first. Then you can make various breeding programs. When you leave the system, the system automatically save the jobs you have done. The next time, you can start from a new project, or from an existing project.

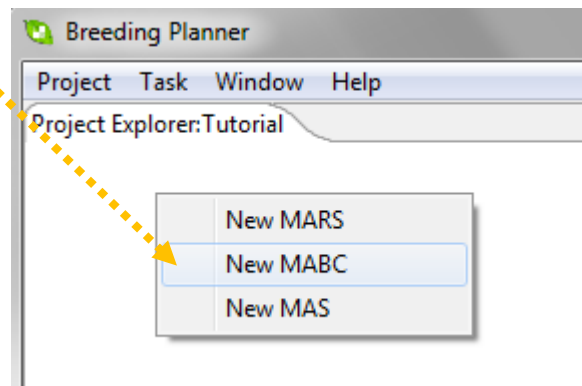
PMABC Tutorial: Start a new MABC breeding program

5. Make a new MABC program to the newly-built project

- Click **Task-> New MABC**
- Or right click in the blank area of project explorer, then choose **New MABC**



- You can use either way to make a MABC program



- Import parameters from an external file

A screenshot of the 'New MABC' dialog box. The 'Input Information' section is active. It contains several input fields and dropdown menus. A yellow dashed arrow points from the text 'Import parameters from an external file' to the 'Import file' field. The fields are organized as follows:

Researcher information		Species information	
Country	China	Crop species	Cowpea
Researcher's name	CAAS	Expected seeds per plant	150

Greenhouse/offseason		Field condition	
Length of each season (months)	6	Seasons per year	1
		1st season starts in	Jan
		2nd season starts in	
		3rd season starts in	

Growing condition		Backcross and self generations	
Parental generation starts in	November, 2013	Backcrossing generations	1
<input checked="" type="radio"/> Greenhouse/offseason	<input type="radio"/> Field condition	Repeated selfing generations	1

At the bottom right, there are 'Finish' and 'Cancel' buttons.

- Or specify the initial parameters by hand

PMABC Tutorial: Parameters required to define a MABC program

Users have to specify a set of parameters before a MABC scheme can given by the system. Below are more details on the required parameters.

1. Researcher information

Country;

Researcher's name;

2. Species information

Crop species: Select one crop species from Cowpea, Rice, Wheat, Maize, Groundnuts, and Cassava

Expected seeds per plant (or propagation rate) **(more on the next page)**

3. Greenhouse/offseason

Length of each season (months).

Note: We assume the crop can be grown across the whole year under the Greenhouse condition. That is, the next season can start in the same month when the previous season is harvested. So the planting time for each season is not needed.

4. Field condition

Seasons per year: the number of seasons per year, select from 1-3

1st season starts in: select a month

2nd season starts in: select a month, after the end of the 1st season

3rd season starts in: select a month, after the end of the 2nd season

Note: The crop cannot be grown across the whole year under the Field condition. So if multiple seasons are possible, the system asks for the planting time for each season.

5. Growing condition

Parental generation starts in: select the start time of the parental generation

Early generation growing condition: select one case "Greenhouse/offseason" or "Field condition"

6. Backcross and self generations

Backcrossing generations:1-3

Repeated selfing generations: 1-3

Minimum and maximum numbers of seeds per plant for each crop under optimum or normal conditions

Crop	Minimum number	Maximum number	Median (used as default in Breeding Planner)
Cowpea	10	300	50
Rice	50	300	200
Wheat	50	250	150
Maize	50	500	200
Groundnuts	30	200	80
Cassava	10	100	50

Notes:

- Expected seeds per plant in Breeding Planner will be used to calculate if there are enough seeds for phenotyping. If not, additional seed increase (by selfing) will be requested.
- The user input must fall into the min-max range for the selected crop! Otherwise, when the input number is smaller than the minimum number, the minimum number will be assumed. When the input number is greater than the maximum number, the maximum number will be assumed.
- The number of seeds required is calculated from settings for “Multi-locational phenotyping”. Say, when genotyping is conducted in F2, multi-locational phenotyping is only possible when each F2 plant can give enough seeds. Otherwise, phenotyping will be delayed until the required seeds are produced.

