# Annotator Manual – CRAPome version 1.0

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## 1: Overview

The CRAPome is a repository of negative controls performed in affinity purification coupled with mass spectrometry (AP-MS) experiments. Negative controls are collected from various studies (published or unpublished), processed, annotated and made available for download and analysis via an online interface. See User Manual for details.

An Annotator is usually the contributor of mass spectrometry data to the CRAPome. Contributors first submit raw mass spectrometry files to the CRAPome administrator. The administrator processes them to yield protein identifiers and spectral counts, assigns an experiment number to each of the files that passed a quality control step (these experiments are labeled CCx; CRAPome Control x), and releases them for annotation. The Annotator defines protocols to describe the experimental procedures and links the protocols to each experiment. Protocols include controlled vocabularies and free text.

## 2. Accessing the system as an Annotator and viewing existing experiments and protocols

Annotators are assigned a higher level of privileges than regular registered users. They can create protocols and link protocols to experiments. Annotator-level login access can be requested by emailing the CRAPome administrator. Use the login credentials to enter the CRAPome (**Fig. 2.1**). The Annotator menu bar will look like **Fig. 2.2**.

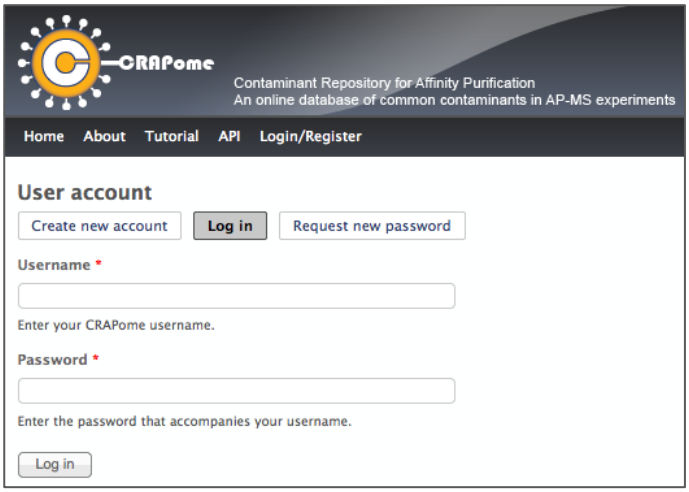



**Figure 2.1. Welcome screen at the [www.CRAPome.org](http://www.CRAPome.org) database**. Enter username and password as prompted.


**Figure 2.2. Annotator menu bar.** "Experiments" lists all the experiments that the annotator has access to (those that have been contributed by their laboratory). "Protocols" lists all the protocols in the CRAPome, but enables editing only those protocols belonging to the Annotator. "Define Experiment" and "Define Protocol" enable the creation of new data.

Select the “Experiments” tab to view the list of all experiments contributed by the Annotator laboratory (**Fig. 2.3**). Click on an Experiment Name (here CC40) to view the associated details (**Fig. 2.4**). Similarly, select the “Protocols” tab to view the list of all protocols available in the CRAPome (only those protocols contributed by the Annotator laboratory can be edited; **Fig. 2.5**). Clicking on the name or protocol number opens a new window with the protocol details (**Fig. 2.6**).

