

# KaryoMeasure manual for karyotyping and idiogram drawing

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**KaryoMeasure is a free and user-friendly karyotype analyzing software that facilitates data collection from different digital images of the metaphase chromosome spreads and calculates a wide variety of chromosomal and karyotypic parameters along with the related standard errors. Moreover, KaryoMeasure can draw adjustable ideograms of diploid, allopolyploid, monocentric, and holocentric species into a vector-based PDF or SVG file.**

## Highlights

- 1. PDF and SVG format ideograms:** KaryoMeasure can draw idiogram of both diploid and allopolyploid and monocentric and holocentric species into a vector-based PDF or SVG file. There are a few controls to adjust the output ideograms.
- 2. Chromosomal parameters:** KaryoMeasure calculates the mean and standard deviation of chromosomal parameters of each homologous group.
- 3. Karyotype parameters:** KaryoMeasure calculates a wide variety of karyotype parameters and asymmetry indices.

Download KaryoMeasure software at: <http://karyomeasure.uok.ac.ir/>

## Overall software design and architecture

KaryoMeasure software is used for the calculation of chromosomal and karyotypic parameters and to build ideograms. For this, a project is created at first, in which chromosome pictures are uploaded for karyotype analysis or ideogram generation via the 'Image' button in the menu bar. The added pictures and upcoming activities on the pictures are automatically saved to the project, so it is possible to continue analysis later after closing and reopening the project. Chromosomal and karyotypic parameters represented in Table 1, are extracted by KaryoMeasure. The nomenclature used for the description of type or morphology of monocentric chromosomes is that proposed by Levan *et al.* (1964) in which the chromosome type is defined based on the mean of arm ratio (Table 2). Stebbin's asymmetry index is according to Table 3.

**Table 1.** Chromosomal parameters and karyotypic asymmetry indices along with their formula are extracted by KaryoMeasure software.

<b>Chromosomal parameter or asymmetry index</b>	<b>Formula</b>	<b>Reference</b>
Chromosomal parameters		
Arm ratio	$AR = L/S$	
Chromosome length	$CL = L + S$	
r-value	$S/L$	
Relative length of chromosome	$RL\% = (CL/\sum CL)\times 100$	
Centromeric index	$CI = S/CL$	
Chromosome type	Defined Terms in Table 2	(Levan et al., 1964)
Form percentage of chromosome	$F\% = (S/\sum CL)\times 100$	
Karyotypic parameters or asymmetry indices		
Total chromosome length of the haploid complement	$HCL = \sum CL$	
Total form percentage	$TF\% = (\sum S/\sum CL)\times 100$	(Huziwara, 1962)
Stebbins asymmetry index	A-C, 1-4 (Table 3)	(Stebbins, 1971)
Arano index of karyotype asymmetry	$AsK\% = (\sum L/\sum CL)\times 100$	(Arano, 1963)
Intrachromosomal asymmetry index	$A_1 = 1 - [\sum_{i=1}^n (S_i/L_i)/n]$	(Romero-Zarco, 1986)
Interchromosomal asymmetry index	$A_2 = s_{CL}/x_{CL}$	(Romero-Zarco, 1986)
Symmetry index	$S\% = (CL_{min}/CL_{max})\times 100$	
Mean centromeric index	$x_{CI} = \sum CI/n$	
Degree of karyotype asymmetry	$A = [\sum_{i=1}^n (L_i - S_i)/(L_i + S_i)]/n$	(Watanabe et al., 1999)
Mean Centromeric Asymmetry	$x_{CA} = A\times 100$	
Coefficient of variation of chromosome length	$CV_{CL} = (s_{CL}/x_{CL})\times 100 = A_2\times 100$	(Paszko, 2006)
Coefficient of variation of centromeric index	$CV_{CI} = (s_{CI}/x_{CI})\times 100$	(Paszko, 2006)
Asymmetry Index	$AI = (CV_{CL}\times CV_{CI})/100$	(Paszko, 2006)

L: Length of long arm, S: Length of short arm, i: homologous group number, s: standard deviation, x: mean, n: haploid chromosome number of an individual or a taxon.

