WE ARE READY TO LAUNCH !

Improving the time-efficiency and quality of your results

Implementation of a standardized sample quality control workflow.

ARBRE MOBIEU
BETWEEN ATOM AND CELL

CA15126

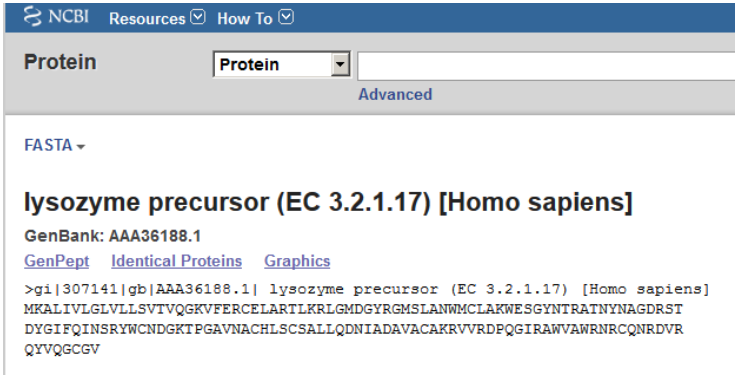
The international position

The guideline:

The minimal information that needs to be provided or known

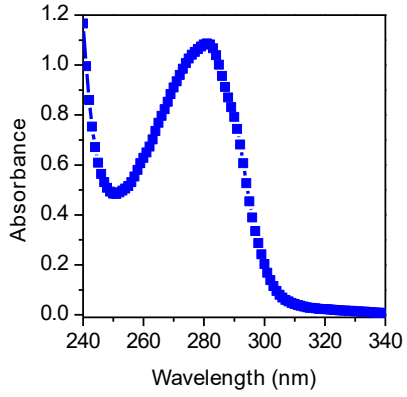
Protein name and full primary structure, by providing a NCBI or UniProt accession number and cloning strategy

i.e. the source of the DNA (species), expression vector and host strain, including the tags and cleavage sites used, accompanied by the full amino acid sequence of the final protein, or sufficient details to derive the full amino acid sequence of the final protein.



Protein concentration

Specifying the method used for quantification and the molar extinction coefficient at 280nm, if applicable



Storage conditions

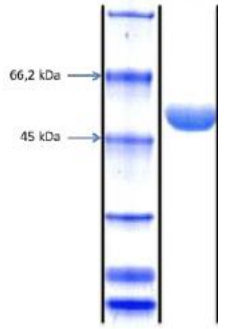
i.e. final buffer composition (pH, buffers, salts and additives), storage temperature or lyophilization conditions

The guideline:

The minimal quality control parameters

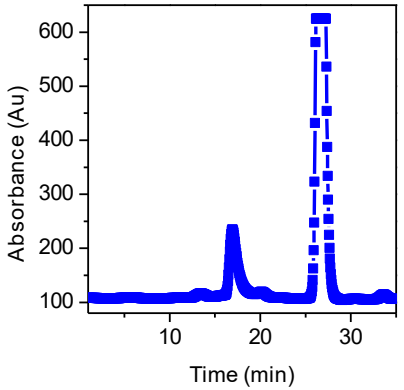
Purity

Checked by SDS-PAGE, Capillary Electrophoresis or Reverse Phase HPLC



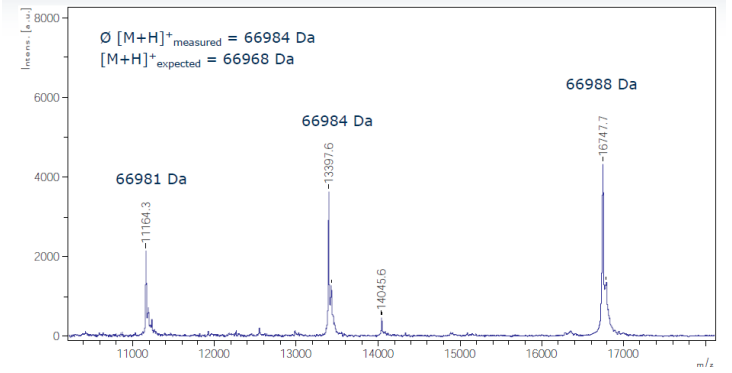
Homogeneity (aggregation sate)

checked preferably by Size Exclusion Chromatography (SEC) and/or dynamic Light Scattering (DLS) or by SEC with Multi Angle Light Scattering (SEC-MALS), Flow Field-Flow Fractionation (F4), F4-MALS or analytical ultracentrifugation (AUC)



Identity

checked preferably by intact protein mass, peptide mass fingerprint, or Edman sequencing.

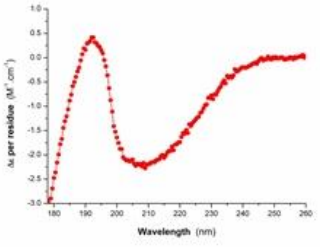


The guideline:
Extended quality control parameters

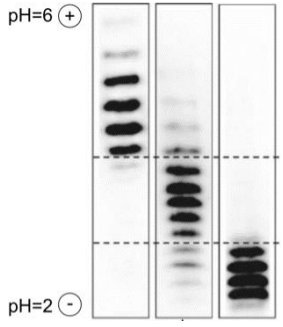
Nucleic acid content **Mandatory if the protein binds nucleic acid**
checked by UV-Vis spectroscopy.

Batch-to-batch consistency **Mandatory if more than one batch is used**
Use some of the methods listed in the minimal test.

Conformational stability/folding state
Checked by Circular Dichroism(CD), Differential Scanning Calorimetry (DSC) or NMR

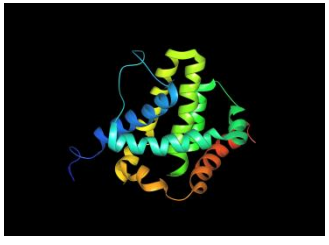


Homogeneity
Checked by analytical IEX, analytical hydrophobic interaction chromatography, isoelectric focusing



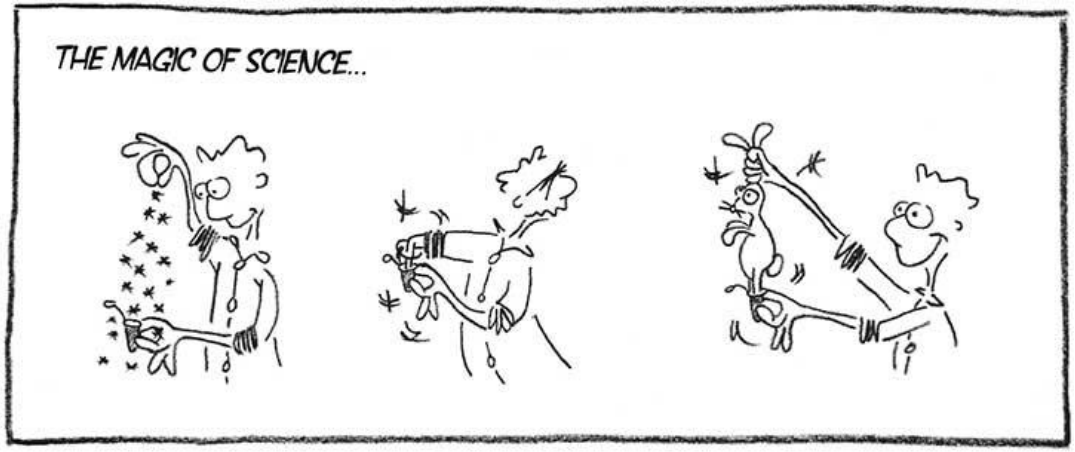
Protein competent fraction
i.e. the relative amount of functionally active protein
Measured as specific activity, by active-site titration or other suitable methods

Optimization of storage conditions
Long term stability, activity assay, thermal shift assay



Convincing Researcher in our institution!