PMABC Tutorial: The interface

6. Overview of the MABC functionality:

There are four windows in MABC functionality

- **Project Window:** List all molecular breeding programs you have planned. Three distinct MB programs can be considered: MARS, MABC and MAS.
- **Parameter Viewing Window:** You can edit/view your breeding parameters in this window.
- **Breeding Scheme Window:** Once the breeding parameters are specified, a breeding flowchart will be demonstrated in this window.
- **Plan-making Window:** You can select the current stage/generation of your breeding programs in this window. A detailed plan for the near future will be made by the BP system.

When the required parameters are set in the Parameter setting/viewing Window, the defined MABC breeding program is graphed in the Breeding Scheme/flowchart Window. A set of output files are listed in the Project Window.

The screenshot displays the Breeding Planner application window. The interface is divided into several sections:

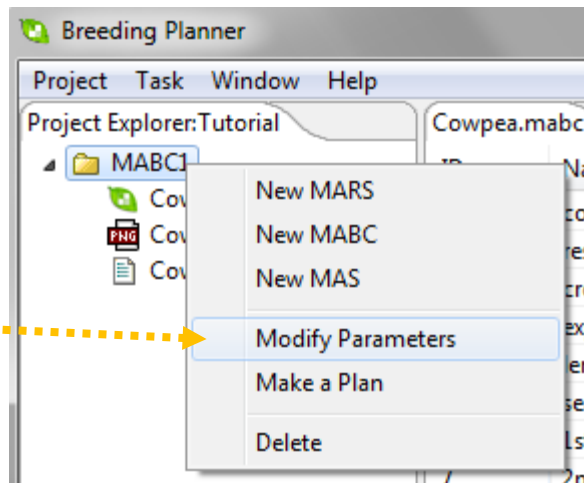
- Project Explorer:** Located on the left, it shows a tree view with 'MABC1' selected, containing files 'Cowpea.mabc', 'Cowpea.png', and 'Cowpea.txt'.
- Parameter viewing Window:** A table titled 'Cowpea.mabc' with columns 'ID', 'Name', and 'Value'. It lists parameters such as country (China), researcher (CAAS), crop species (Cowpea), expected seeds per plant (150), length of each season (6 months), seasons per year (1), and start dates for three seasons.
- Breeding scheme/flowchart window:** Titled 'Cowpea.png', it shows a flowchart of the breeding process. It starts with 'Recurrent parent' and 'Donor parent' crossed to produce 'F1'. The 'F1' is then crossed with the 'Recurrent parent' to produce 'BC1F1'. The 'BC1F1' is then selected as the 'Best BC1F1'. The flowchart includes instructions for each step, such as 'Grow the two parents; make a few hybrid seeds by hand pollination' and 'Grow BC1F1 plants and genotype them with chosen markers'.
- Plan-making Window:** At the bottom, it contains a 'Make a Plan' section with a 'Current status starts in' dropdown (set to November) and a 'Select current status' dropdown (set to 0. Select parental line). A 'Make a Plan' button is also present.

Red boxes are overlaid on the screenshot to highlight the four main windows: Project Window, Parameter viewing Window, Breeding scheme/flowchart window, and Plan-making Window.