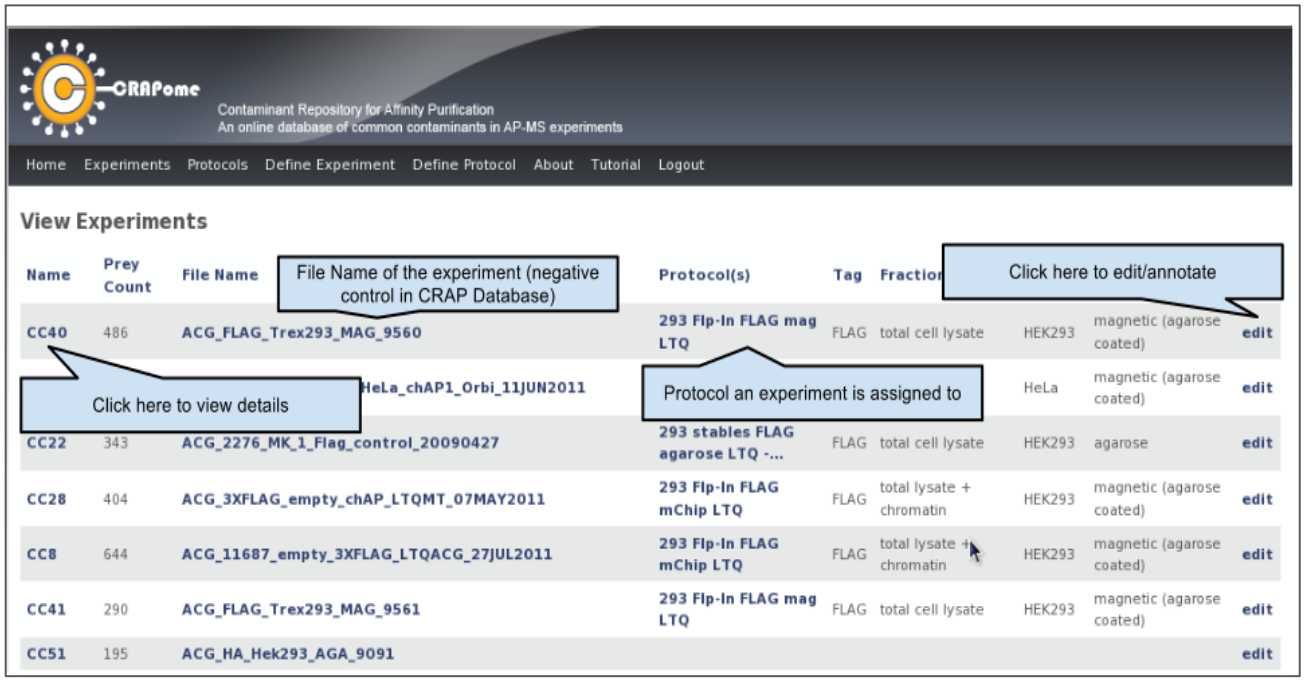



**Figure 2.3. Experiment View.** The procedure for creating and editing protocols will be described in section 3.

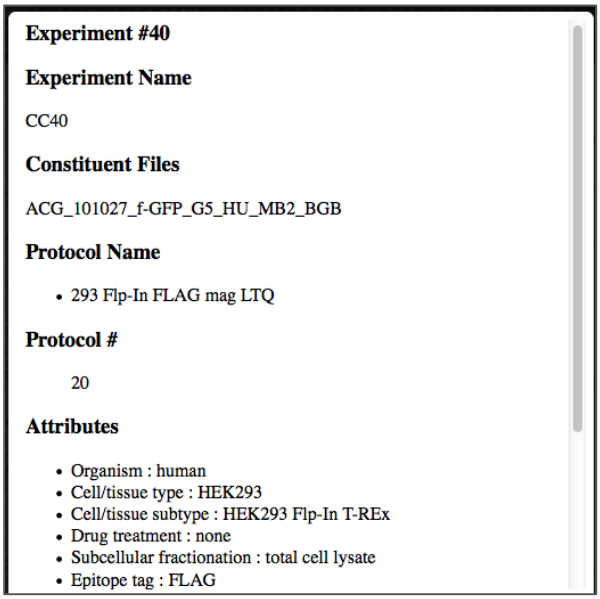



**Figure 2.4. Experiment details.** Only the top portion of the Experimental details view is shown.

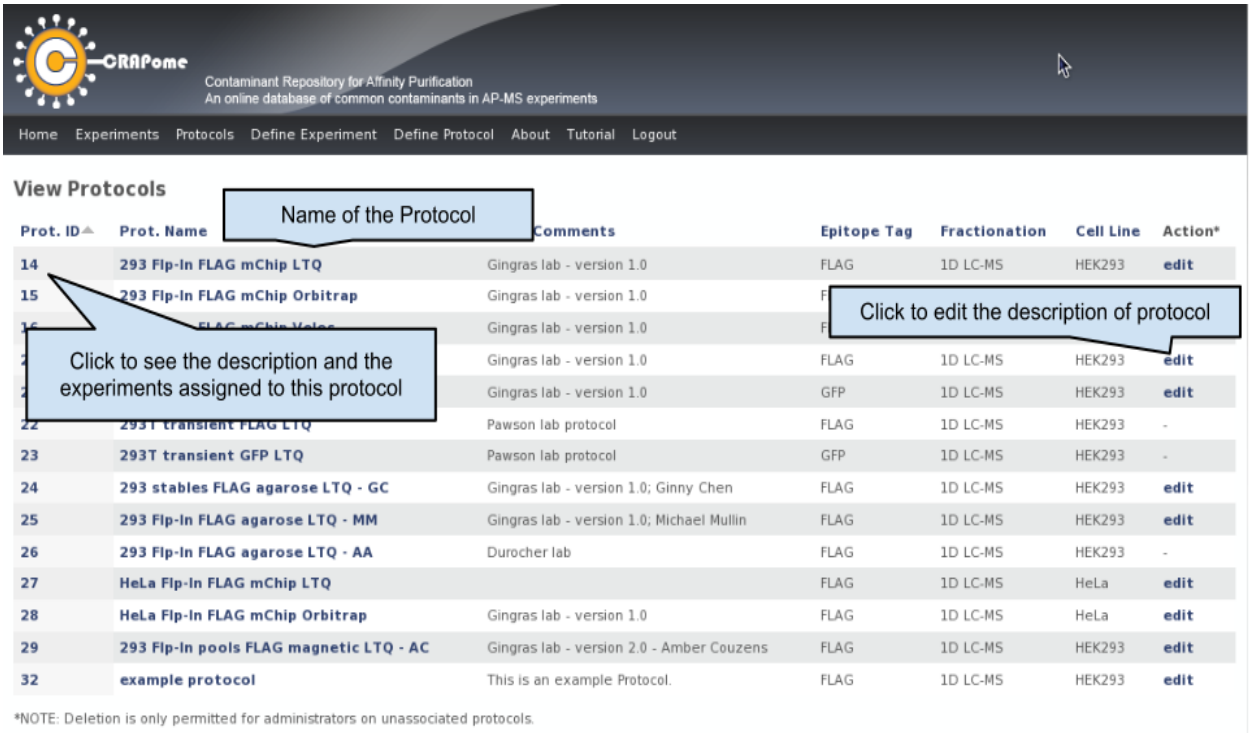
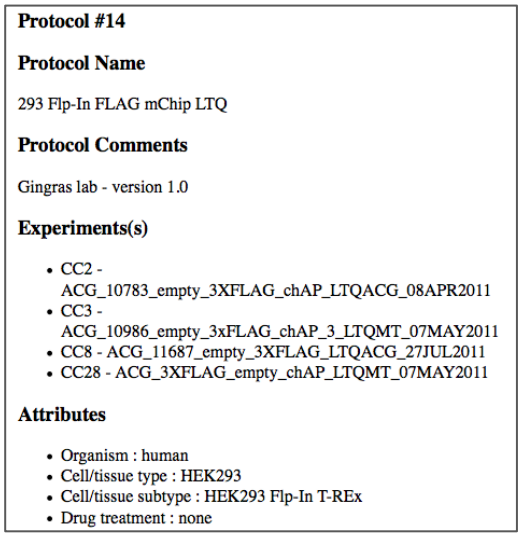



**Figure 2.5. Protocol View.** The procedure for creating and editing protocols will be described in section 3.



**Protocol #14**

**Protocol Name**

293 Flp-In FLAG mChip LTQ

**Protocol Comments**

Gingras lab - version 1.0

**Experiments(s)**

- CC2 - ACG_10783_empty_3XFLAG_chAP_LTQACG_08APR2011
- CC3 - ACG_10986_empty_3xFLAG_chAP_3_LTQMT_07MAY2011
- CC8 - ACG_11687_empty_3XFLAG_LTQACG_27JUL2011
- CC28 - ACG_3XFLAG_empty_chAP_LTQMT_07MAY2011

**Attributes**

- Organism : human
- Cell/tissue type : HEK293
- Cell/tissue subtype : HEK293 Flp-In T-REx
- Drug treatment : none

**Figure 2.6. Protocol Details**. Only the top portion of the Experimental details view is shown.

## 3. Creating and editing protocols and experiments

The main responsibility of the Annotator is to define Protocols and link Experiments to the protocols. An annotator can also edit protocols and experiments that belong to their research group. **Fig. 3.1** summarizes the different steps of the annotation process.

