Instructions for Requesting Functional Genomics Cherry Picks at ICCB-L

All cherry-pick requests must be submitted to Dave Wrobel (david_wrobel@hms.harvard.edu)

- You must submit the following information to Dave **before** making a request:
  1. An annotated primary screening data template(s). Please see Step 4 of the Data Deposition Guidelines.
  2. A completed Primary Screen Report, which will include a summary of the screening protocol, the controls used during the screen, a description of the analysis method, and the criteria for scoring screening positives.

- Included in your full-genome Functional Genomics screening fee is a cherry pick of 300 genes (1200 duplexes for the Dharmacon library, 900 duplexes for the Ambion library). If you would like to cherry pick more genes than this, you will be charged $1/duplex ($4/gene for Dharmacon and $3/gene for Ambion). You do not have to wait until all screening is complete to request cherry picks, but you must submit data for all library plates up to that point to make a request.

- Once your request has been submitted to Dave with all of the above conditions and in the format described below, it will be forwarded to the screening room. The screening room staff will prepare a cherry-pick microplate with the volume necessary for the primary screening assay to be repeated with the individual duplexes. For example, if your primary assay screened each SMARTpool at 2 ul x 3, there will be sufficient volume in the cherry-pick microplate for you to screen each individual duplex at 2 ul x 3.

- The standard format for cherry pick plates is to have the outer two columns (1, 2, 23, 24) and rows (A, B, O, P) empty. If you would like additional columns or wells to be left empty for controls, please specify this to Dave at the time you request your cherry picks.

- The screening room will notify the researcher via email when the cherry-pick plates are ready. This will generally be within two weeks of receiving the cherry-pick request from Dave.

  Note: Please use your cherry pick samples carefully. It is often advisable to not use all of your cherry pick plates in a single experiment. The screening group generally does not repeat cherry pick requests.

Required Format for Cherry Pick Requests

Screeners must make their own data file in Microsoft Excel in the following format:
Example: cherry pick request file for screen # 101

Screen 101
20 cherry-picks
Dharmacon human library screened

Plate	**Well**	**Positive**	**Cherry Pick**	**Comment**	**Gene Symbol**	**Entrez Gene ID**	**Catalog #**
50001	I09	W	C		AK1	203	M-006811-02
50001	L11	W	C		DUSP6	1848	M-003964-01
50004	I19	M	C		CCL23	6368	M-007835-01
50004	K13	M	C		CCR5	1234	M-004855-02
50005	O07	W	C		OR2W1	26692	M-014666-00
50006	A20	M	C		DACH1	1602	M-013222-00
50006	G20	M	C		CHAF1A	10036	M-019938-01
50006	P18	M	C		HIST1H1C	3006	M-006630-01
50007	A15	M	C		M96	22823	M-012796-00
50008	P15	M	C		ETV6	2120	M-010510-01
50009	A19	W		Very weak	HOXC5	3222	M-013381-00
50009	B08	W	C		TLX2	3196	M-017174-00
50009	K11	M	C		LBX1	10660	M-012289-00
50011	A16	M	C		NNT	23530	M-009809-00
50011	C13	W	C		GLULD1	51557	M-008430-00
50012	A19	M	C		UEV3	55293	M-008494-00
50012	K08	W	C		CAPN1	823	M-005799-00
50015	P18	S	C		KIF26A	26153	M-022010-01
50016	A08	W	C		KCNH8	131096	M-006238-01
50016	B06	W	C		ASIP	434	M-011587-00

Cherry pick request files should include all positives annotated as a W, M, or S. The columns should be **Plate**, **Well**, **Positive**, **Cherry Pick**, **Comment, Gene Symbol, Entrez Gene ID,** and **Catalog #**. The file must contain the **Screen Number** for which the cherry picks are being requested. The file must also contain the **number of cherry picks** being requested and the **RNAi library screened.**

- Place a C in the Cherry Pick column for those wells that you decide to cherry pick.
- Enter a comment for all positives that you decide not to cherry pick.
- The Plate and Well columns are listed in ascending order, first by Plate, then by Well.

Important Contact Information:

Jennifer Smith, Ph.D.
Director
617-432-5735
jennifer_smith@hms.harvard.edu

David Wrobel
Data Analyst
617-432-6266
david_wrobel@hms.harvard.edu

Jen Splaine
Data Curator
617-432-6266
jennifer_splaine@hms.harvard.edu

ICCB-L Screening Facility Staff
617-432-5815
iccb_screen@hms.harvard.edu