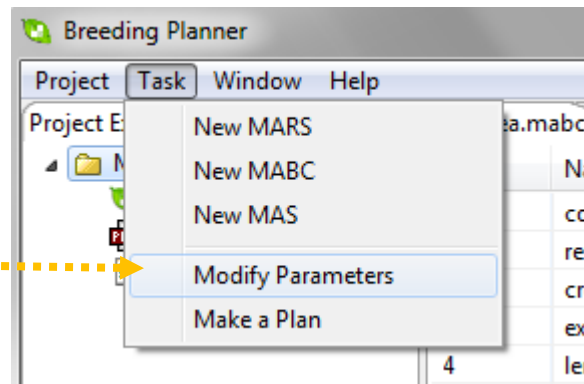
PMABC Tutorial: Modify your parameters

7. Modify Parameters

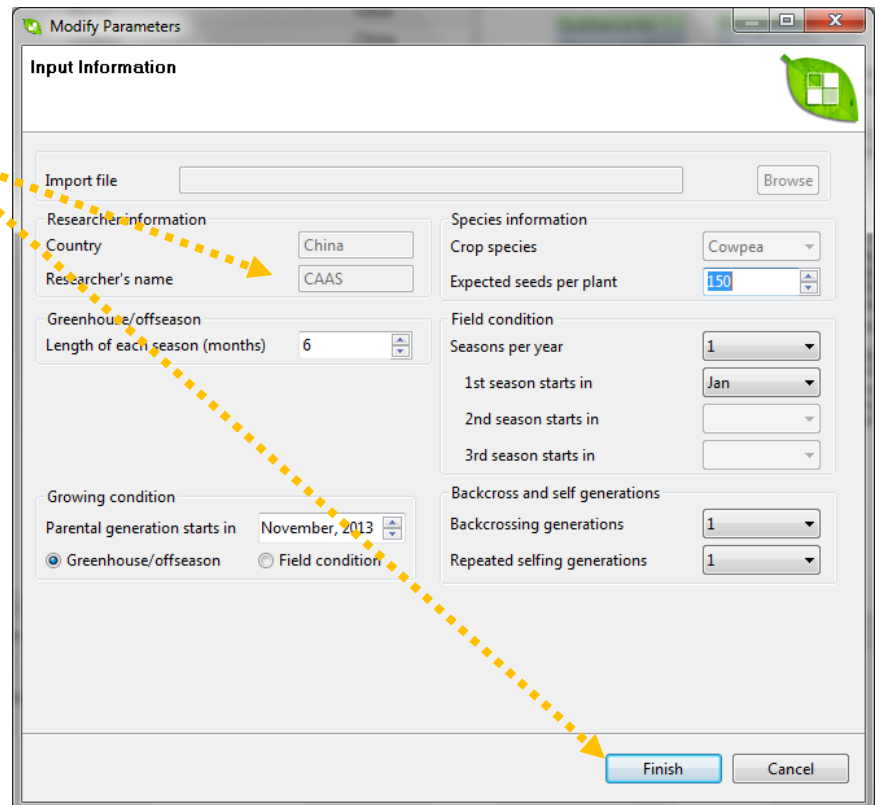
- Right click the MABC1 folder, then choose **Modify Parameters**



- Or click **Task-> Modify Parameters**



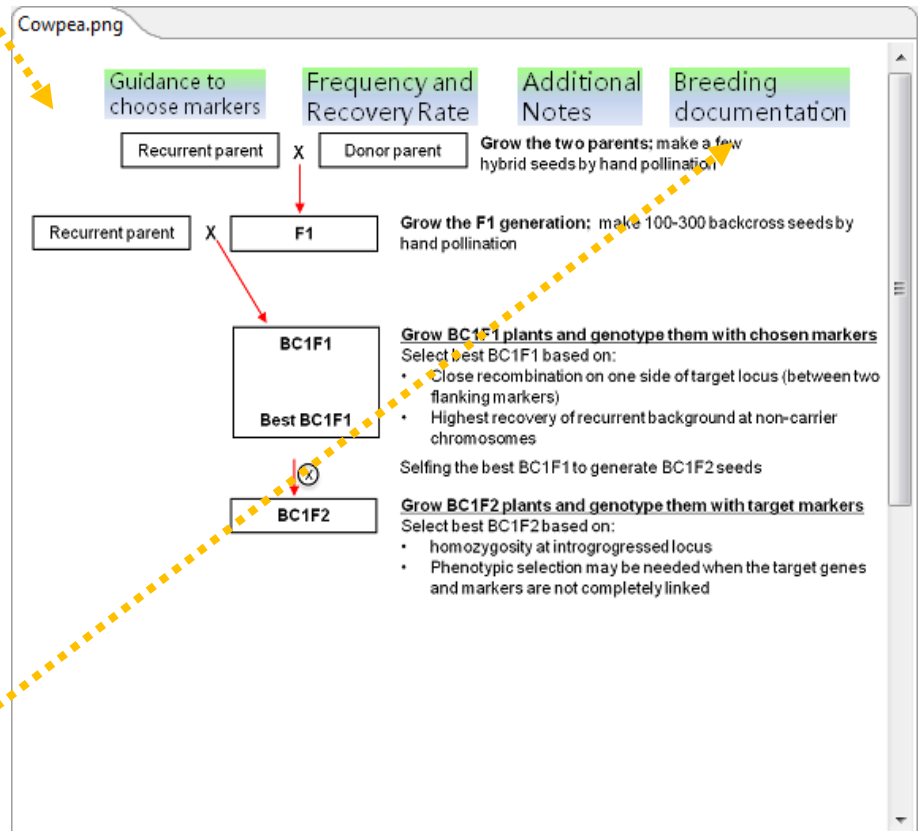
- You may modify the parameters in the Dialog Window, and then click Finish button to save your modifications. The breeding scheme will be automatically modified.