- **“I do not have time…”**
- **“My boss thinks it is a waste of time…”**
- **“It is the way we have prepared samples in the lab for the last ten years….”**
- **“But some experiments have worked with this sample…”**
- **“I do not know how to do it…”**
- **“I will do the experiment anyway it may work…”**



Convincing Researcher in our institution!

The magic of quality control

Our analysis of the situation as core facilities in different European institutions

- A lot of time is spent on poor quality samples

- The best experiments in the world will turn garbage in expensive garbage

Improving the quality of the samples is essential to improve the quality of the results we produce

The international position

Researcher opinion !

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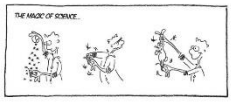

The reality:

Quoting people's experience with sample not fully validated

- " We expressed a human protein in insect cells. We visualize by Coomasie stain in PAGE-SDS a protein band with a "correct" MW. We succeed to crystallize, and only then we "discover" that we did not crystallize our target. It was an ubiquitous insect cell protein "
- " We expressed a yeast protein. We visualize by Coomasie stainning in SDS-PAGE a protein band with a "correct" MW. We did interaction studies and could not reproduce our previous data. After a test by mass spectrometry we have discovered that we were missing 4 amino acids at one end that were essential for the interaction."
- " I have done many unsuccessful tests of crystallization with my protein. I finally decide to do a simple quality test. I noticed that the protein was not homogenous in the buffer I used for purification. After buffer optimization, I have obtained an homogenous protein that crystallized."

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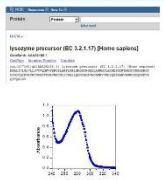

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Specifying the method used for quantification and the molar extinction coefficient at 280nm, if applicable

Storage conditions
i.e. final buffer composition (pH, buffers, salts and additives), storage temperature or lyophilization conditions

Our proposal:

The minimal quality control parameters

Purity
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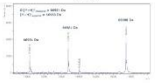

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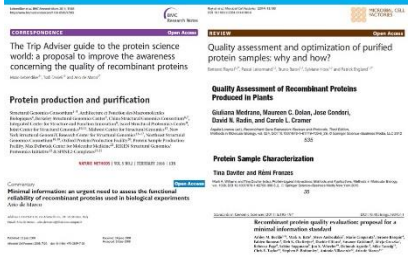
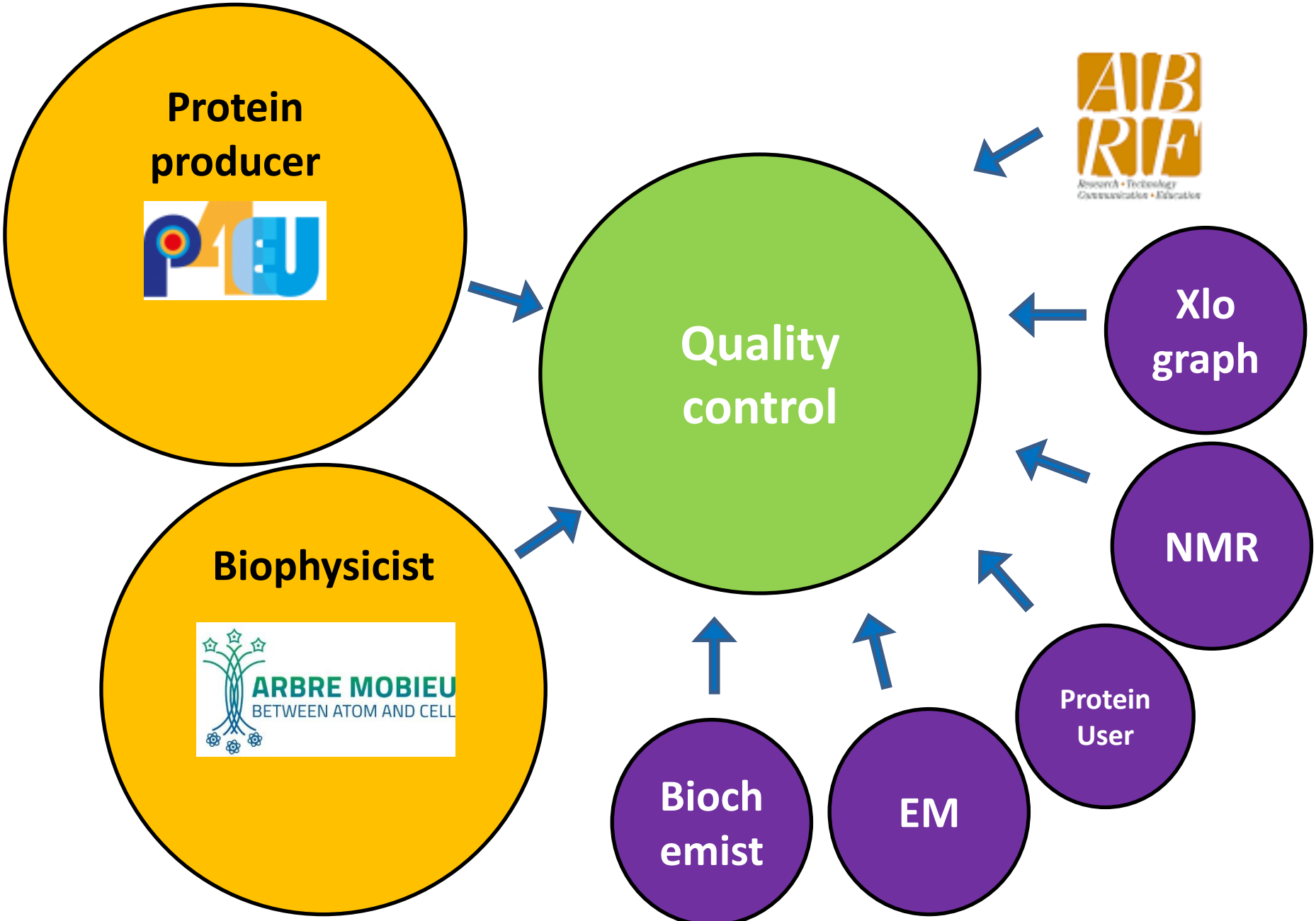
Long term stability, activity assay, thermal shift assay

