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DRAWID V0.26

Make your karyotyping easier!

Manual

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Content

Introduction

Chromosome number and morphology are an important source of information for comparative cytogenetic and phylogenetic studies. Discrepancies in chromosome morphology between individuals often result from inter- and intra-chromosomal rearrangements being a major force of evolution and speciation. Knowledge about chromosome rearrangements and basic chromosome parameters (e.g. centromere index, arm ratio, relative length) can be useful for integration of physical and genetic maps, study of speciation and evolution and tracing desirable traits during the plant breeding process. Chromosome number and morphology is typically schematically represented as an Idiogram. An Idiogram is an informative way to represent chromosome length, centromere index and chromosome arm ratio providing general information about karyotype structure. Typically, to build an Idiogram a couple of metaphases is measured and average values of chromosome length and centromere index are then used. To determine correct chromosome order and homologous pairs additional chromosomal markers such as banding patterns and FISH signals are often required. Model plant species such as Arabidopsis, wheat and maize often have standardized karyotype and Idiograms facilitating cytogenetic studies of their genomes. However, most of the plant species still lack this information and chromosome measurements are needed to build a standardized karyotype. Moreover, cytology-based ecological studies of genome variability require measurements of chromosomes from a large number of individuals. To accelerate karyotypic studies in plants few software programs have been published for chromosome measurements, including MicroMeasure (Reeves, 2001), IdeoKar (Mirzaghaderi and Marzangi, 2015) and KaryoType (Altınordu et al., 2016). However, there are no freely available, user-friendly and cross-platform programs for simultaneous measuring of chromosome parameters, chromosomal landmark positions (e.g. band, FISH signals) and Idiogram construction. Here we present the DRAWID (DRAWing Idiogram) – program for karyotype analysis and Idiogram construction. DRAWID is user-friendly and freely available (under GNU General Public License license) java-based software that facilitates basic as well as FISH-based karyotype analysis. DRAWID is equipped with nicely designed and intuitive graphical user interface. It takes an image file or data table generated by DRAWID as input files and generate Microsoft XL (2010) table, containing measurement details (Centromere index, Arm ratio, relative and absolute length of chromosome and chromosome arms, signal and band positions and size if available), and Idiogram. The Idiogram can be easily adjusted to prepare a high-quality image suitable for journal publication. In addition, to facilitate high-throughput karyotyping the program allows to collect data from numbers of metaphases, store it and use for average Idiogram construction decorated by standard deviation bars. DRAWID speeds up the process of chromosome measurements and makes ready-to-publish Idiogram construction much simpler. Current version of DRAWID is v0.2

and can be downloaded from web site of Russian State Agrarian University-MTAA (Department of plant genetics, biotechnology and breeding): http://plantgen.com/DRAWID/.

General procedure of DRAWID application

1. Upload jpeg image file - by  button
   New window for chromosome measurements will appear.

2. Start chromosome measurements by clicking along the chromosome length and choosing chromosomal landmarks if necessary (e.g. gene, centromere, band). There are several options available (Table 1).

Table 1. Buttons of the measuring window and their functions

Button	Hot key	Explanation
	F	Finish measurement of current chromosome
	-	Draw Idiogram
	-	Remove one of the chromosome measurements
	-	Join broken chromosome. Note! Firstly, a part possessing centromere must be measured followed by second part without centromere.
	C	Mark position as a centromere
	B	Band measurements. A first click on the button starts a band measurement while a second click denotes the end of the band. After the second click the name of the band is announced.
	D	Mark as a single point feature (e.g. single gene signal). The name of the feature is asked.
	-	Undo
	-	Remove all measurements
	-	Use last measured segment as a bar. It will allow to scale the length of the measured chromosomes according to the bar length.
-	S	Satellite measurements. The first click on the button starts a satellite measurement while the second click denotes the end of the measurement.