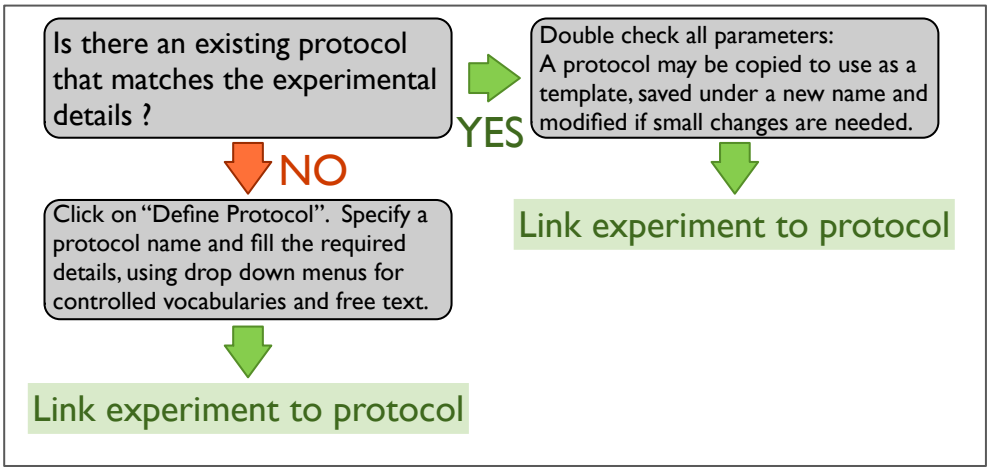



**Figure 3.1. Flowchart of the Annotator tasks.**

### Task 1: Define/Edit Protocol

The first task of the Annotator is to define a protocol that corresponds to the experiments to be annotated. Create a new protocol by clicking the "Define Protocol" tab: Fill in the requested information, including a descriptive name for the protocol associated with optional protocol notes. Select the controlled vocabulary by using the drop down "Attributes" (**Fig. 3.2;** See **Fig. 3.3** for current CV terms; contact the CRAPome administrator if the controlled vocabulary is inadequate), and add text-based experimental details (see **Fig. 3.4** for an example). Before creating a new protocol, review the list of the existing protocols to prevent duplication. Note, however, that since even minor changes in experimental procedures can lead to observable changes in the composition of the background contaminants, new protocols should be created that fully describe protocol details without creating obvious redundancies.