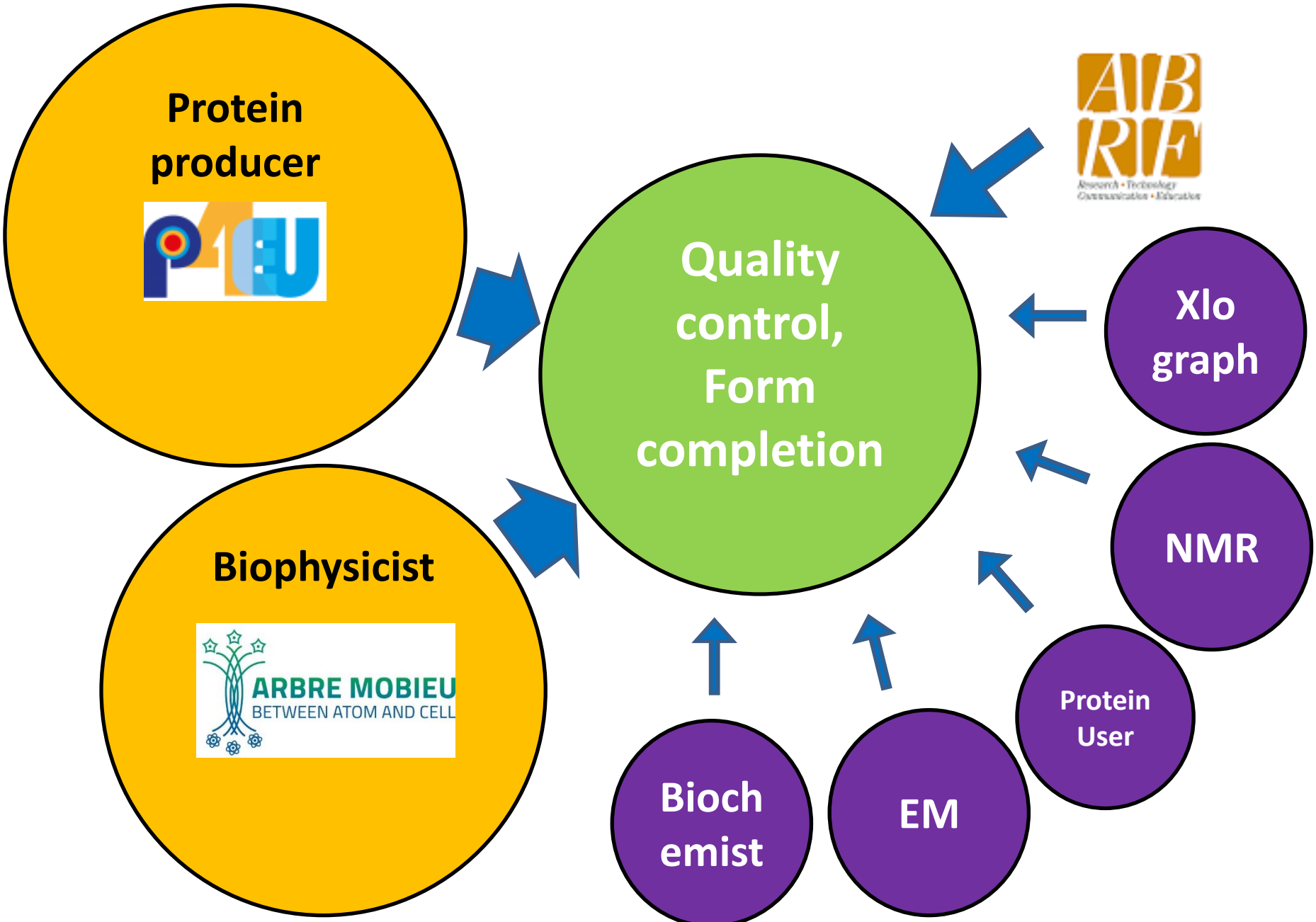
Reference on the subject



Who should perform quality control?



Who should fill the form?



Completing the form!

Implementation of a standardized sample quality control workflow

This form is a joint initiative between the ARBRE-MOBIEU and P4EU community to improve data quality.
For any enquiries concerning the form please contact wg4.mobieu@gmail.com or consult our FAQ here:
https://docs.google.com/document/d/1tGGdBBc68gIDfYOPBOS5nsZ1jODGT_D8mIpY-0pYdyY/edit?usp=sharing

*Obligatoire

Are you registered? *

- ( ) Yes
- (•) No

Continuer »

Terminé à 3 %

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Signaler un cas d'utilisation abusive - Conditions d'utilisation - Clauses additionnelles

Frequently Asked Questions
FAQ

1. My name is not in the list, but I have registered. What can I do?
Wait one hour, for the website to make its update. If you still have problem contact wq4.mobieu@gmail.com

2. I want to fill the form but I would like the sample to be anonymous.
Give a name to the sample that is anonymous like "AlbertE1".

3. I have already tested the sample, should I enter the data of this sample again?
Yes, enter the sample with the same name and enter yes to the question "Have you tested this sample before?". You will be able to detail what you have change or not change in your process.

4. I have already tested the sample, should I enter the data of this sample again?
Yes, enter the sample with the same name and enter yes to the question "Have you tested this sample before?". You will be able to detail what you have change or not change in your process.

5. How long does it take to fill the form?
Around 5 min the first time, and less after a few utilisation.

6. There is a test in the minimal list that I have not done. How do I fill the form?
Fill the "Sadly none", but you should make all the effort to perform the test. These tests are essential.

7. How many times should I fill the form

information as many times as possible.

8. Why do you ask to use the data in publication?
The objective of this form is to published statistics about quality control. We may need some real data to illustrate our conclusion

Implementation of a standardized sample quality control workflow

*Required

Registration

You will receive an E-mail in the next few days to confirm your registration to the survey with the access code

First Name and Name *

eg: Albert Einstein

Institution *

eg: Relativity Institut

E-Mail *

eg: albert.einstein@mail.org

Network *

Tick all your network affiliation

- [ ] ARBRE-MOBIEU
- [ ] P4EU
- [ ] ABRF
- [ ] None

« Back Submit

100%: You made it.

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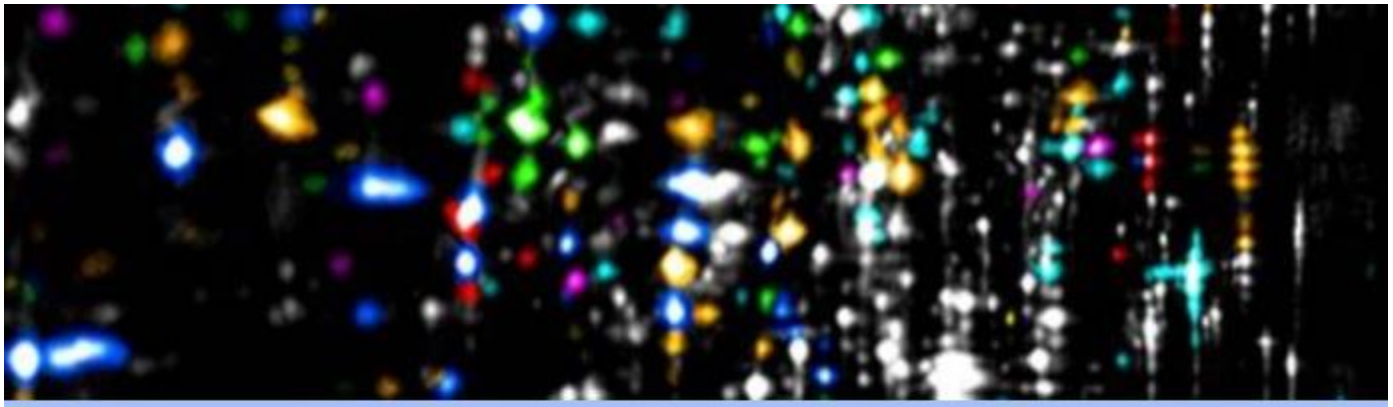