3. Click  button to draw Idiogram

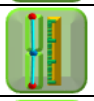
4. Data retrieval. To get data click **Data -> Show Chromosome (metadata) table -> Save as xslx ->** chose folder and name. It will save Microsoft XL file with two pages insight: 1 – Chromosome, 2- Metadata

5. Save Idiogram picture. To save Idiogram picture, click **File -> Save Figure**

Idiogram settings and functions

When the Idiogram has been constructed and it is visible in the main window different settings can be applied to adjust Idiogram parameters (Table 2). Color and name of an individual chromosome can be changed by selecting the chromosome and clicking on the right button of the mouse.

Table 2. Buttons of the main window and their functions

Button	Explanation
	Change chromosome color for all chromosomes
	Change centromere color for all chromosomes
	Load image for measurements
	Change chromosome names to the numbers sorted according to the current chromosome order
	Add current Idiogram to the database
	Draw average Idiogram from set of Idiograms stored in the database. The function will take chromosomes with the same number (homologous chromosomes) and find mean and SD. Then new plot will be drawn.
	Show chromosome legend (if any gene or bands were measured)
	Build 1n Idiogram. It will merge neighbor chromosomes according to the ploidy level (by 2, 3, 4 ..) into one and draw mean and SD for each arm and centromere index.
	Switch to other Idiogram from the database
	Show all Idiograms stored in the database
	Legend (if any genes or bands were measured)

FAQs

a. How to draw Idiogram from previously saved DRAWID xlsx table. Data tables generated by DRAWID can be used as input file to draw Idiogram again. For this click  and choose file.

b. How to change chromosome order. To change chromosome order, select a chromosome by left click and use left and right arrows on the keyboard to move the chromosome.

c. How to change chromosome size, distance and width. Now, this is only possible if no signals were measured. Select **Settings -> Chromosome parameters**

Bug reports.

Please send your proposals, comments and bug reports to Kirov Ilya (kirovez@gmail.com)

Examples

Conventional Idiogram construction

1. Measurement of chromosomes (Figure 1):

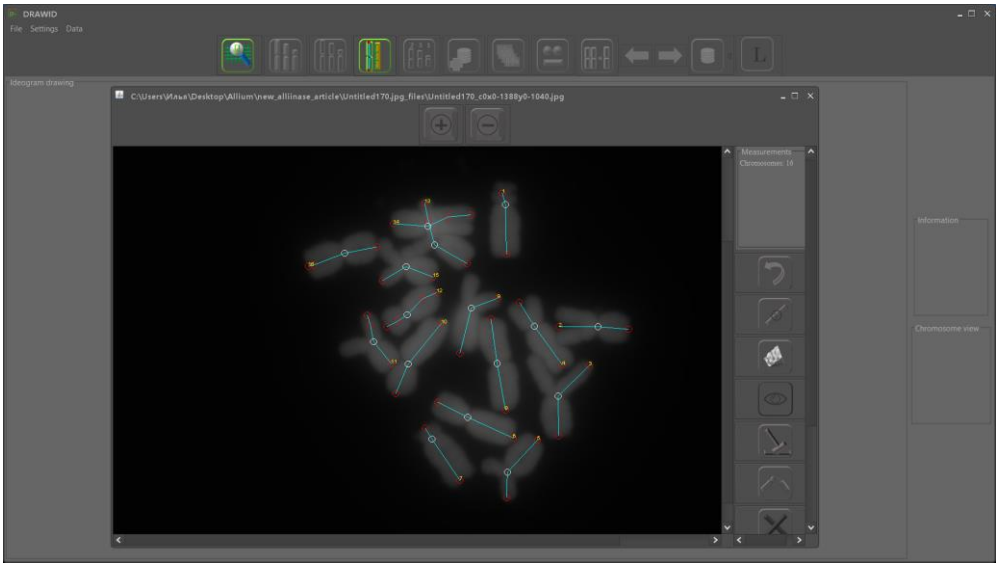


Figure 1. Measuring of metaphase plate of *Allium fistulosum* by DRAWID.

