# Sample handling @ CryoEM Shared Resource Howard Hughes Medical Institute Janelia Research Campus

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#### HHMI Janelia CryoEM Shared Resource

-One of the first shared cryoEM facilities set up for a large user community Serve the entire HHMI community (within the US and also internationally)

-Have been in operation since late 2012, reached mature production phase In 2016, over 25 papers published with data collected at the facility

-State-of-the-art instrumentation

-Efficient Operation and top quality service by technically savvy, professional, and dedicated staff

More information @ janelia.org/support-team/cryo-electron-microscopy



#### A wide variety of biological samples imaged at the Janelia CryoEM Facility

- -- Two different experimental techniques: single particle analysis and electron cryotomography
- -- a wide variety of samples with size ranging from a few nm to ~800nm



Doudna lab Nogales lab HHMI/UC Berkeley Science 2015



Ribosome complex



Frank lab HHMI/ ColumbiaUniversity Science Advances 2015



**Grigorieff** lab **HHMI** Janelia Elife 2015

#### **Bacterial MACs complex**



**Grant Jensen** HHMI / Caltech Science 2014



### Two types of work and two types of samples

1, High quality high throughput data collection service only on user-provided ready-toimage cryo grids.



2, Accept solution sample and carry out the entire cryoEM workflow: sample calibration, cryo grids preparation/screening, data collection and image processing.





## Quality control on receiving cryogrids

1, Users should systematically screen a couple of cryo grids from the same batch that will be sent to Janelia at their own institute beforehand.

2, Send representative cryoEM images from the screening to Janelia cryoEM facility

- --a few high magnification images to show how sample look like and particle density and distribution
- --a few low magnification images to show ice quality and distribution over different grid squares
- --further proof that sample and grids are suitable for data collection (such as 2D class averages) is helpful but optional.





3, No significant amount of Krios time at Janelia should be used for grids screening.

up to 8 cryo grids for one sample should be loaded in one load and there should be a suitable one for large quantity data collection.



## Shipping Cryo grids





An instruction document on shipping cryo grids is provided to users if needed. Each week we may receive up to four shipments as users typically get 3-day data collection session. Most time the shipper arrives overnight.

--There were a few cases where the shipper arrived 3-4 days late but grids were still OK.



### Dealing with/storing cryo grids

Typically cryo grids should be received 1-5 days before the scheduled data collection session. -- shipper is filled with LN2 and grids stored in shipper till data collection session

Unused cryo grids are either sent back to user or stored in our cryo storage for future use -- Within 12 months, grids should used, sent back or discarded.

Cryostorage with automatic liquid nitrogen filling-- Fisher Scientific CryoPlus Model 7402



Automatic filling LN2

Holds ~120 Cylinders

Each Cylinder holds 4 tubes

Cap is labeled

Chen Xu, Dan Shi and Jason de la Cruz

## Logging cryo grids

Cryo Grid Storage internal1 internal2 CryoT12 Vitrobot CP3									
Cryo Grid Storage Record with Sample Descriptions, Page 1 of 7 Not logged in hhmi janelia									
Find   Help   Login									
Full   Summary   Threaded								<b>1</b>	26 Entries
Goto page 1, 2, 3 5, 6, 7 Next All									
ID	Owner	Cylinder	Tube	Start Date	Sample Description		Text		0
124		48	2	11/10/16					
95		70	1	10/21/16					
127		102	4	09/02/16					
126		102	3	08/31/16					
125		102	2	08/04/16					
123		63	1	07/29/16					
122		65	4	06/28/16	-				
121		62	1	06/10/16					
66		55	1	05/20/16	_				
120		9	4	05/13/16					

We recommend the stored grids be used at the user's next session. No long term storage policy.

Within 12 months, grids should used, sent back or discarded.

Logging program by Jason de la Cruz.



#### **Receiving solution samples**

- 1, samples shipped at 4C on cold pack or wet ice
- -- we coordinate the shipment with the availability of freezing equipment, screening scope and staff time.
- -- work on the sample upon receiving





- 2, samples shipped frozen on dry ice
- -- flexible on when to ship the sample.
- -- upon receiving sample is typically stored in -80C freezer first and we work on the sample at the earliest available time.



#### Requirements on solution samples

1, Sample needs to be biochemically pure.

2, sample should be sent in reasonably high concentration. Dilution buffer need to be included.

3, Sample should be sent in 2-3 small aliquots (say 15-20  $\mu$ L) per sample.

4, If sent at 4C, no more than 2 samples should be sent a time.

5, Ideally, there should be no glycerol or sugar in the buffer and salt concentration is not too high (below 300 mM).

6, If glycerol has to be included in frozen sample, buffer exchange will be carried out at the facility before making cryo grids.



#### Sample calibration, cryo grids preparation and screening

Negative stain EM is first performed on new sample to quickly check suitability.

Then test freeze cryo grids and image on screening scope.

Might take multiple rounds of iterations. close interactions between facility and collaborators.









#### Acknowledgement

Many factors contributing to the success of a shared facility: equipment, user base, management, good policy/proctcol and more importantly, <u>the team members</u> who carry out the day to day work and interact with users.







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- All users