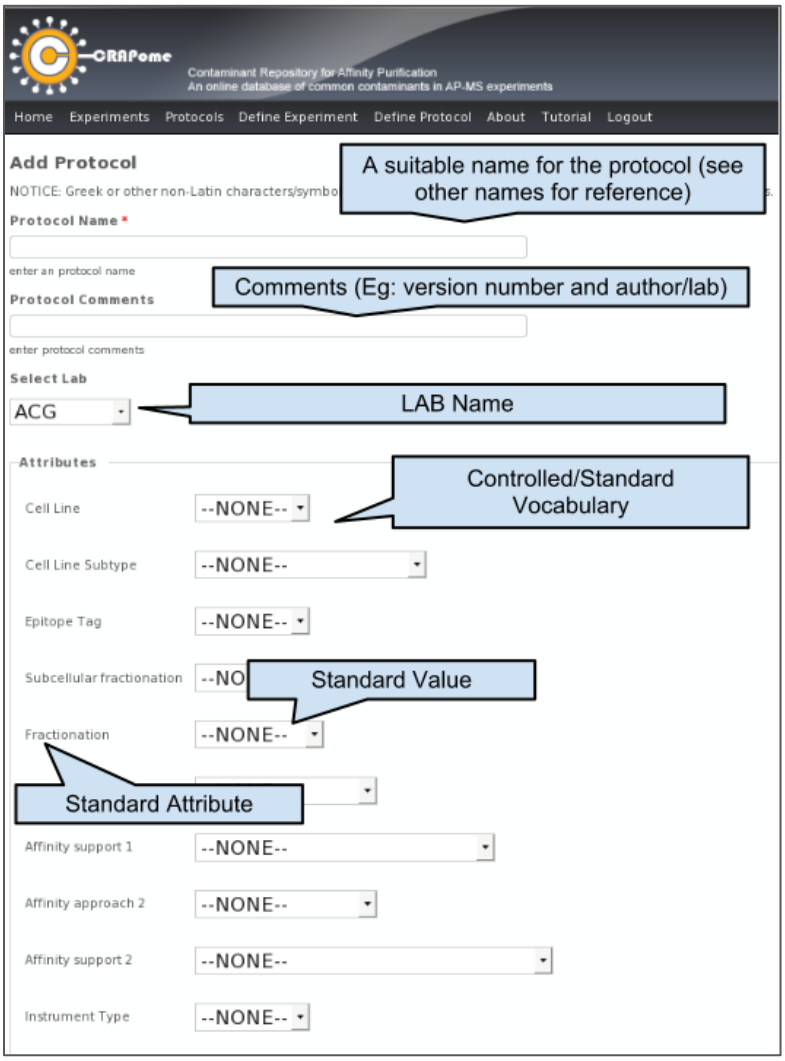
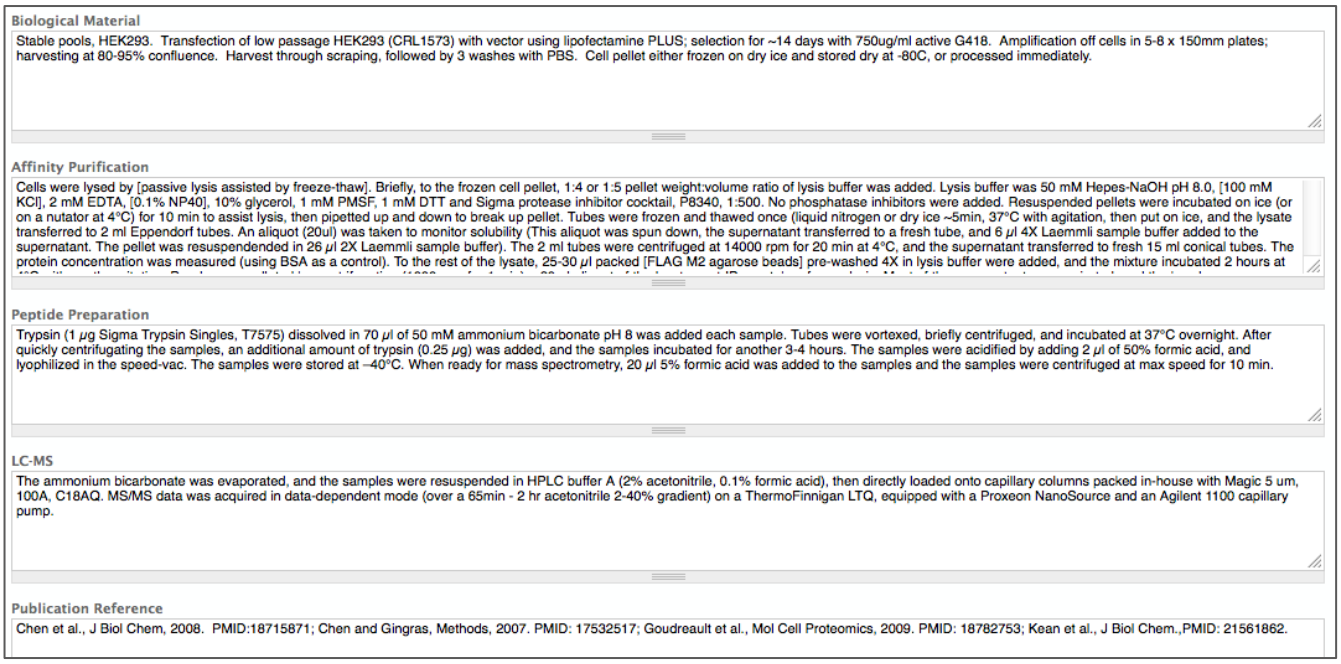