Implementation of a standardized sample quality control workflow

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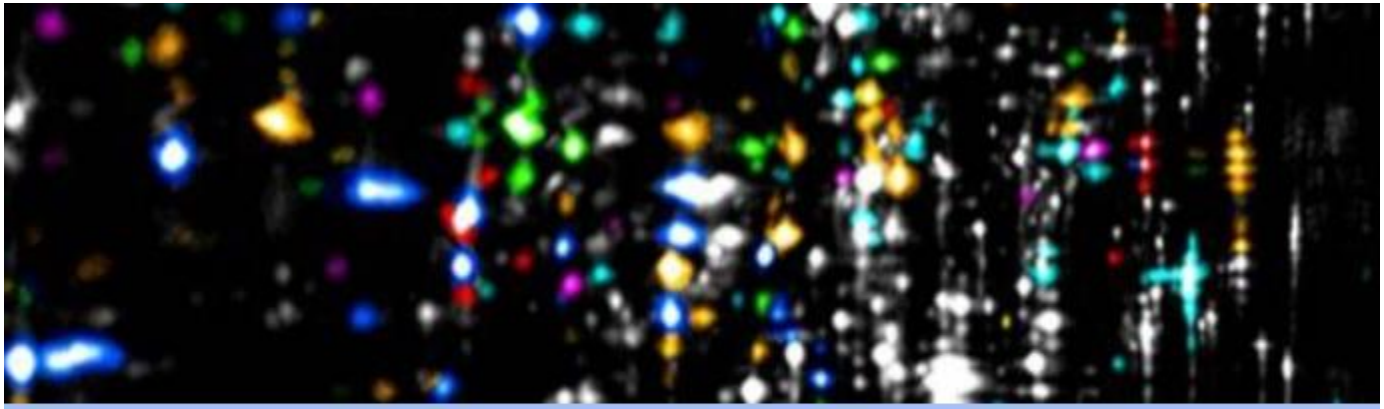
*Obligatoire

Are you registered? *

- ( ) Yes
- (•) No

Continuer »

Terminé à 3 %



Implementation of a standardized sample quality control workflow

*Required

Submission information

Please complete the form to establish statistics about sample quality in research laboratories and Core facilities

First Name and Name *

You need to wait 1 hour for your name to appear in the list, please come back later if your name does not appear

Bertrand Raynal

« Back Continue »

11% completed

General information about the sample

Date of quality control test *

March 3 2016

Name of protein *

If you want to keep the protein anonymous put a name not related to the real name of the protein

Protein DNA source organism *

- Bacterial
- Fungi
- Human
- Insect
- Mouse
- Phages
- Plant
- Viral
- Yeast
- Other:

Type of produced protein construct *

- Soluble secreted
- Soluble cytoplasmic
- Membrane protein whitout any trans-membrane domain
- Membrane protein including part or all the trans-membrane domain
- Other:

Did you or the sample provider measured a full UV spectrum (230nm to 340nm) as a simple quality test *

Have you tested this protein previously? *

Implementation of a standardized sample quality control workflow

*Required

Previous test of this protein *

Give the number of times in total you have performed the quality control test on this protein

6

*

Tick the parameter that has been changed?

- [ ] Expression vector
- [ ] Host strain
- [ ] Protein Concentration
- [ ] Buffer composition
- [ ] Storage condition
- [ ] Storage time
- [ ] Purification Protocol
- [ ] None same preparation
- [ ] New identical preparation
- [ ] Other:

« Back Continue »

23% completed

Implementation of a standardized sample quality control workflow

Minimal information known by you or the sample provider

These informations should be provided in publications.

Our proposal:

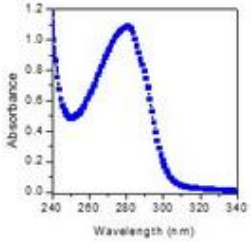
The minimal information that needs to be provided or known

Protein name and full primary structure, by providing a NCBI or UniProt accession number and cloning strategy

i.e. the source of the DNA (species), expression vector and host strain, including the tags and cleavage sites used, accompanied by the full amino acid sequence of the final protein, or sufficient details to derive the full amino acid sequence of the final protein.

Protein concentration

Specifying the method used for quantification and the molar extinction coefficient at 280nm, if applicable



Storage conditions

i.e. final buffer composition (pH, buffers, salts and additives), storage temperature or lyophilization conditions

« Back Continue »

26% completed

Minimal information known by you or the sample provider

These informations should be provided in publications.

Protein ID and origin *

Tick the information that is known by you or the sample provider

- [ ] Protein name
- [ ] Full Primary structure
- [ ] NCBI or Uniprot accession number
- [ ] Cloning strategy
- [ ] Theoretical extinction coefficient
- [ ] Measured extinction coefficient
- [ ] Not known

Protein concentration *

Specify the method used by you or the sample provider

- [ ] 280nm with cuvette spectrophotometer
- [ ] 280nm with Nanodrop
- [ ] Amino Acid Analysis
- [ ] Fourier Transform InfraRed (Direct detect...)
- [ ] Bradford assay
- [ ] BCA assay
- [ ] Lowry assay
- [ ] Refractometry
- [ ] Not performed
- [ ] Other:

Storage conditions *

Tick the information that is known by you or the sample provider

- [ ] Final buffer composition
- [ ] Storage temperature (-80°C, -20°C, 4°C, 20°C...)
- [ ] Lyophylisation condition
- [ ] Not known

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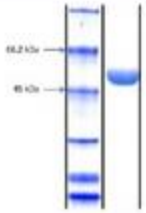
30% completed

Implementation of a standardized sample quality control workflow

Minimal quality control parameters

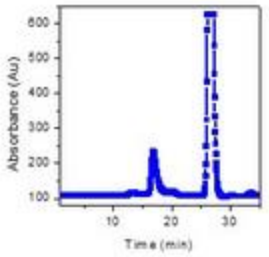
Our proposal:

The minimal quality control parameters



Purity

Checked by SDS-PAGE, Capillary Electrophoresis or Reverse Phase HPLC



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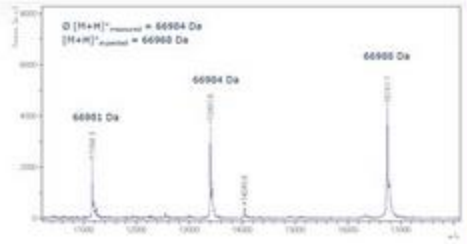

Identity

checked preferably by intact protein mass, peptide mass fingerprint, or Edman sequencing.

« Back Continue »

34% completed

Implementation of a standardized sample quality control workflow

*Required

Minimal quality control parameters

Purity test *

Which method have you used?

- [x] SDS-PAGE
- [ ] Capillary Electrophoresis
- [ ] Reverse Phase HPLC (RP-HPLC)
- [ ] Sadly, none
- [ ] Other:

Did the sample pass the test? *

A single species must be detected to pass the test

Failed

Comments if applicable

« Back Continue »

38% completed

Minimal list of quality control parameters

Homogeneity (Aggregation test) *

Which method have you used?

- [ ] Analytical Ultracentrifugation (AUC)
- [ ] Dynamic Light Scattering (DLS)
- [ ] F4 with Multi Angle Light Scattering (F4-MALS)
- [x] Flow Field-Flow Fractionation (F4)
- [ ] Size Exclusion Chromatography (SEC)
- [ ] SEC with Multi Angle Light Scattering (SEC-MALS)
- [ ] Sadly, none
- [ ] Other:

Did the sample pass the test? *

A single species must be detected to pass the test, aggregates must be absent of the preparation

Failed

Comments if applicable

« Back Continue »

42% completed

Implementation of a standardized sample quality control workflow

*Required

Minimal quality control parameters

Identity test *

Which method did you use?