**Table 2.** Nomenclature of chromosomes based on Levan *et al.* 1964.

Term	Centromeric position	Arm ratio	CI × 100
M	Median point	1	50
m	Median region	1-1.7	50-39.5
sm	Submedian region	1.7-3	39.5-25
st	Subterminal region	3-7	25-12.5
t	Terminal region	7-∞	12.5-0
T	Terminal point	∞	0

**Table 3.** The classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971).

Ratio	Proportion of chromosomes with arm ratio < 2			
	1.00 (1)	0.99–0.51 (2)	0.50–0.01 (3)	0.00 (4)
CL <sub>max</sub> /CL <sub>min</sub>				
< 2 (A)	1A	2A	3A	4A
2 – 4 (B)	1B	2B	3B	4B
> 4 (C)	1C	2C	3C	4C

The chromosomal and karyotypic parameters are extracted after click-wise tracing of the chromosomes in KaryoMeasure. After that, all the parameters are automatically stored in a text file in the output folder of the project. The output text file includes karyotypic parameters, chromosomal parameters, and raw data which can be opened in Excel software as well. KaryoMeasure calculates the mean and standard deviation of chromosomal parameters for all chromosomes of the same homologous group. These homologous chromosomes may be from one image or may belong to different images already opened in the project.

## 2 Materials

### 2.1 Software and hardware requirements

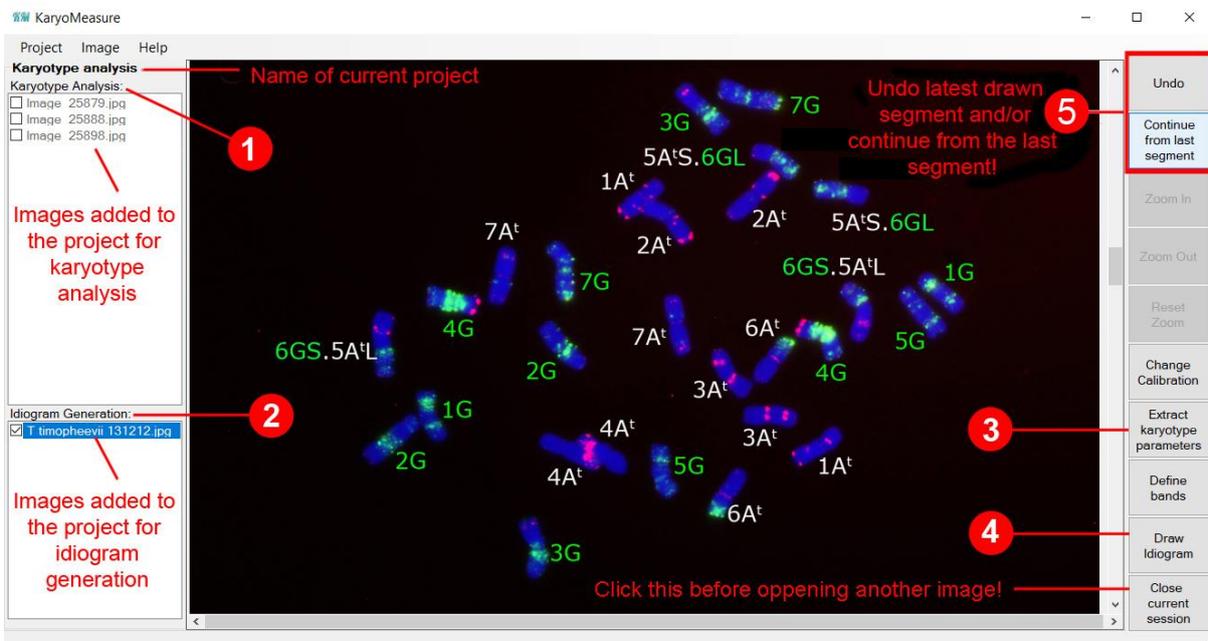
1. Mitotic metaphase chromosome pictures belonging to the same individual, same or different accessions or species depending on the aim of the study are required (*see Note 1*). KaryoMeasure supports input files with anyone of the jpg, tiff, bmp, and gif formats so there should be no need to convert the originally acquired image. It is possible to define a scale for all the opened images at once or a scale can be defined for each image separately, therefore it is not necessary that all the pictures be in the same magnification.
2. KaryoMeasure cannot identify homologous chromosomes automatically. Therefore, one needs to identify the homologs in advance. Homologs can be identified using a primary round of