For each chromosome, the following actions are repeated:

   a. Start measurement by clicking in the beginning of a chromosome;
   b. Click on the centromere and press C or  button.
   c. Finish measurement by clicking in the end of a chromosome;

2. Measure the bar (Figure 2):

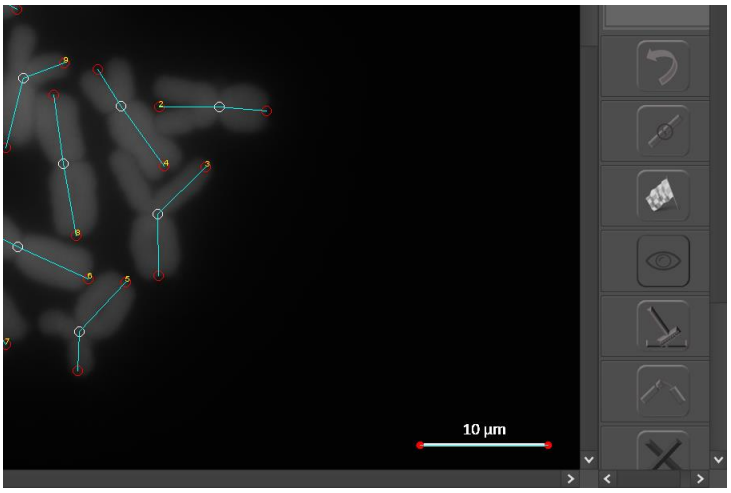
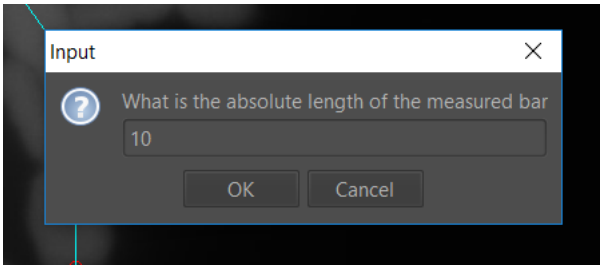


Figure 2. Bar measuring

   a. Select the bar by clicking in the beginning and in the end

b. Press 

c. Type real bar length in opened window and click OK:



3. Click  to get Idiogram picture (Figure 3).

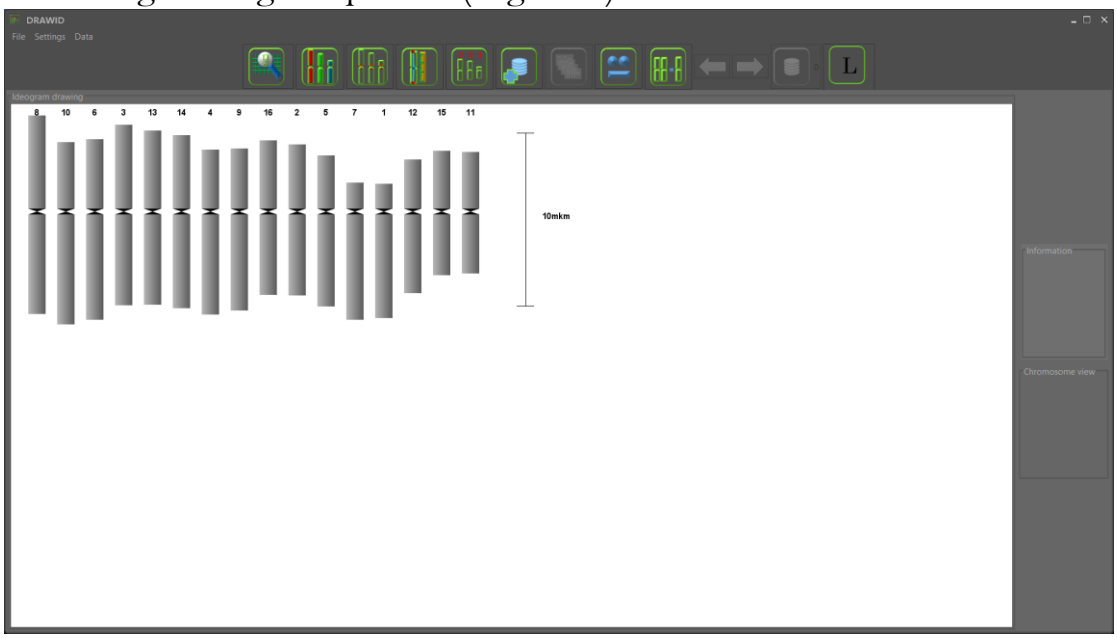
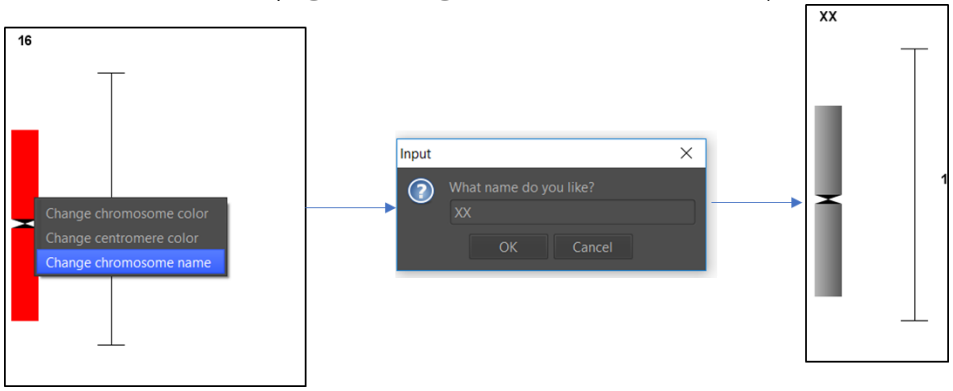


