Supporting Online material for:

Genes Required for Mitotic Spindle Assembly in *Drosophila* S2 Cells

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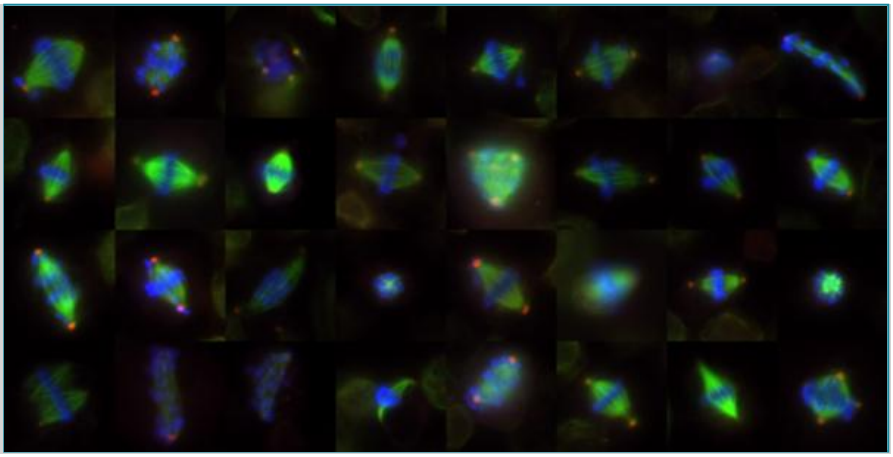
- Online database
- How to use the database
- Hit List (summary, pdf)
- Hit List (database)
- GFP Localization
- Analysis code



Note: Always compare phenotypes in the database to neighboring wells as there is significant variation between plates.

[Vale lab RNAi updates] [Database enquiries] [Phenotype enquiries]

http://rnai.ucsf.edu/mitospindlescreen/

February 22, 2010, 2:56 pm

--Links-- --Databases-- --System--

Now Viewing Table: **MitoSpindleScreen**

16394 Records found. Showing 1 through 12. 12 Records per page

name	symbol	CG	plate	row	col	RNAi probe	repeats	cells/image	mitotic index	hit	re-imaged	positive control	cd RN
	CG13589	13589	113	G	2	probe info		86.07	0.00%	No			
			146	F	2	probe info		123.44	6.93%	No			
			146	F	1	probe info		141.11	9.00%	No			
folded gastrulation	fog	9559	146	E	11	probe info		56.47	12.25%	No			
	CG12446	12446	146	E	10	probe info		183.69	4.96%	No			
	CG17601	17601	146	E	9	probe info		204.33	6.80%	No			
	CG10677	10677	116	G	11	probe info	Repeat	148.79	6.39%	No		No	
	CG14617	14617	146	E	8	probe info		213.58	2.58%	No			
	CG12576	12576	146	E	7	probe info		187.83	6.33%	No			
	CG14615	14615	146	E	6	probe info		191.61	3.70%	No			
DISCO Interacting Protein 1	DIP1	17686	146	E	5	probe info		58.47	6.51%	No			
	CG32822	32822	146	E	4	probe info		162.61	6.99%	No			

Table S1: List of mitotic genes identified in the screen

Centrosome/γ-Tubulin

Phenotype	Novelty	CG #	*Drosophila* name	Human homologue	Function	S2 metaphase localization	Other phenotypes
Anastral & Monastral bipolar	Expected	7186	Sak	Plk4	Kinase	Centriole (C)	
		10061	DSas-4	CPAP		Centriole (N & C)	
		15524[W]	*Sas-6*	Sas-6			
	Novel	6631	*Ana1*			Centriole (N) Whole cell(C)	
		8262[W]	*Ana2*			Centriole (N & C)	
		13162[W]	*Ana3*				
Dim γ-tubulin (Centrosome)	Expected	4832	Cnn		γ-tubulin recruitment	Centrosome[∃]	
		12306	Polo	Plk1	Kinase		Pole detachment
		17286 (18217)	*Spd-2*	Cep192			
	Unexpected	32782[W∀]	Tlk	Tousled-like kinase	Kinase		Misalign
	Novel	6118[W]			Transcription factor		
Dim γ-tubulin (Centrosome + Spindle)	Expected	γ-Tubulin small complex (γTuSC) (CG3157/γTub23C, CG3917/Dgrip84, CG10988/dd4/Dgrip91)			MT nucleation	Centrosome[∃] Spindle[∃]	Monopolar Long SP
	Unexpected	1135[W∀]		Microspherule protein 1	rRNA transcription	Whole cell(N)	Long SP
	Novel	18041[W∀]	*Dgt1*	LOC54934		Whole cell (N & C)	
		16969	*Dgt2*			Spindle (N & C)	
Dim γ-tubulin (Spindle)	Expected	γ-Tubulin ring complex (γTuRC) outer subunits (CG5688/Dgrip163, CG6176/Dgrip75, CG9201/Dgrip128, CG10346/Dgrip71)			MT nucleation	Centrosome[∃] Spindle[∃]	
	Novel	3221	*Dgt3*			Whole cell (N & C)	
		4865	*Dgt4*			Spindle (N & C)	
		8828	*Dgt5*				
		11881	*Dgt6*	FAM29A			
Dim MT	Expected	Tubulin (3)				Spindle[∃]	Monopolar Short SP
		Tubulin chaperone (8)					