**Figure 3.2. Creating a new CRAPome protocol / part A, define controlled vocabulary.**

Current Attributes

| Attribute Name | Attribute Values |
|---|---|
| Organism | human |
| Cell/tissue type | HEK293, HeLa, U2OS, PBMC, Jurkat, CEM-T, MRC-5, LS174 |
| Cell/tissue subtype | --, HEK293T, HEK293 Flp-In T-REx, Jurkat-Flp-In |
| Drug treatment | aphidicolin, rapamycin, nocodazole, MG132, none, IFN-beta, DMSO, okadaic acid, doxycycline+thymidine, tetracycline+thymidine, thymidine+nocodazole |
| Subcellular fractionation | total cell lysate, total lysate+chromatin, nuclear fraction, cytosolic fraction |
| Epitope tag | FLAG, HA, GFP, TAP, HaloTag, Strep-HA |
| Control protein | RFP, GFP, FLAG, mCherry, tag alone, untransfected, uninduced, RFP |
| AP steps | single, tandem |
| Affinity approach 1 | M2 anti-FLAG, anti-GFP camel, anti-GFP rabbit, HA-7 anti-HA, HaloLink, IgG, Streptactin, 2xFLAG, SBP, anti-GFP mouse |
| Affinity support 1 | agarose, magnetic (dynabead), magnetic (agarose coated), nano-magnetic, microMACS |
| Affinity approach 2 | --, M2 anti-FLAG, anti-GFP camel, anti-GFP rabbit, calmodulin, HA, 2xHA, HA-7 anti-HA, anti-GFP mouse |
| Affinity support 2 | --, agarose, magnetic bead (dynabead), magnetic beads, agarose coated, nano-magnetic beads, microMACS |
| Fractionation | SDS-PAGE, 1D LC-MS, MudPIT, RP-RP, GeLC |
| Instrument type | Velos-Orbitrap, LTQ-Orbitrap, LTQ, LCQ, LTQ-FT, 5600 TripleTOF |

**Figure 3.3. Currently available controlled vocabularies (Attributes)**



Biological Material

Stable pools, HEK293. Transfection of low passage HEK293 (CRL1573) with vector using lipofectamine PLUS; selection for ~14 days with 750ug/ml active G418. Amplification off cells in 5-8 x 150mm plates; harvesting at 80-95% confluence. Harvest through scraping, followed by 3 washes with PBS. Cell pellet either frozen on dry ice and stored dry at -80C, or processed immediately.

Affinity Purification

Cells were lysed by [passive lysis assisted by freeze-thaw]. Briefly, to the frozen cell pellet, 1:4 or 1:5 pellet weight:volume ratio of lysis buffer was added. Lysis buffer was 50 mM Hepes-NaOH pH 8.0, [100 mM KCl], 2 mM EDTA, [0.1% NP40], 10% glycerol, 1 mM PMSF, 1 mM DTT and Sigma protease inhibitor cocktail, P8340, 1:500. No phosphatase inhibitors were added. Resuspended pellets were incubated on ice (or on a nutator at 4°C) for 10 min to assist lysis, then pipetted up and down to break up pellet. Tubes were frozen and thawed once (liquid nitrogen or dry ice ~5min, 37°C with agitation, then put on ice, and the lysate transferred to 2 ml Eppendorf tubes. An aliquot (20ul) was taken to monitor solubility (This aliquot was spun down, the supernatant transferred to a fresh tube, and 6 µl 4X Laemmli sample buffer added to the supernatant. The pellet was resuspendended in 26 µl 2X Laemmli sample buffer). The 2 ml tubes were centrifuged at 14000 rpm for 20 min at 4°C, and the supernatant transferred to fresh 15 ml conical tubes. The protein concentration was measured (using BSA as a control). To the rest of the lysate, 25-30 µl packed [FLAG M2 agarose beads] pre-washed 4X in lysis buffer were added, and the mixture incubated 2 hours at

Peptide Preparation

Trypsin (1 µg Sigma Trypsin Singles, T7575) dissolved in 70 µl of 50 mM ammonium bicarbonate pH 8 was added each sample. Tubes were vortexed, briefly centrifuged, and incubated at 37°C overnight. After quickly centrifugating the samples, an additional amount of trypsin (0.25 µg) was added, and the samples incubated for another 3-4 hours. The samples were acidified by adding 2 µl of 50% formic acid, and lyophilized in the speed-vac. The samples were stored at -40°C. When ready for mass spectrometry, 20 µl 5% formic acid was added to the samples and the samples were centrifuged at max speed for 10 min.