Figure 3. "Raw" Idiogram after measuring

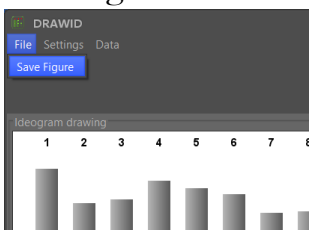
4. Idiogram settings:
   a. Change the chromosome order if it is required.
   Note! The DRAWID orders the chromosomes according to the length only and centromere index is not taken into account.
   b. Click  button to rename the chromosomes according to their order.
   c. Move down all chromosomes by choosing one of the chromosomes and pressing down button.

d. Chromosome and centromere colors can be changed using  and  , respectively. In this example chromosome and centromere colors were changed to blue and grey, respectively.

e. Individual chromosome color, name, and centromere color can be changed by selecting the chromosome, right click and choosing the respective button (e.g. "Change chromosome name):



f. Save figure: **File -> Save Figure**



Final Idiogram picture after cropping (Figure 4):

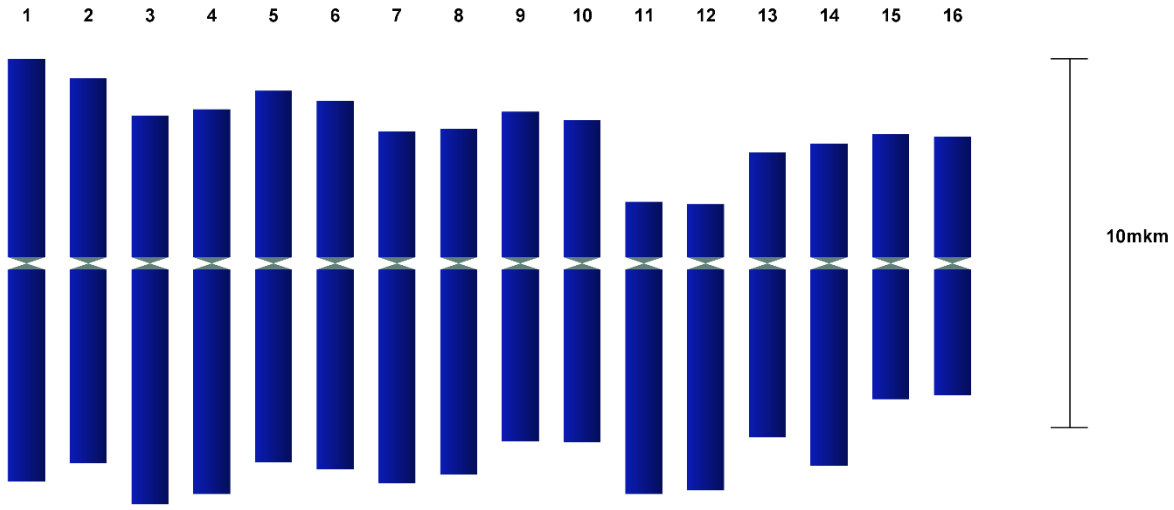


Figure 4. Final Idiogram

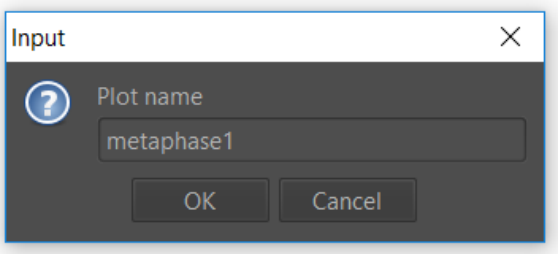
Average Idiogram construction

To an Idiogram using data from several metaphases button  is used.

Initially this button is inactive because you need to add > 1 Idiogram to the database. Follow these steps for each metaphase plate:

1. Measure individual metaphase plates and construct Idiogram as it is shown in previous section.

2. Add Idiogram to the database by clicking  and type Idiogram plot name:



After all Idiograms have been added to the database press  and choose the plots you want to include to the calculation. Finally, you will get an "average" Idiogram (Figure 5).

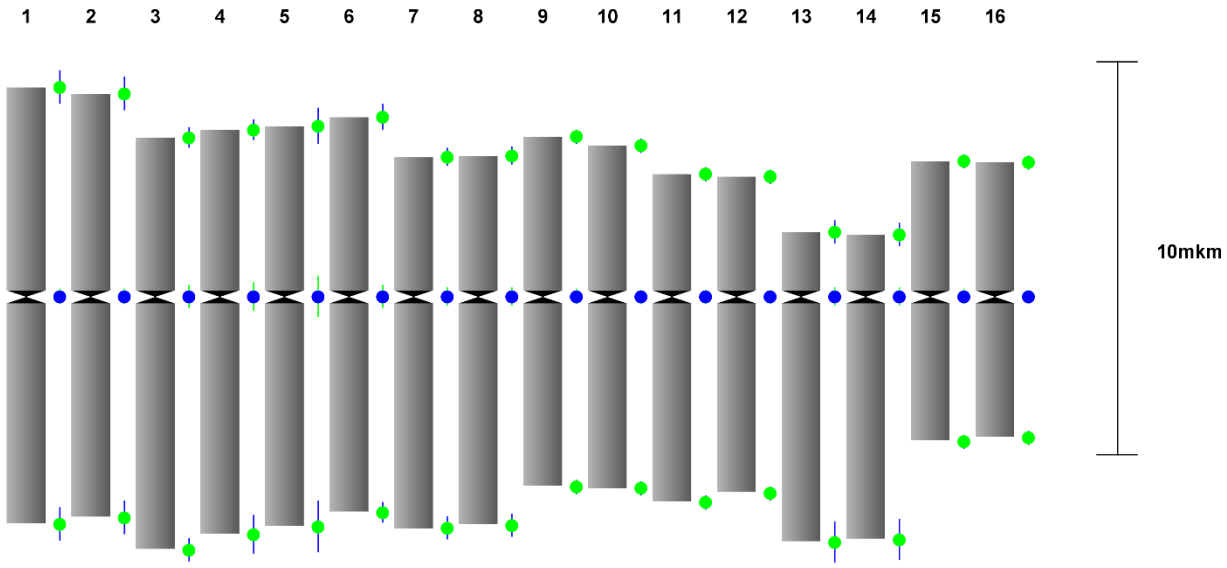
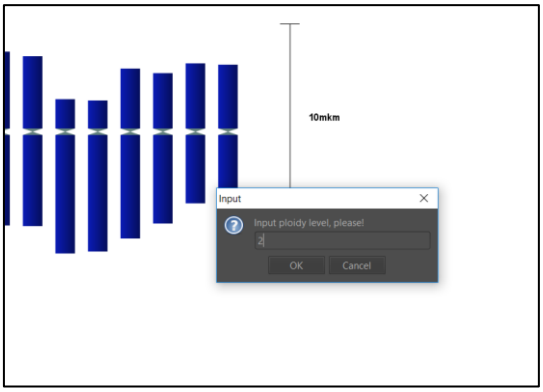