- [ ] Intact protein mass (Preferably)
- [x] Peptide mass finger printing
- [ ] Edman sequencing
- [ ] Sadly, none
- [ ] Other:

Did the sample pass the test? *

Intact mass and sequence must correspond to the expected results from the primary sequence

Comments if applicable

« Back | Continue »

46% completed

Implementation of a standardized sample quality control workflow

Extended quality control parameters

Our proposal:

Extended quality control parameters

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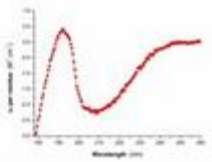

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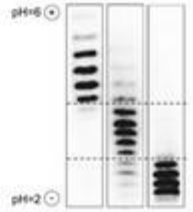

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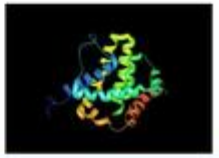

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Long term stability, activity assay, thermal shift assay

« Back Continue »

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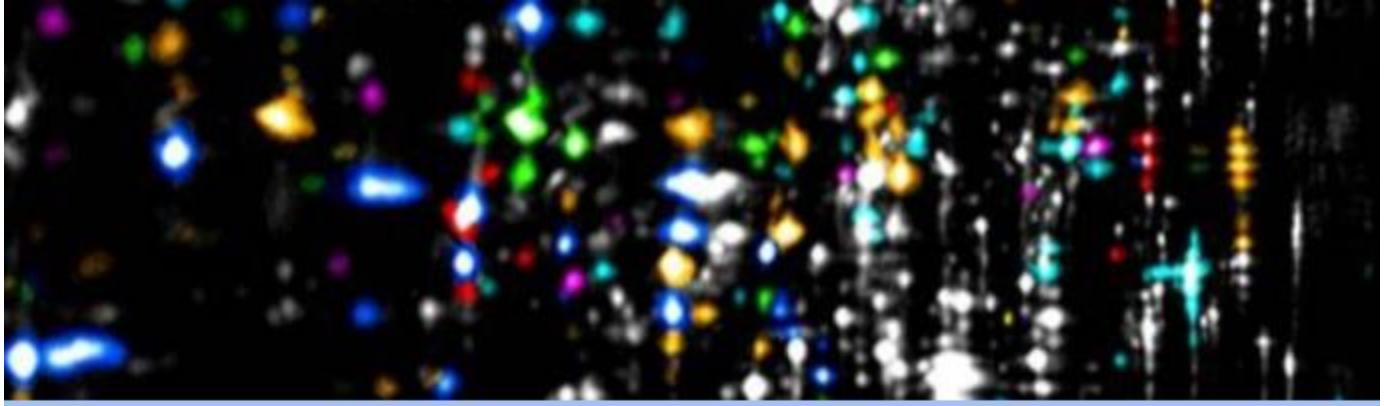

Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Did you test the nucleic acid content? *

Mandatory if the protein binds nucleic acid.

Yes

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53% completed

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Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Nucleic acid content test *

Specify the method used

- ○ UV-Vis spectroscopy
- ○ Other:

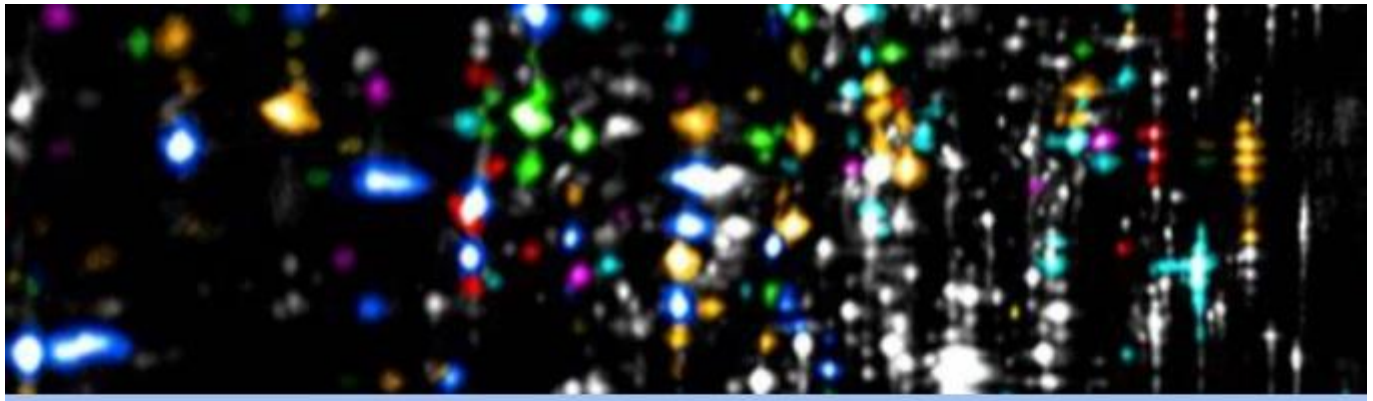
Did the sample pass the test? *

No trace of nucleic acid, ratio of absorbance 260nm/280nm should be around 0.6 to pass the test

Comments if applicable

« Back Continue »

57% completed



Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Did you perform a conformational stability/folding state test? *

Yes

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Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Conformational stability/folding state test *

Specify the method used

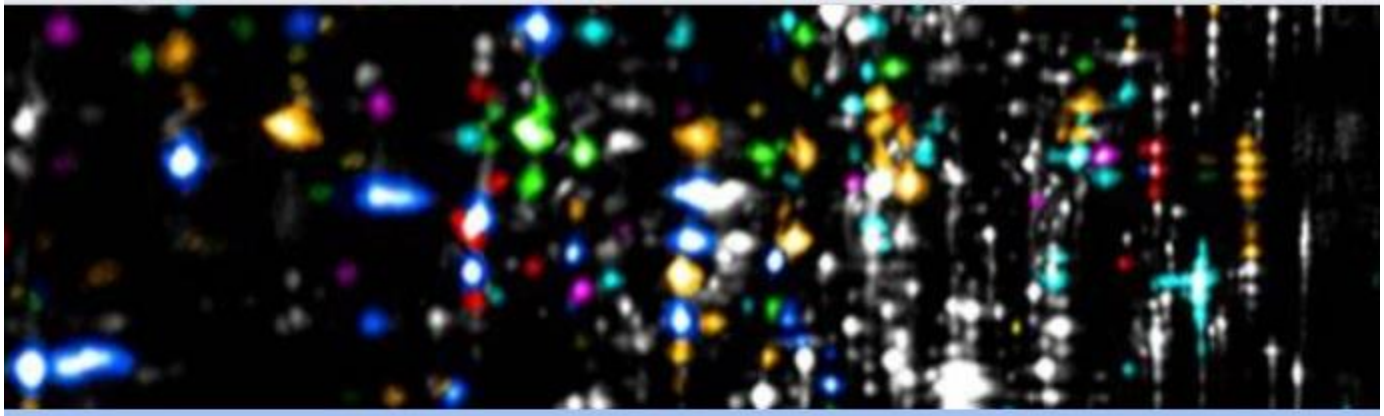
- [ ] Circular Dichroism
- [ ] Differential Scanning Calorimetry
- [ ] NMR
- [ ] Thermal shift assay
- [ ] Other:

Did the sample pass the test? *

Comments if applicable

« Back Continue »

65% completed



Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Did you perform a protein competent fraction test? *

Yes

« Back Continue »

76% completed

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Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Protein competent fraction test *