length measuring and/or by referring to arm ratios, banding patterns, and chromosome landmarks if available (*see Note 2*).

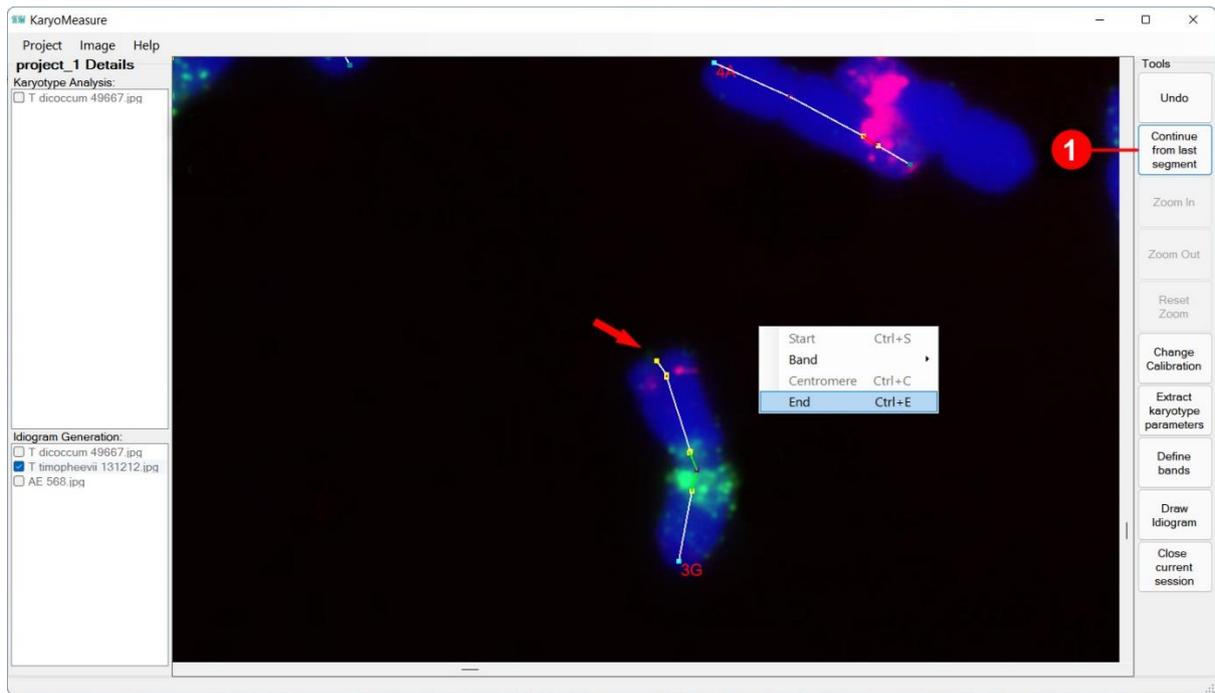
### 3 Methods

#### 3.1 Karyotype analysis

1. **Defining a project:** When the software opened, a project can be created via the Project button of the menu bar and then clicking on New Project (Ctrl + N).
2. **Adding images:** For karyotype analysis, images can be imported via the 'Image' button of the menu bar and then the 'Add image for Karyotype Analysis' button. Different metaphase images (as replicates) can be added to the project for karyotype analysis. The imported images are arranged in the karyotype analysis field on the left side (Figure 1). It is possible to remove any image along with the related data from a project via 'Image' and then clicking Remove Image' button.