Figure 5. Average Idiogram after merging three Idiograms. Bars are SD for short arm, long arm and centromere index.

Build 1n Idiogram

When you need to build 1n Idiogram  button is used after an Idiogram has been constructed as it is shown in Example 1.

1. Build an Idiogram as it is shown in Example 1;
2. Click 
3. Type ploidy level



After these steps new Idiogram will appear (Figure 6).

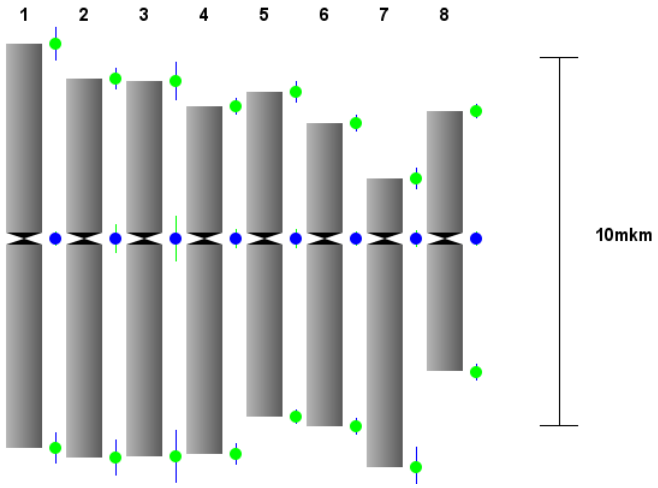


Figure 6. 1n Idiogram of *Allium fistulosum*.

If SD bars are not required you can remove them by unselect checkbox in **Settings -> Stat graphs**

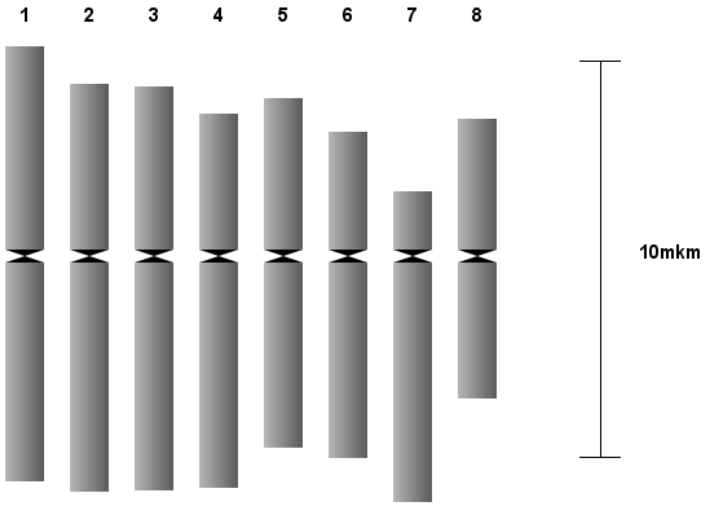


Figure 7. 1n Idiogram of *Allium fistulosum* without SD bars.