Specify the method used

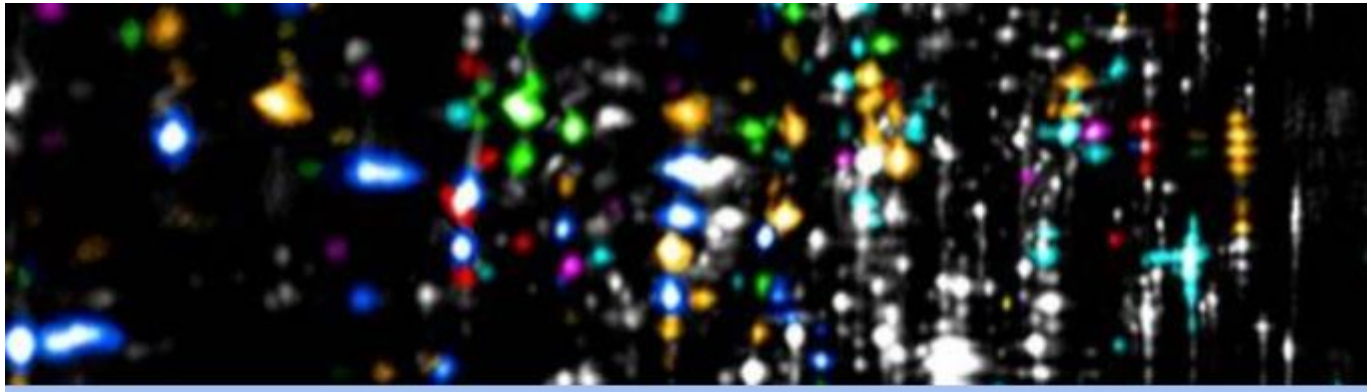
- [ ] Specific activy test
- [ ] Active site titration
- [ ] Other:

Did the sample pass the test? *

Comments if applicable

« Back Continue »

80% completed



Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Have you performed an optimization of storage condition test? *

Yes

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Implementation of a standardized sample quality control workflow

*Required

Extended quality control parameters

Optimization of storage condition *

Specify the method used

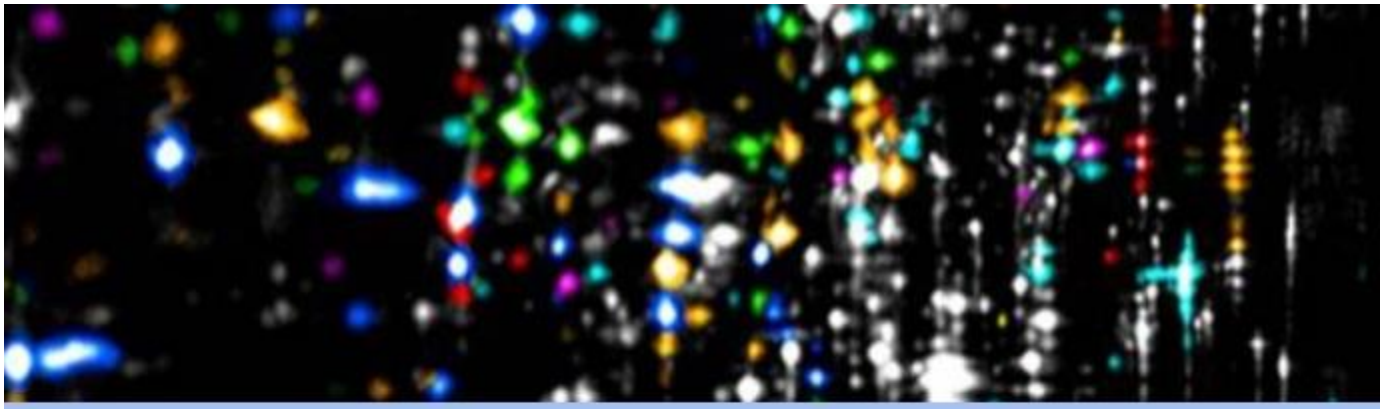
- [ ] Activity assay
- [ ] Long term stability
- [ ] Thermal shift assay
- [ ] Other:

Did you find an optimal storage condition? *

Comments if applicable

« Back Continue »

88% completed



Implementation of a standardized sample quality control workflow

*Required

Downstream application

Do you know the downstream application of the sample *

« Back Continue »

92% completed

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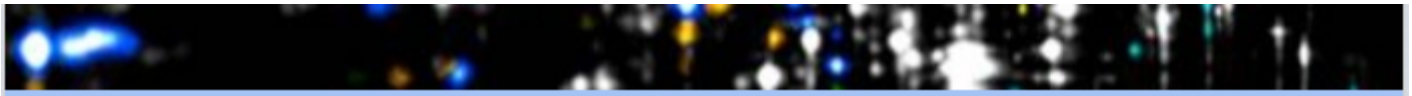


Implementation of a standardized sample quality control workflow

*Required

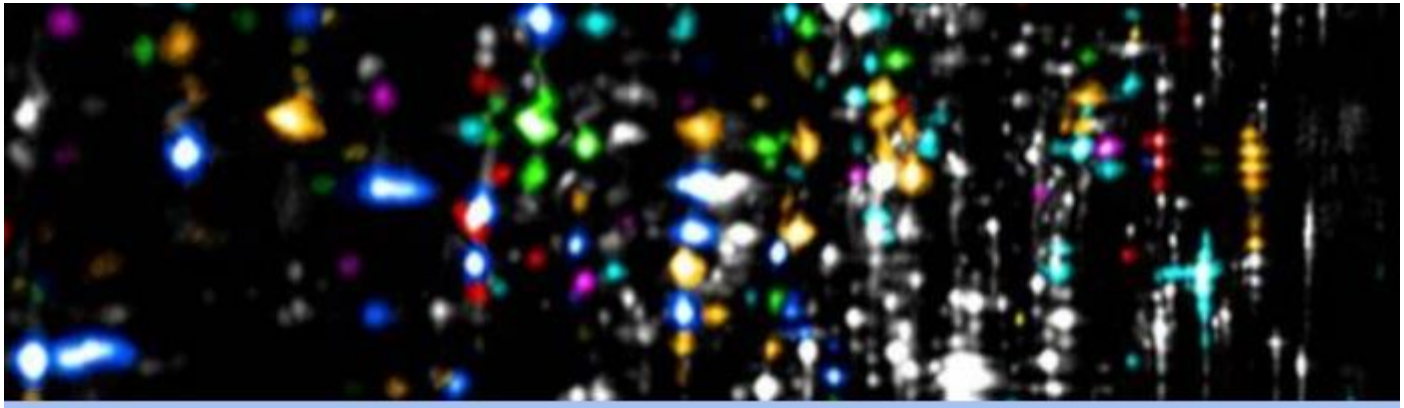
Downstream application

Please tick all the box defining the downstream application *

- [ ] Molecular Biophysics
- [ ] Crystallization
- [ ] Biological test
- [ ] Biochemical studies
- [ ] Other:

Did the downstream applications succeed *

Comments if applicable



Implementation of a standardized sample quality control workflow

Submission

Would you be able to provide raw data on this protein to use in publication?

Yes

« Back | Submit

100%: You made it.