LC-MS

The ammonium bicarbonate was evaporated, and the samples were resuspended in HPLC buffer A (2% acetonitrile, 0.1% formic acid), then directly loaded onto capillary columns packed in-house with Magic 5 um, 100A, C18AQ. MS/MS data was acquired in data-dependent mode (over a 65min - 2 hr acetonitrile 2-40% gradient) on a ThermoFinnigan LTQ, equipped with a Proxeon NanoSource and an Agilent 1100 capillary pump.

Publication Reference

Chen et al., J Biol Chem, 2008. PMID:18715871; Chen and Gingras, Methods, 2007. PMID: 17532517; Goudreault et al., Mol Cell Proteomics, 2009. PMID: 18782753; Kean et al., J Biol Chem.,PMID: 21561862.

**Figure 3.4. Creating a new CRAPome protocol / part B, adding protocol details.** Add information details pertaining to the biological material (How were the cells grown and harvested? How was the recombinant protein expressed? Has a subcellular fractionation been performed?), the affinity purification step, the procedure for preparing the peptides (including fractionation at the protein or peptide level when applicable), and details of the LC-MS/MS analysis. If the Method has been published, add citations in the "Publication reference" box.

**Task 2: Link protocols to experiments**

The general pipeline for the addition of experiments to the CRAPome database begins by the processing of the raw mass spectrometry files by the CRAPome administrator. The CRAPome administrator then defines the experiments with some basic information (such as the name of the spectrum file and the laboratory that deposited the data) and initiates the processing of data.

The role of the Annotator is to "edit" such experiments by linking protocols to them. To do so, the Annotator access the list of his/her experiments by selecting the "Experiments" tab at the top of the page (as in **Fig. 2.3**). Experiments which are already linked to a protocol (e.g. CC5 in **Fig. 3.4**) already have controlled vocabularies associated with them, in addition to the protocol number and protocol name. Experiments which are not yet associated are missing this information (see CC40 in **Fig. 3.4**).

To associate protocols and controlled vocabularies, select the "edit" link on the right. This will open a new window: information entered by the administrator is displayed, but not editable (please contact the CRAPome administrator to report any errors). The Annotator should change the "Experiment Status" to "Annotated" (from the default "newly added"), and link the experiment to a protocol via the drop-down menu. When the annotator selects a protocol for an experiment (see **Fig. 3.5**), all the attributes of the experiment ("controlled vocabularies") are populated with the attributes of the protocol (see **Fig. 3.6**).