**Figure 1.** Using KaryoMeasure to trace extract karyotype parameters or generating idiograms. Images added to the project for karyotype analysis or Ideogram Generation are listed under Karyotype Analysis (1) and Ideogram Generation (2), respectively. After manual chromosome tracing, karyotypic parameters can be extracted (3) and idiograms can be generated (4). After reopening a project and selecting an image, it is possible to undo the latest drawn segment and/or continue from the latest point on the active image (5).



**Figure 2.** If the latest point is a chromosome end, clicking on the ‘Continue from last segment’ key, reopens the end so that one can continue that chromosome. If you want to start a new chromosome, the previous chromosomes should be reclosed first (by Ctrl + E) because clicking on the ‘Continue from last segment’ key has already opened its end.

3. **Introducing scale:** Double click on one of the images and it will be opened. A scale needs to be defined by clicking on the start and endpoints of the scale bar of the image. A popup window appears asking for the actual length of the scale bar. Enter the corresponding size in  $\mu\text{m}$  and click on OK. It is possible to redefine the scale bar any time later via ‘Change calibration’ key of the right sidebar.
4. **Assigning a name to each chromosome:** For tracing each chromosome, it is required to define the chromosome number and its genome (or subgenome) at first by right-clicking and pressing Start (or Ctrl + S). In the case of allopolyploid species, it is possible to assign the corresponding subgenome to each chromosome (*see Note 3*).
5. On each image, the user needs to trace the chromosomes using mouse clicking. The centromere and end point of the chromosome is defined via right clicking or pressing Ctrl + C and Ctrl + E, respectively (*see Note 4*).
6. After tracing all chromosomes in an image, tracing can be continued in the other images, but the current session (e.g. the current image) must be closed before opening another image. The current session needs to be closed after tracing all chromosomes and before any further activity be conducted in the current project.
7. After tracing chromosomes in an image, ‘Close current session’ and double click on another image from the left pane to be opened. After tracing chromosomes in all images, karyotypic