A screenshot of the 'Modify Parameters' dialog window. It is titled 'Input Information' and contains several sections of input fields. The 'Researcher information' section includes 'Country' (set to 'China') and 'Researcher's name' (set to 'CAAS'). The 'Species information' section includes 'Crop species' (set to 'Cowpea') and 'Expected seeds per plant' (set to '150'). The 'Field condition' section includes 'Seasons per year' (set to '1'), '1st season starts in' (set to 'Jan'), '2nd season starts in', and '3rd season starts in'. The 'Growing condition' section includes 'Parental generation starts in' (set to 'November, 2013') and radio buttons for 'Greenhouse/offseason' (selected) and 'Field condition'. The 'Backcross and self generations' section includes 'Backcrossing generations' (set to '1') and 'Repeated selfing generations' (set to '1'). At the bottom right, there are 'Finish' and 'Cancel' buttons. A yellow dashed arrow points from the text 'then click Finish button' in the list to the 'Finish' button.

PMABC Tutorial: The breeding scheme and documentation

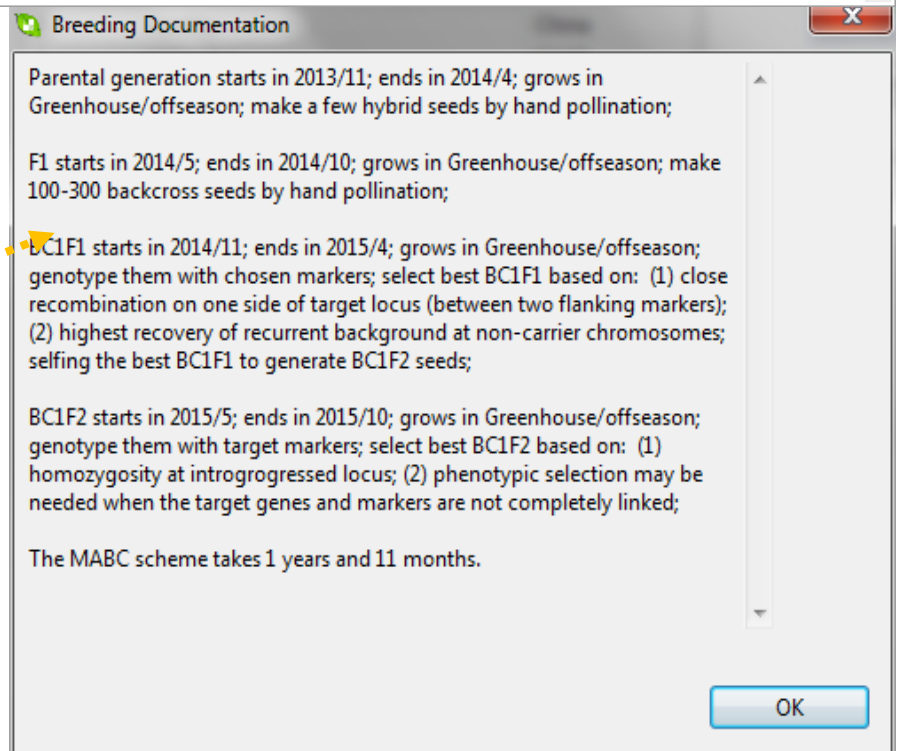
8. View the flowchart

- It is in the right window



9. View breeding documentation

- Click the **Breeding Documentation** button

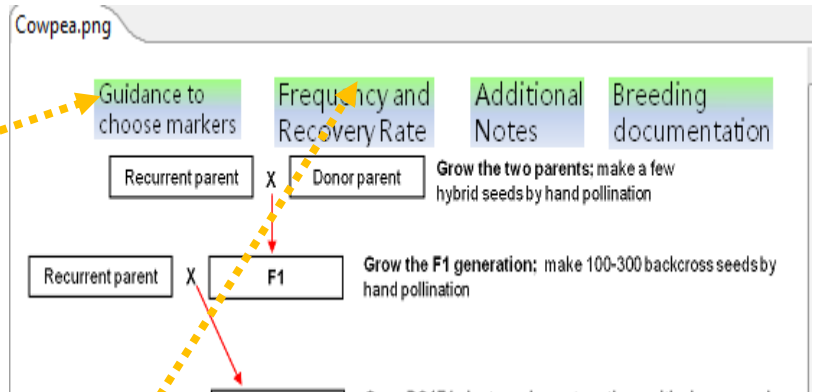


- The **Breeding Documentation** is in the pure text describing the whole flow of the MABC program.