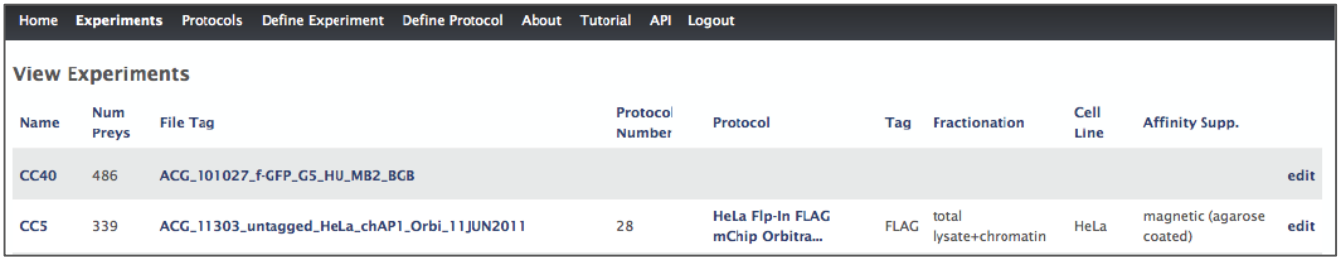



**Figure 3.4. Experiments view.** Clicking on "edit" on the right enable linking a protocol to the experiment.

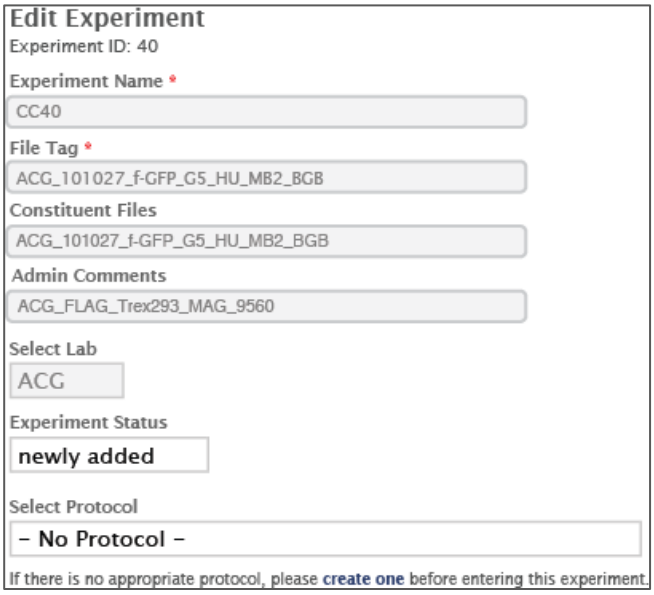



**Figure 3.5. Edit experiment view.** Data entered by the administrator is greyed out. Select a protocol to link to the experiment. Create new protocols as needed, as described above.

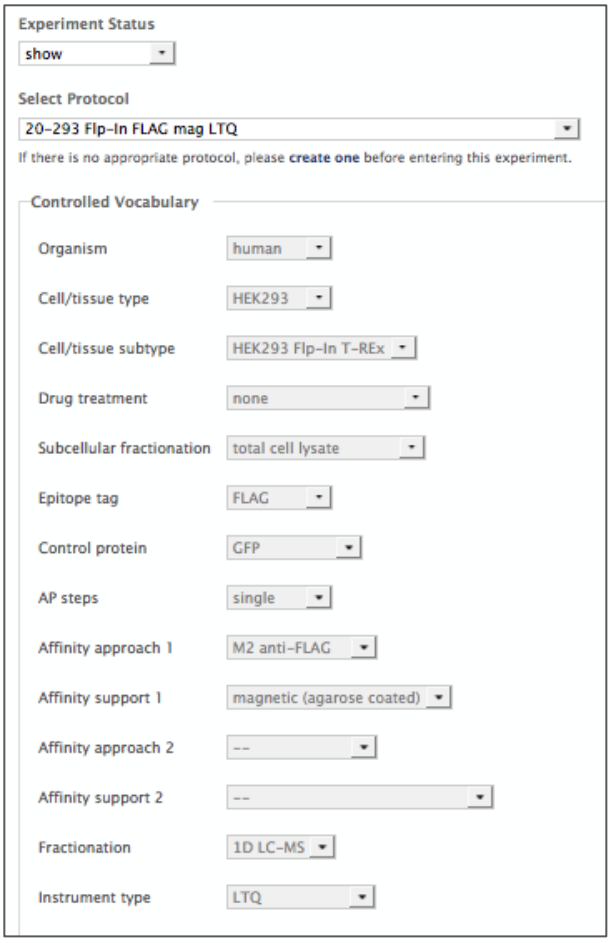



**Figure 3.6. Controlled vocabularies are populated from the selected protocols.**

**Deleting experiments and protocols:**
The annotator can only define or edit new experiments (or protocols) but cannot delete them. Each newly defined experiment has an attribute called 'status' (see **Fig. 3.4**) which can be one of a) newly added, b) annotated, c) ready for release, d) show, or e) retire. If the CRAPome administrator adds a new experiment, he/she sets the status to "newly added". The annotator can change the status to "annotated", once the annotation is complete. The status is set to "ready for release" once the spectrum file(s) are processed and the database is updated. Finally, the status is set to "show" when the data is released to the end user. Only those experiments with a status "show" can be viewed by the end user. If the annotator accidently created a wrong entry, he/she can set the status to retire. All retired experiments will be periodically purged from the database by the CRAPome administrator.

**Requesting new controlled vocabularies:**
The annotator can only use pre-defined controlled vocabularies (attributes): new CVs can be requested to the CRAPome administrator.