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Statistical objectives

Aims: 95% confidence

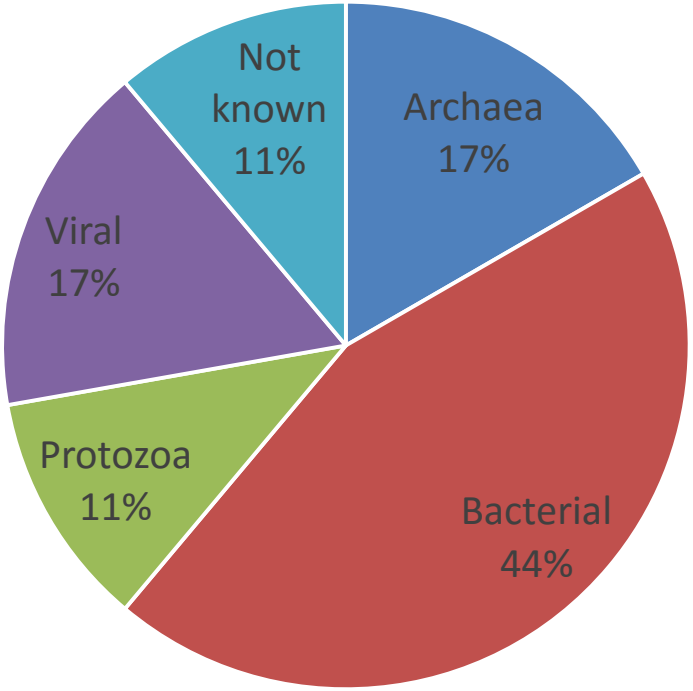
		Sample size	
Type of parameter	Population	3% error	5% error
Total Protein known	115000	1060	385
Human Protein	25000	1023	378
Membrane protein	5500	894	359
Group Participating	100	91	79

Aims: 99% confidence

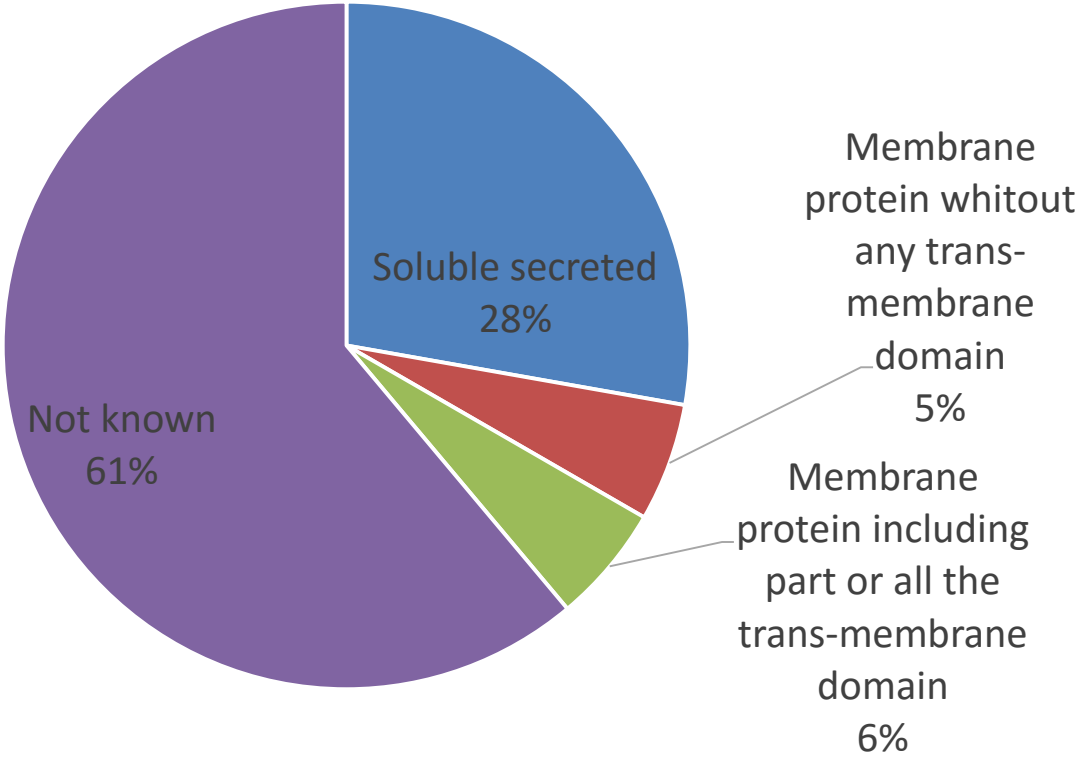
		Sample size	
Type of parameter	Population	3% error	5% error
Total Protein known	115000	1820	662
Human Protein	25000	1772	648
Membrane protein	5500	1384	594
Group Participating	100	95	87

Small study in Institut Pasteur

Protein DNA source organism

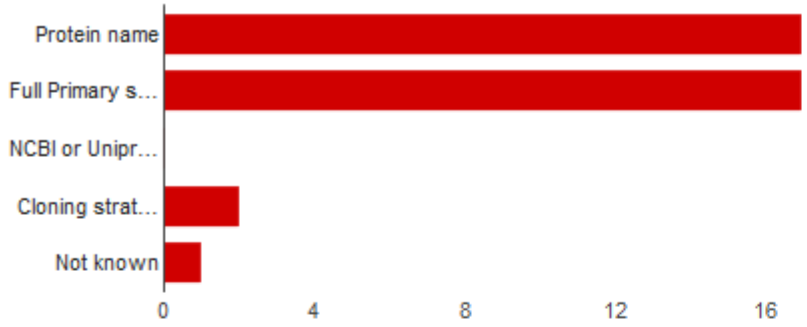


Type of expressed protein



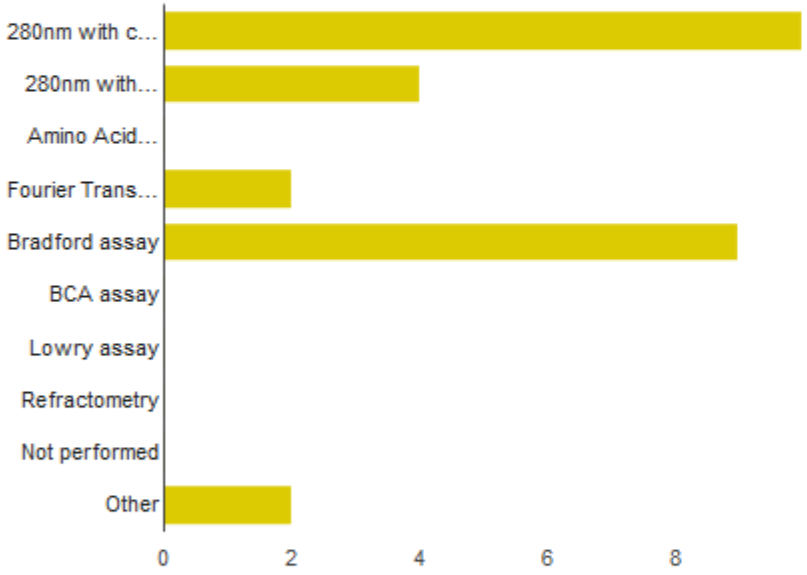
Small study in Institut Pasteur

Protein ID and origin



Protein name	**17**	94.4%
Full Primary structure	**17**	94.4%
NCBI or Uniprot accession number	**0**	0%
Cloning strategy	**2**	11.1%
Not known	**1**	5.6%

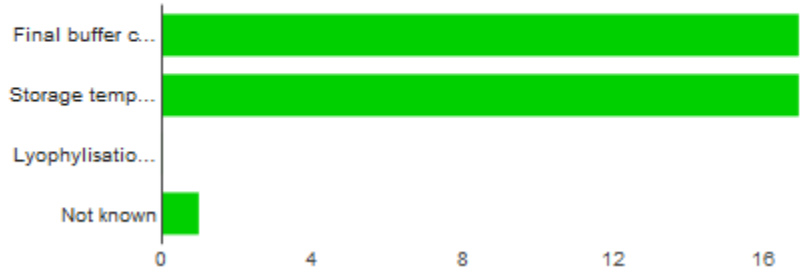
Protein concentration



280nm with cuvette spectrophotometer	**10**	55.6%
280nm with Nanodrop	**4**	22.2%
Amino Acid Analysis	**0**	0%
Fourier Transform InfraRed (Direct detect...)	**2**	11.1%
Bradford assay	**9**	50%
BCA assay	**0**	0%
Lowry assay	**0**	0%
Refractometry	**0**	0%
Not performed	**0**	0%
Other	**2**	11.1%