Idiogram with FISH signals

Dot-like signals

There are few hotkeys and buttons designed to show signal/bands/NOR regions positions on chromosomes. Example below show measurements of the chromosomes stained by DAPI following FISH with 5S rDNA (red signals).

Chromosomes without signals are measured as it is explained in the example 1. Chromosomes possessing FISH signals are measured by the same way but FISH signals then selected by clicking on the signal followed by pressing **D** or  button. Then signal name is typed (Figure 8).

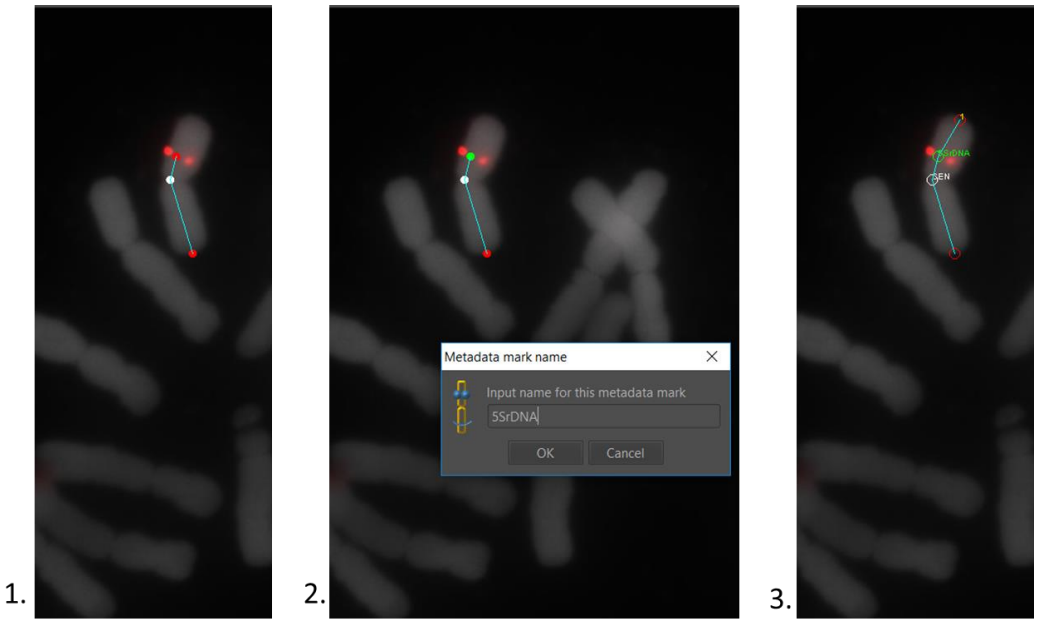


Figure 8. Measurement of chromosome with FISH signal.

Band-like signals

Bands as well as NOR regions have start and end coordinates. Both are required to correctly measure the positions. For these you need click in the beginning of the band (or NOR region) and press **B** (or **S** in case of NOR) or  button then you will be asked to type name of the band (not for **S** button) and then you can continue measurements of the band and when you finish press **B** (or **S** in case of NOR) again (Figure 9).

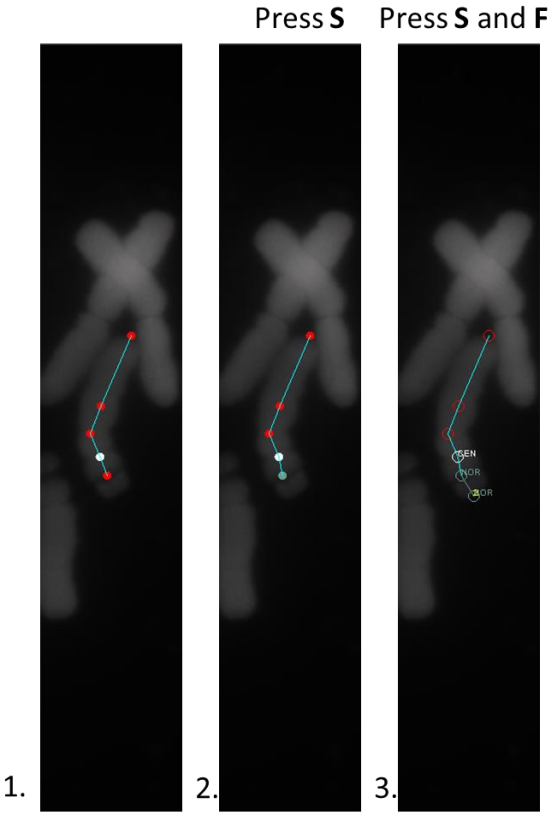


Figure 9. Measurement of chromosome with NOR.