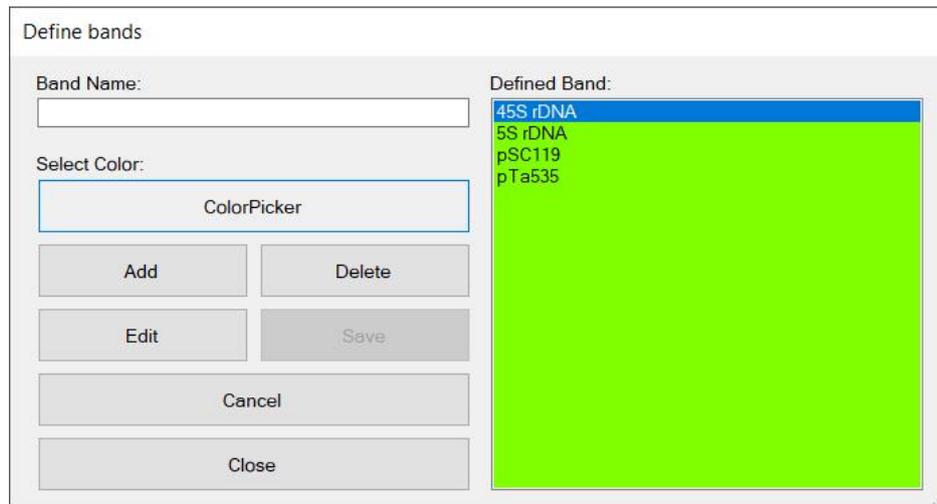
data can be stored in a text table file by pressing the ‘Extract karyotype parameters’ icon. The output text file has three parts: karyotype parameters, chromosomal parameters, raw data and abbreviations. Standard errors are also represented for each chromosomal parameter in the output table. The Notepad output file can also be opened in Excel software (Figure 3).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Karyotype Parameters															
2	HCL	TF%	Stebbins	AsK%	A1	A2	S%	Xci	A	Xca	CVcl	CVci	AI			
3	22.23467	37.16444	1A	62.83556	0.339581	0.298683	65.12544	0.387974	0.224051	22.40514	29.86825	28.18392	8.418043			
4	Chromosomal Parameters															
6	Name	L	SE_L	S	SE_S	CL	SE_CL	AR	SE_AR	r-value	SE_r-value	RL%	CI	SE_CI	Chromosc	F%
7	1A	4.689024	0.154085	4.080324	0.176871	8.769348	0.298685	1.149179	0.871173	0.870186	0.870186	39.43998	0.465294	0.592166	m	18.35118
8	2A	9.282254	0.115804	4.183066	0.220901	13.46532	0.207522	2.219007	0.524233	0.450652	0.450652	60.56002	0.310655	1.064472	sm	18.81326
9	Raw Data															
11	Chromoso	L	S	CL												
12	1A	4.966179	4.400322	9.366502												
13	1A	4.433761	4.050902	8.484664												
14	1A	4.667132	3.789747	8.456878												
15	2A	9.082449	4.532877	13.61533												
16	2A	9.483596	4.24186	13.72546												
17	2A	9.280715	3.774459	13.05517												
18	Abbreviations															
20	Chromoso	Formula	Reference													
21	L:	Mean length of long arm														
22	S:	Mean length of short arm														
23	CL:	Chromosome length = L + S														
24	AR:	Arm Ration = L/S														
25	r-value:	S/L														
26	RL%:	Relative length of chromosome = (CL/Sum(CL))×100														
27	CI:	Centromeric index = S/CL														
28	Chromoso	Defined Te	Levan et al., 1964													
29	F%:	Form percentage of chromosome = (S/Sum(CL))×100														
30	x:	mean														
31	n:	haploid chromosome number of an individual or a taxon														

**Figure 3.** By adding images for karyotype analysis followed by manual tracing of chromosomes, a text file including karyotypic parameters, chromosomal parameters, raw data and abbreviations will be added to the Output folder of the project.