Spindle shape

Phenotype	Novelty	CG #	*Drosophila* name	Human homologue	Function	S2 metaphase localization	Other phenotypes
Monopolar	Expected	9191	Klp61F	Eg5/Kinesin-5	MT sliding	Spindle[∃]	
Multipolar	Expected	7831	Ncd	HSET/Kinesin-14	MT sliding	Spindle[∃] MT plus-end[∃]	Unfocused spindle MT
		4454	Borealin	Borealin	Passenger	Centromere[∃]	Monopolar Few M
		6620	Ial	Aurora-B			
		12165	Incenp	INCENP			
	Unexpected	1258	Pav-KLP	MKLP1/Kinesin-6	MT bundling	Centrosome[∃]	Cytokinesis failure
		13345	RacGAP50c		GAP		

Monastral bipolar	Unexpected	9045	Myb	Myb	Transcription factor	Whole cell (N & C)	Long SP Pole detachment Few M
		3480[W]	Twit/ Mip130	Lin-9 family	Myb-interacting		Long SP
		2013	UbcD6	E2A/hRad6	E2		
		Proteasome (10)				Whole cell [Mov34] (N & C)	Dim γ-tub Short SP Few M
		17291	PP2A-29B	PR65	PP2A regulatory subunit		Defective overall Few M
		4494[∀]	Smt3	SUMO			
Unfocused spindle MTs	Expected	6875	Asp	ASPM	MT binding	Spindle pole[∃]	Pole detachment
	Unexpected	8472	CaM	Calmodulin	Ca binding	Spindle pole (N & C)	
Pole detachment	Expected	Dynein/dynactin (CG1938/Dlic2, CG7507/Dhc64C, CG8269/Dmn, CG9206/Glued)				Whole cell[∃] Unattached kinetochore[∃]	
	Unexpected	7007[W]	VhaPPA1-1		Vacuolar H - transport		

Spindle length

Phenotype	Novelty	CG #	*Drosophila* name	Human homologue	Function	S2 metaphase localization	Other phenotypes
Long spindle	Expected	1453	Klp10A	MCAK/Kinesin-13	MT depolymerization	Spindle pole[∃] Kinetochore[∃]	
	Unexpected	5785	Thr	Separase	Protease		Few M
		11006	Sap130				
		4654[∀]	Dp		Transcription factor		
		10800[∀]	Rca1	Rca1	APC inhibition		Few M Defective overall
		RNA polymerase II (7) [ΠA]				Whole cell [RpII33] (N & C)	Misalign Few M
Short spindle	Expected	5000	Msps	Dis1/XMAP215	MT polymerization	Centrosome[∃]	Monopolar
		32435	Mast/Orbit	CLASP		Kinetochore + Spindle (N) Whole cell(C)	
		3265	EB1	EB1		Centrosome[∃] MT plus-end[∃]	
	Unexpected	3992[W∀]	Srp		GATA-like		Monopolar Few M
		Ribosome & translation factors (54)[Π]				Whole cell [RpL11, RpL12, RpL19] (N & C)	
		10648	RBM13	Mak16	Ribosome biogenesis		Monopolar
		12396	Nnp-1				
		5650	Pp1-87B	PP1	Phosphatase		
		8222[W]	Pvr		Receptor-related		
	Novel	14781	*Ssp1*			Spindle (N)	Monopolar
		9028	*Ssp2*				
		18397	*Ssp3*			Spindle (N & C)	
		33130	*Ssp4*				
		14735	*Ssp5*				