PMABC Tutorial: Additional information

10. Additional information on the flowchart

- Click **Guidance to choose markers**



- The table for Guidance to choose markers will be popped up to help to choose markers to use in MABC process

Guidance to choose markers

Choose markers to use in MABC process

- 2-4 well spread polymorphic markers per chromosome for background selection
- 2-3 flanking markers on each side of locus to introgress (2-5 cM apart if major gene, if QTL involved the whole confidence interval needs to be spanned (4-5 markers))

OK

- Click **Frequency and Recovery Rate**

Frequency and Recovery Rate

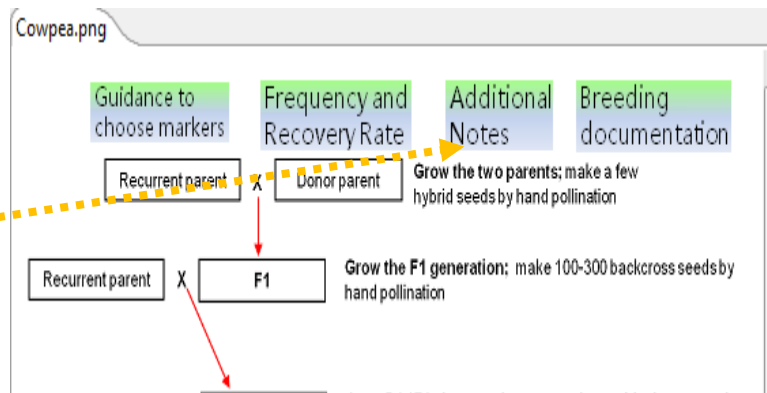
Rate (%) of homozygosity during repeated selfing								
Generation	Number of independent loci (higher value for linkage)							
	1	2	3	4	5	10	20	30
F2	50.00	25.00	12.50	6.25	3.13	0.10	0.00	0.00
F3	75.00	56.25	42.19	31.64	23.73	5.63	0.32	0.02
F4	87.50	76.56	66.99	58.62	51.29	26.31	6.92	1.82
F5	93.75	87.89	82.40	77.25	72.42	52.45	27.51	14.43
F6	96.88	93.85	90.91	88.07	85.32	72.80	52.99	38.58
F7	98.44	96.90	95.39	93.89	92.43	85.43	72.98	62.35
F8	99.22	98.44	97.67	96.91	96.15	92.46	85.48	79.03
F9	99.61	99.22	98.83	98.45	98.06	96.16	92.47	88.92
F10	99.80	99.61	99.42	99.22	99.03	98.06	96.17	94.30

OK

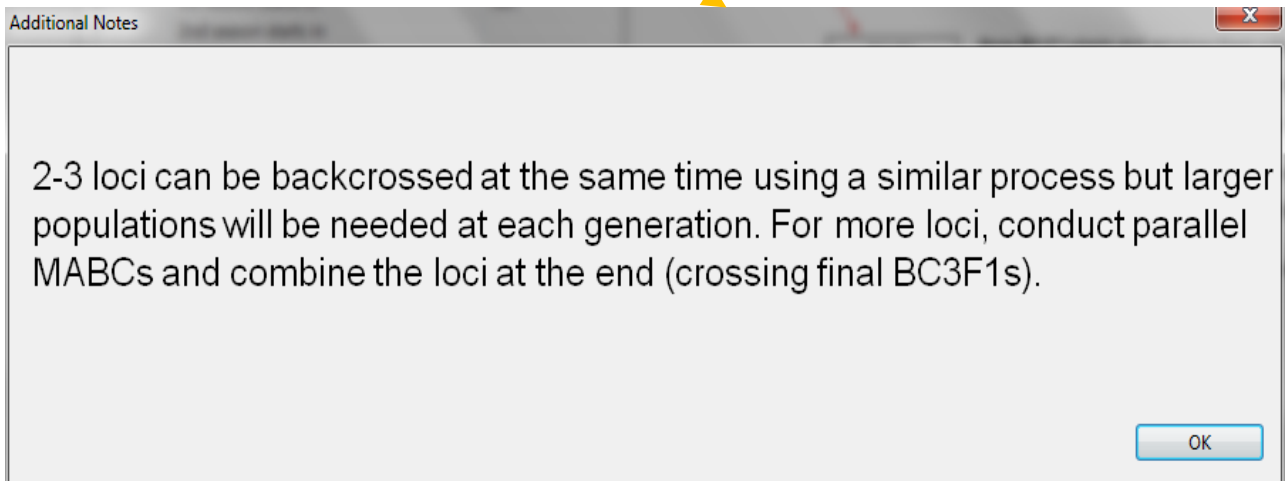
- The table for Frequency and Recovery Rate will be popped up to help to get the Rate (%) of homozygosity during repeated selfing