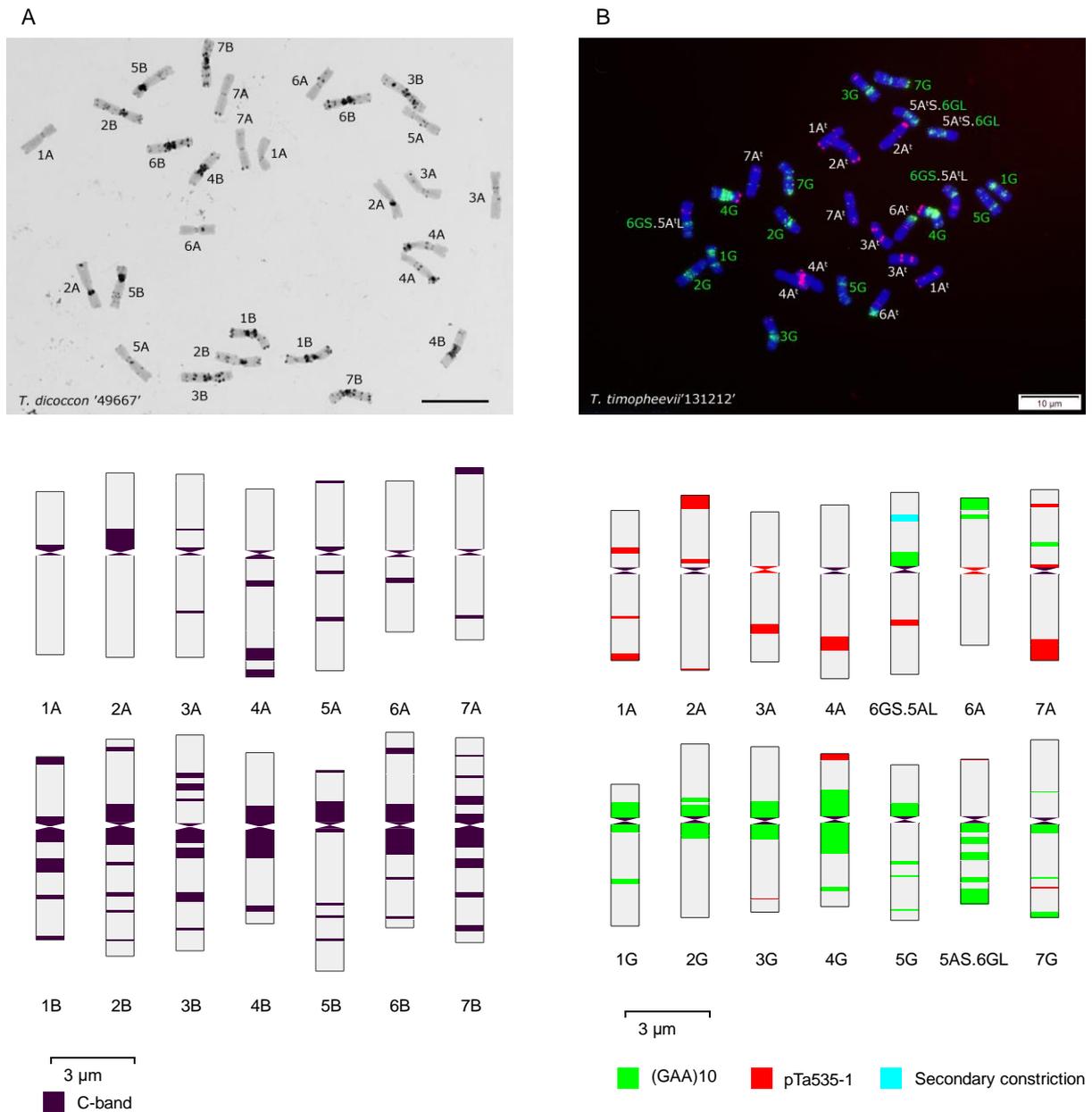
### 3.2 Idiogram generation

1. **Adding images:** For idiogram generation, image(s) should be added to the project via the ‘Image’ button of the menu bar and then the ‘Add image for idiogram generation’ button (Figure 1).
2. Different chromosome images can be added to the project for idiogram generation. The imported images are arranged in the ‘Idiogram Generation’ pane on the left side of the software (down area). Only one idiogram plate can be generated from each of these images.
3. Open one of the added images by double-clicking and introduce the image scale as mentioned in paragraph 3 of section 3.1.
4. If specific chromosomal landmarks such as centromere, C- or FISH bands, secondary constriction, 5S rDNA and 45S rDNA, etc. need to appear on the idiogram, a colour should be assigned to each of these landmarks via the ‘Define bands’ icon before chromosome tracing (Figure 4).



**Figure 4.** A color picker window will be appeared after clicking the 'Define bands' icon where different colors can be assigned to different bands.