Chromosome structure and alignment

Phenotype	Novelty	CG #	*Drosophila* name	Human homologue	Function	S2 metaphase localization	Other phenotypes
Chromosome misalignment	Expected	13329	Cid	CENP-A	Histone H3 variant	Kinetochore$^{\exists}$	Long SP
		31258	CENP-C	CENP-C	Kinetochore assembly	Kinetochore(N)	Long SP Pole detachment
		7838	Bub1	Bub1			
		7581	Bub3	Bub3			
		10923	Klp67A	KIF18/Kinesin-8	MT depolymerization	Kinetochore$^{\exists}$ Spindle$^{\exists}$	Long SP Monastral bipolar
		4008^{W}	Rad21	hRad21/hScc1	Chromatid cohesion		
		8598^{W}	Deco	EFO1			
		1763^{W}	Nod	Kid/Kinesin-10	DNA-bd kinesin		
		6392^{W}	CENP-meta	CENP-E/Kinesin-7	Kinetochore kinesin		
		8590^{W}	Klp3A	KIF4/Kinesin-4	Chromokinesin		
	Unexpected	9633	RpA-70				Long SP
		8374	Dmt			Chromatin (N & C)	
		7269	Hel25E		RNA helicase		
		5838^{W}	Dref		Transcription factor		
		7626^{W}	Spt5				
		6513^{W}	Endosulfine				
		RNA splicing factor (21)$^{\Pi}$				Whole cell [U2af38] (N & C)	Long SP Few M
		1242	Hsp83		Heat shock protein		
		6546$^{\forall}$	Bap55	Arp6	Nuclear actin-like		
		10377$^{\forall}$	Hrb27	Daz-associated	Ribonucleoprotein		
		10385$^{W\forall}$	Msl-1		Dosage compensation		Long SP
		3241$^{W\forall}$	Msl-2				
		15095$^{W\forall}$			Phosphate transporter	Membrane (N & C)	
		4029$^{W\forall *1}$	Jumeau			Whole cell + Chromatin (N)	Long SP Multipolar
		1081$^{\forall}$	RheB	RheB	GTPase		Few M
		4320$^{\forall}$	Raptor	Raptor	mTOR associated		
		5380$^{W\forall}$			RPase III		Few M
	Novel	9938	*Ndc80**	Ndc80/HEC1		Kinetochore (N & C)	Long SP
		8902	*Nuf2**	Nuf2		Whole cell(N) Kinetochore(C)	
		7242	*Cal2*	Spc25?		Kinetochore(C)	
		18156	*Mis12**	Mis12		Kinetochore (N & C)	
		5148	*Cal1*				
		13550					
		11451					Defective overall
Chromosome condensation defect	Expected	1911	CAP-D2	hCAP-D2	Condensin	Chromosome$^{\exists}$	Misalign
		10212	SMC2	SMC2			
		10726	Barren	hCAP-H			
		11397	Glu	SMC4			
		17054	CAP-G	CAP-G			
		10223	Top2	Topo II	Topoisomerase		
Compact chromatin	Unexpected	8171	Dup	Cdt1			Few M

Long SP = Long spindle: Short SP = Short spindle: Misalign = Chromosome misalignment: Few M = Few mitotic cells (low mitotic index and/or low cell number): N = GFP was tagged at N-terminus of the gene: C = GFP was tagged at C-terminus of the gene.

w: Weak phenotype

★: *Drosophila* homolog identified after the screen by bioinformatics

∀: Phenotype was observed only in the presence of Cdc27 dsRNA

∃: Localization was determined in prior studies

Π: Likely indirect effects of general defects in protein synthesis

( ): Numbers in parenthesis after gene name indicates how many subunits showed RNAi phenotypes. See Data S1 for the gene list.

*1: CG4029/Jumu RNAi showed misalignment phenotype in the presence of Cdc27 dsRNA, but predominantly multipolar spindles in the absence of Cdc27.

Note: This table only lists genes that produced identical phenotypes in the primary screen and one or more secondary screens. Unexpected/novel genes were tested in the secondary screen with a non-overlapping dsRNA (see Methods section in this Supplemenraty Materials). This inventory of mitotic genes is undoubtfully incomplete as only a small subset of the weak phenotypes in the primary screen were pursued. In addition, a number of clear phenotypes from the primary screen are not included here because the secondary screen did not yield an identical, unambiguous phenotype. All of the data from primary and secondary screening (as well as GFP localization) can be accessed at our www site (http://rnai.ucsf.edu/mitospindlescreen).