PMABC Tutorial: Additional information

- Click **Additional Notes**



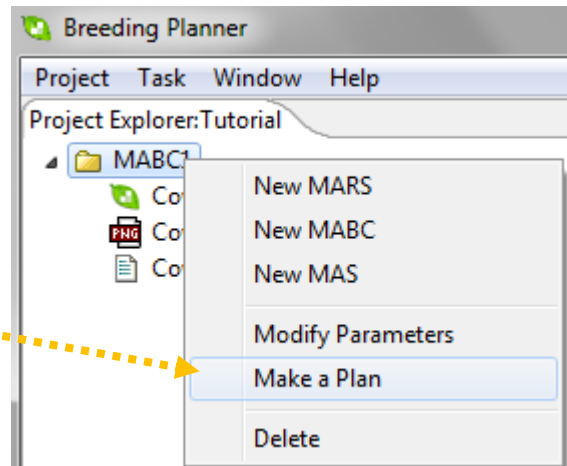
- The table for **Additional Notes** will be popped up to help to conduct the MABC process.



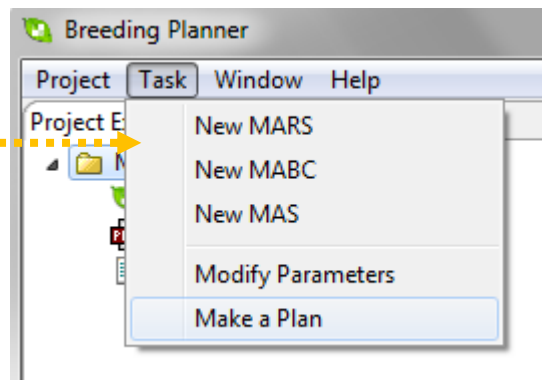
PMABC Tutorial: Make a plan from wherever you are

11. Make a plan

- Right click the **MABC** folder in the **Project Window**, then choose **Make a Plan**



- Or click **Task-> Make a Plan**
- The window for making a plan will be initialized. Some dialog information will appear in the Plan-making Window.



Make a Plan

Current status starts in

Select current status

PMABC Tutorial: Tell the system where you are

- Tell the system when the current season starts. The system will know the current season is grown in Greenhouse or in Field from the breeding parameters you specified.
- Tell the system where you are by selecting:
 0. Select parental lines and prepare for planting
 1. Parental lines is growing
 2. F1 generation is growing
 3. BC1F1 generation is growing
 4. BC2F1 generation is growing
 5. BC3F1 generation is growing
 6. BC3F2 generation is growing
 7. BC3F3 generation is growing
 8. BC4F4 generation is growing

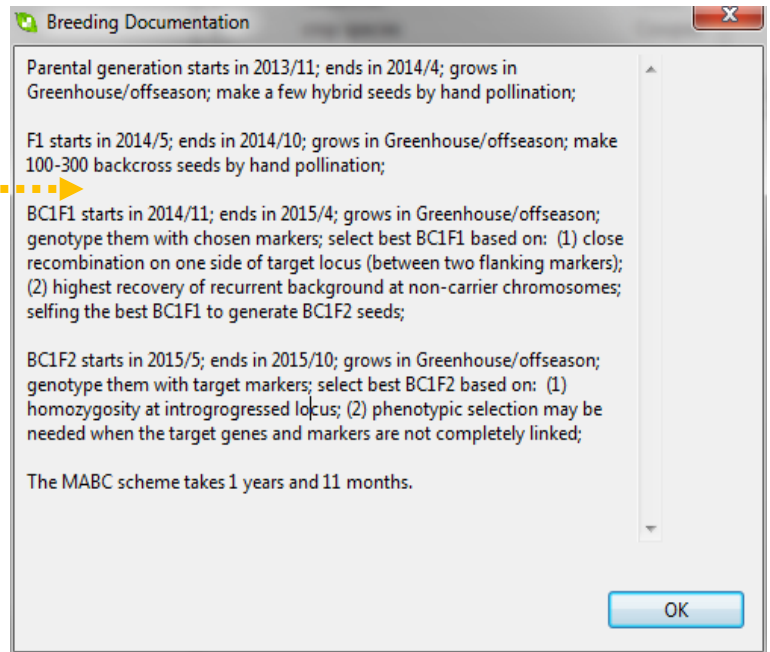
The generation name will depend on the number of backcrossing and repeated selfing generations.