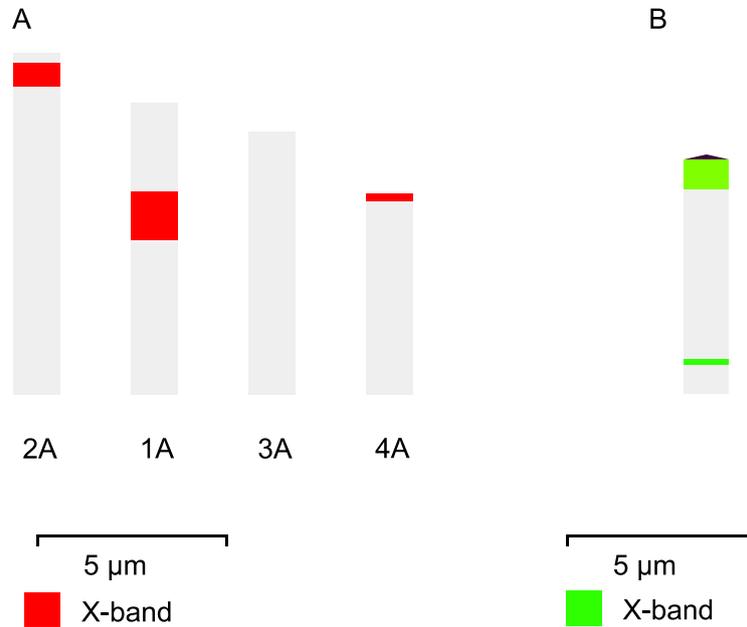
5. Again, chromosome number and subgenome need to be defined for each chromosome by right-clicking and pressing Start (or Ctrl + S) before tracing starts (*see Note 5*).



**Figure 5.** Generating idiogram of allopolyploid species using KaryoMeasure. C-banded idiogram of the *T. dicoccon* '49667' (A) and FISH-banded idiogram of *T. timopheevii* '131212' (B) generated by KaryoMeasure after tracing chromosomes. A scale bar is placed on the idiogram. From each homologous pair, only one chromosome has been traced.

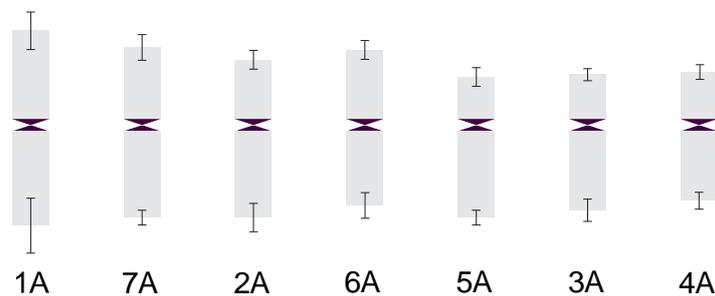
- After tracing all chromosomes, clicking on the 'Draw idiogram' icon will display the idiogram control page where a number of adjustable controls are available for idiogram generation. In this page, the user can adjust chromosome height, chromosome width, centromere size and colour and scale bar size. It is also possible to sort the chromosomes by name or height in descending or ascending order (Figure 9). Usually, only one chromosome from each homologous pair is traced for idiogram generation (*see Note 6*).

7. In the case of species with holocentric chromosomes, no centromere is defined during chromosome tracing in KaryoMeasure. For telocentric chromosomes, the centromere and one chromosome end are overlapping. If tracing is started from the centromere end, the centromere is defined immediately after the start point (Figure 6).

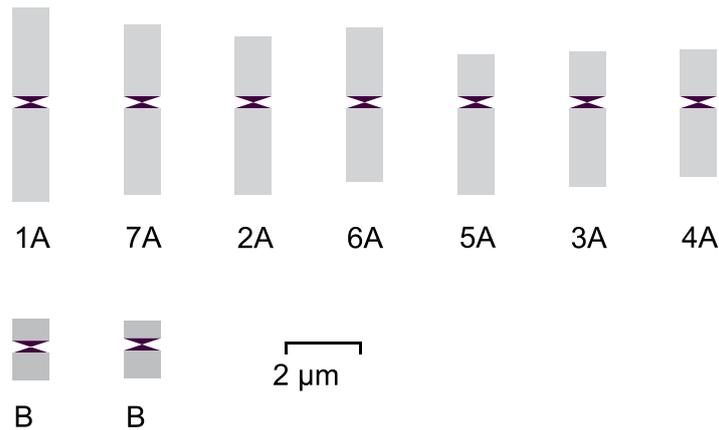


**Figure 6.** A) For the species with holocentric chromosomes, no centromere is defined during chromosome tracing in KaryoMeasure. B) For telocentric chromosomes, centromere and one chromosome end are overlapping. If tracing is started from the centromere end, centromere is defined immediately after the start point.