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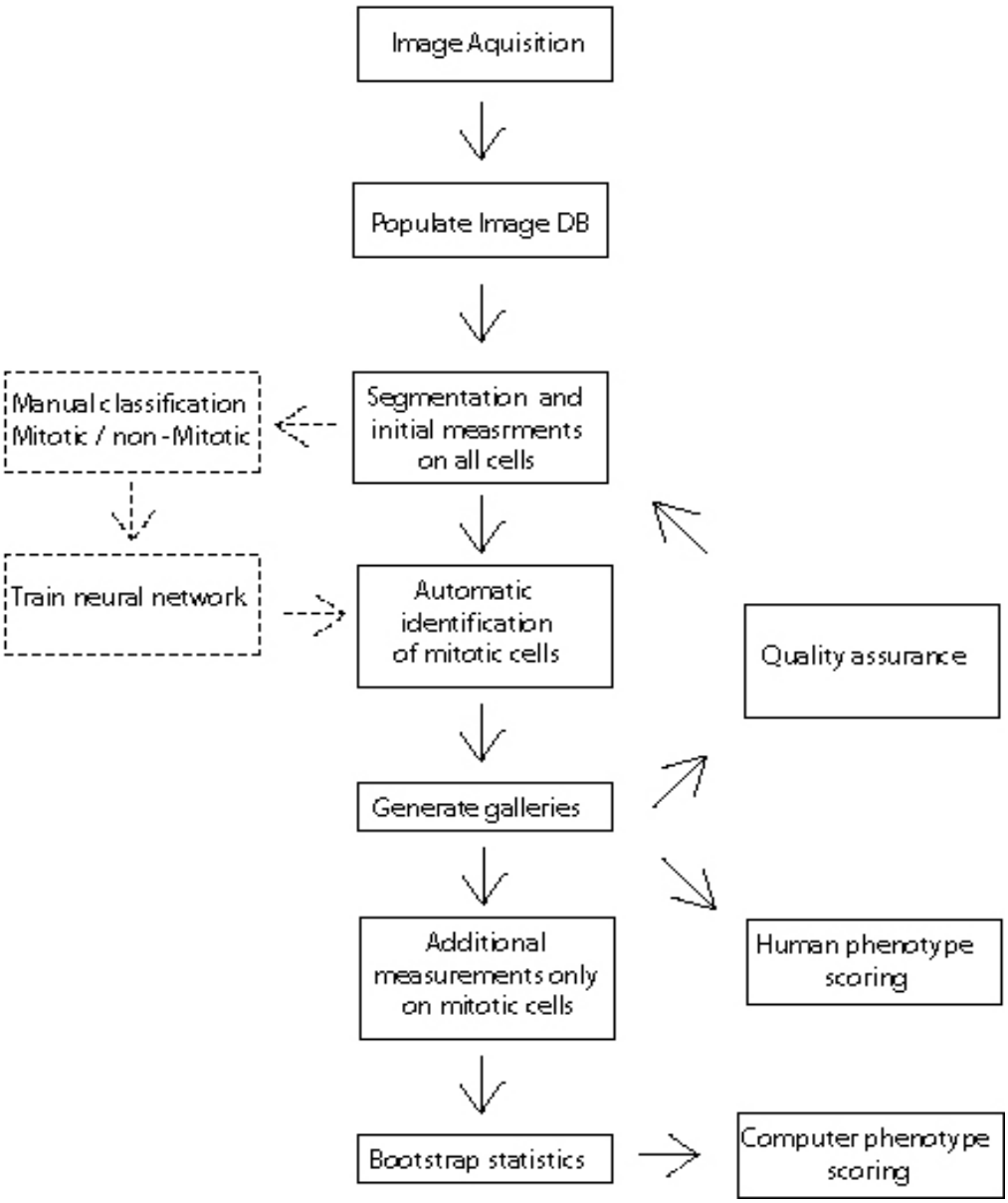
MitoSpindleScreen code

After working with Gohta Goshima and Nico Stuurman from the Vale lab on the spindle length project, we decided to continue the collaboration and extend our work to a genome wide screen. You can read about this screen in a recent Science article here.

Below you can find all the source code that was used for image storing and analysis in the MitoSpindleScreen project.
For the web interface that was used in the screen, please check out Nico's excellent tool: phplabware

This is an overview of the analysis pipeline:



This pipeline was implemented using multiple matlab scripts and functions that perform the different analysis steps.
Overall the code includes many aspects of the computational infrastructure needed for a genome wide screen:

- Database utilities
- Segmentation
- Classification
- Gallery generation
- Graphical User Interface for phenotype assignment
- Quality assurance (QA) and html reports
- Quantitative measurement and statistics
- Data explortation

html docs of all the matlab source code is here.

An overview of how database table structure can be seen here.

The code was developed for the purpose of this screen only, which has pros and cons. We made many 'shortcuts' because we were concentrated on a specific solution and did not aim to create a generatl tool for genome wide screens. Because of that, our >6500 lines of code will to be hard to reuse. To help with its future reusability I tried to organize the files into meaningful folders, rename some files, and add documentation where appropriate. That might have 'broken' a few things, but since I don't expect this code to be usable 'as is' in any case I thought that would be better then just a pile of unreadable code...

a tarball of all m-files is here.

For the biological results of this screen, go to: http://rnai.ucsf.edu/mitospindlescreen