The screenshot shows a web interface for the PMABC system. At the top, there is a grey bar with the text 'Make a Plan'. Below this bar, there are two main input areas. The first area is labeled 'Current status starts in' and contains a date selector showing 'November, 2013'. The second area is labeled 'Select current status' and contains a dropdown menu with the selected option '0. Select parental lines and prepare for planting'. Below these two areas, there is a button labeled 'Make a Plan'. Three yellow dashed arrows point from the text above to the interface: one from the first bullet point to the date selector, one from the second bullet point to the dropdown menu, and one from the final bullet point to the 'Make a Plan' button.

- Click the **Make a Plan** button to complete the on-going MABC breeding program.

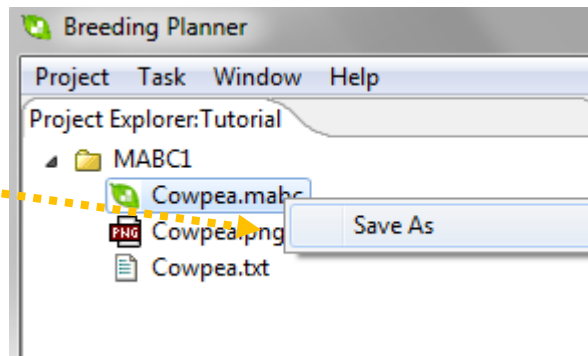
PMABC Tutorial: See the breeding plan the system makes

- New window will show the **Breeding Documentation** for the remaining status, and the time to complete the MABC breeding program

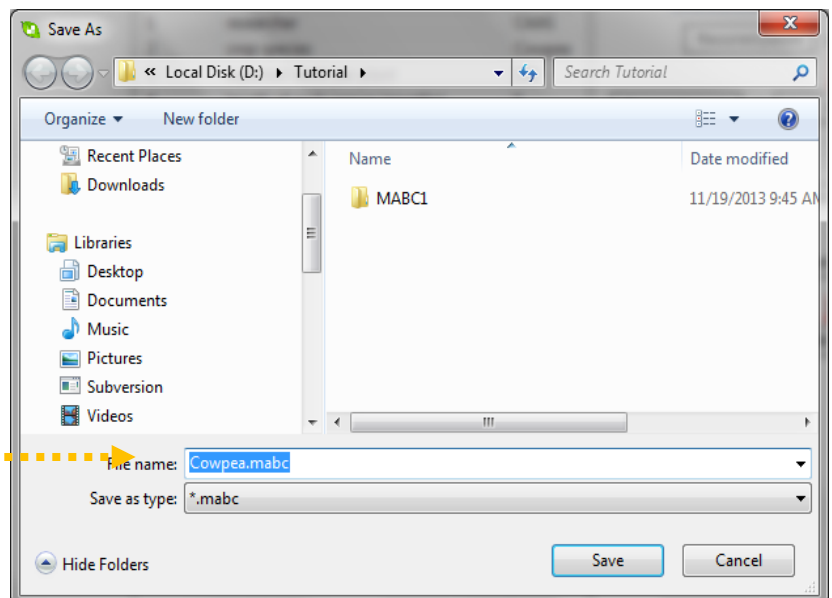


12. Save the result files

- Right click the file name, and then select **Save As** to save the breeding documentation on your computer



- Specify the path and rename of the output file



BP-MABC Tutorial: The contact information

- Any comments or suggestions? You may contact any one on the BP development team
 - Dr. Jiankang Wang, CIMMYT China and CAAS, wangjiankang@caas.cn or jkwang@cgiar.org
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