Signal and band color as well as names can be changed after Idiogram was constructed. Use  button to open signal menu where you can do such changing. Left and right columns re used to change name and color, respectively (Figure 10).

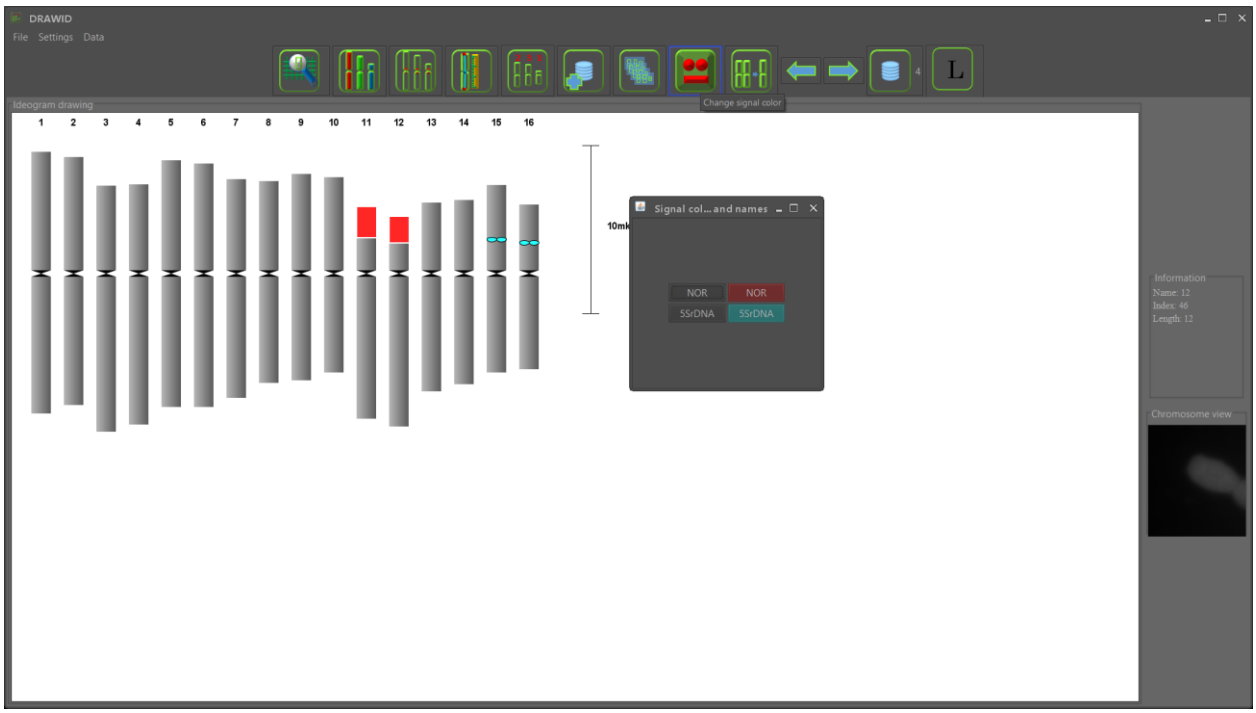


Figure 10. Idiogram with FISH signals and NOR positions Menu for signal correction is shown.

In current version of DRAWID legend is not added to the main plot but can be saved as a separate file and then merged by another program (Photoshop, Power Point etc.) with Idiogram plot. To open Idiogram legend press  button.