Small study in Institut Pasteur

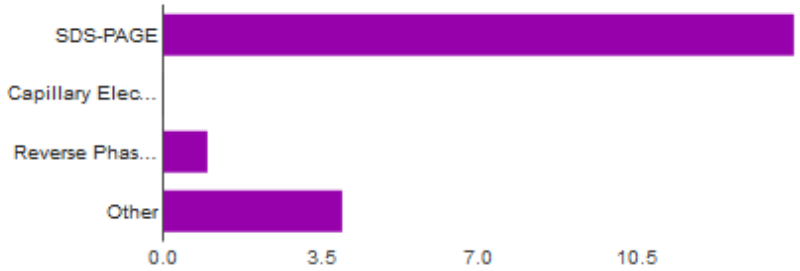
Storage conditions



Final buffer composition	**17**	94.4%
Storage temperature (-80°C, -20°C, 4°C, 20°C...)	**17**	94.4%
Lyophylisation condition	**0**	0%
Not known	**1**	5.6%

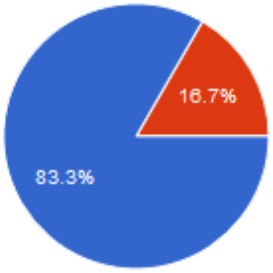
Minimal list of quality control parameters

Purity test



SDS-PAGE	**14**	77.8%
Capillary Electrophoresis	**0**	0%
Reverse Phase HPLC (RP-HPLC)	**1**	5.6%
Other	**4**	22.2%

Did the sample pass the test?

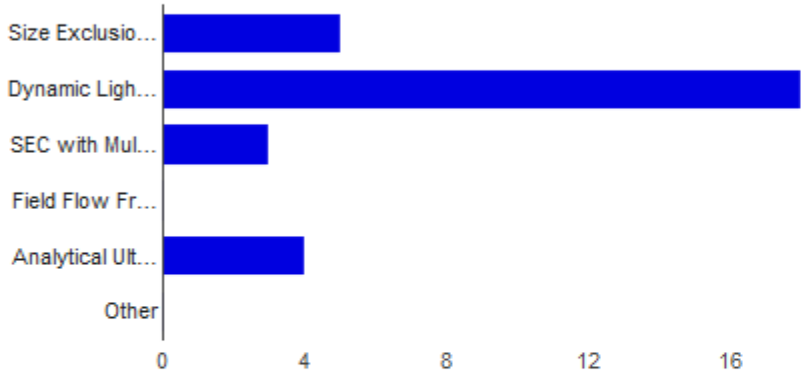


Passed	**15**	83.3%
Failed	**3**	16.7%

Small study in Institut Pasteur

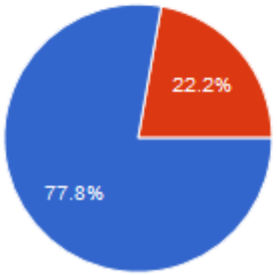
Minimal list of quality control parameters

Homogeneity (Aggregation test)



Size Exclusion Chromatography (SEC)	**5**	27.8%
Dynamic Light Scattering (DLS)	**18**	100%
SEC with Multi Angle Light Scattering (SEC-MALS)	**3**	16.7%
Field Flow Fractionation with Multi Angle Light Scattering (F4-MALS)	**0**	0%
Analytical Ultracentrifugation (AUC)	**4**	22.2%
Other	**0**	0%

Did the sample pass the test?

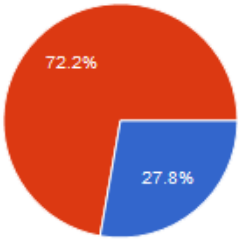


Passed	**14**	77.8%
Failed	**4**	22.2%

Small study in Institut Pasteur

Extended list of quality control parameters

Did you test the nucleic acid content?



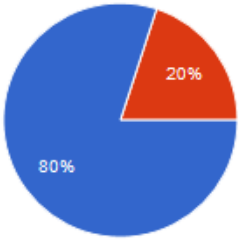
Yes	**5**	27.8%
No	**13**	72.2%

Extended list of quality control parameters

Nucleic acid content test

UV-Vis spectroscopy	**5**	100%
Other	**0**	0%

Did the sample pass the test?

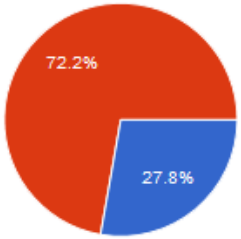


Passed	**4**	80%
Failed	**1**	20%

Small study in Institut Pasteur

Extended list of quality control parameters

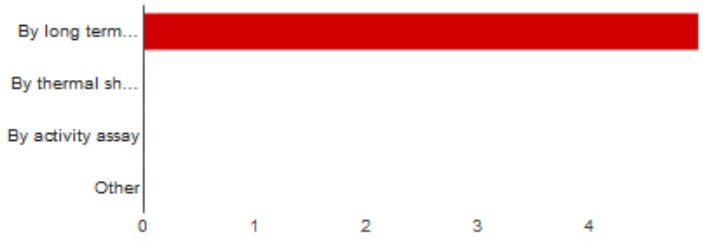
Have you performed an optimization of storage condition test?



Yes	5	27.8%
No	13	72.2%

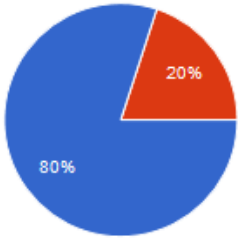
Extended list of quality control parameters

Optimization of storage condition



By long term stability	5	100%
By thermal shift assay	0	0%
By activity assay	0	0%
Other	0	0%

Did you find an optimal storage condition?

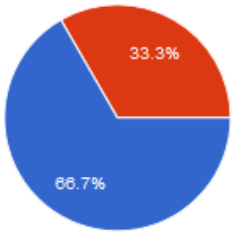


Yes	4	80%
No	1	20%

Small study in Institut Pasteur

Downstream application

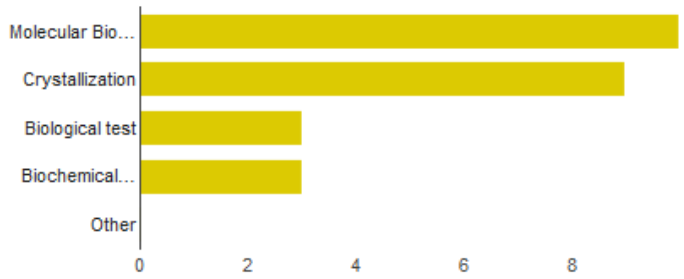
Do you know the downstream application of the sample



Yes	**12**	66.7%
No	**6**	33.3%

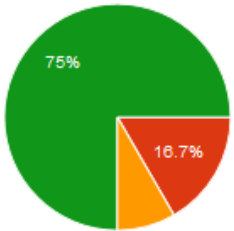
Downstream application

Please tick all the box defining the downstream application



Molecular Biophysics	**10**	83.3%
Crystallization	**9**	75%
Biological test	**3**	25%
Biochemical studies	**3**	25%
Other	**0**	0%

Did the downstream applications succeed



Yes fully	**0**	0%
Not at all	**2**	16.7%
Partly	**1**	8.3%
Do not know	**9**	75%

When and how?
Points to discussed

- Launch next week if we are all ok
- First analysis in December-Janauary
- We will need to go after each team on individual basis
- FAQ will be adapted with the incoming question.
- List will be completed with the others comment that have been filled
- Convincing teams in your institutions to do the tests
- Next step?