8. Another interesting option of idiogram generation is to use the karyotyping output table for making idiogram. By selecting the karyotyping table in the idiogram control page, automatically, its idiogram can be produced (Figure 7).



**Figure 7.** It is possible to use an output karyotype analysis table for idiogram generation in KaryoMeasure. An option is also available that adds standard errors of the arm length to the chromosome endpoints.



**Figure 8.** Idiogram of a species with 2 B chromosomes produced with KaryoMeasure and the Bs labels were edited in Inkscape (see Note 5 and Note 6).

Idiogram based on:	Image_25829.jpg
<input type="checkbox"/> Karyotype Analysis Table	<input checked="" type="checkbox"/> Add error bars
Idiogram width (px):	215
Number of Chromosome in a row:	5
Chromosome width (px):	15
Scale bar (μm):	2
Chromosome height (px):	20
Centromere size (px):	4
<input type="checkbox"/> Fill centromere with color	ColorPicker
Sort settings:	<input type="radio"/> Sort by name <input checked="" type="radio"/> Sort by height    Descending

**Figure 9.** Control page for idiogram production different parameters such as the number of chromosomes in a row, chromosome width and length, centromere size and colour and type of chromosome sorting can be adjusted. It is also possible to draw an idiogram (with or without error bars) based on the karyotype analysis table that has been previously generated during karyotype analysis.

## Notes

1. The whole or arm length of homologous chromosomes in the same or different cells can be very different because they are affected by a lot of factors such as pretreatment time and chromosome preparation method. Ideally, at least three high-quality metaphase spreads from three different cells or individuals should be analyzed for each sample.
2. Usually, it is not possible to identify all chromosomes only based on conventional staining techniques except the cases where chromosomes are morphologically enough heteromorphic. Looking for chromosomal landmarks such as chromosome sizes, arm ratios, secondary constrictions, size and position of heterochromatic knobs, and banding patterns resulting from

Giemsa C-banding, N-banding, and FISH using repetitive sequences helps to identify all the chromosomes.

3. Allopolyploidy is very common among plant species. An allopolyploid has two or more different subgenomes in each cell. If the corresponding subgenome is assigned to each chromosome, chromosomes of each subgenome appear in a separate row in the output idiogram image.
4. During tracing the chromosomes, the colour of startpoints and endpoint of chromosomes are green and dark green, respectively. The centromere point purple and the midpoints are yellow by default. However, users can change these default colours in Project > Properties.
5. If the species under study has B chromosomes, a letter different from that of normal chromosomes should be assigned to them. Consequently, Bs will be arranged under the normal chromosomes in the output idiogram picture (Figure 8).
6. Producing SVG and pdf format is an advantage of KaryoMeasure because they are high quality and low weight and can further be processed in vector-based graphics software such as Inkscape or Adobe Illustrator. Editing colours and fonts and repositioning of objects are simply possible.

## References

- Arano, H. (1963). Cytological studies in subfamily Carduoideae (Compositae) of Japan. *IX. Bot Mag* **76**, 32.
- Huziwara, Y. (1962). Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. *American Journal of Botany*, 116-119.
- Levan, A., Fredga, K., and Sandberg, A. A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* **52**, 201-220.
- Paszko, B. (2006). A critical review and a new proposal of karyotype asymmetry indices. *Plant Systematics and Evolution* **258**, 39-48.
- Romero-Zarco, C. (1986). A new method for estimating karyotype asymmetry. *Taxon*, 526-530.
- Stebbins, G. L. (1971). "Chromosomal evolution in higher plants," Edward Arnold (Publishers) Ltd., London, UK.
- Watanabe, K., Yahara, T., Denda, T., and Kosuge, K. (1999). Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae): statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. *Journal of Plant Research* **112**, 145-161.