ARBRE-P4EU Consensus Protein Quality Guidelines for Biophysical and Biochemical Studies

Minimal information to provide

- Protein name and full primary structure, by providing a NCBI (or UniProt) accession number and cloning pathway, 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 and, where applicable, freezing or lyophilization conditions.

Minimal quality control parameters

- **Purity**: checked by SDS-PAGE, Capillary Electrophoresis (CE) or Reversed-Phase-HPLC (RP-HPLC).
- **Homogeneity** (aggregation state): checked preferably by Size Exclusion Chromatography (SEC) and/or Dynamic Light Scattering (DLS) or by Size Exclusion Chromatography in combination with Multi Angle Light Scattering (SEC-MALS), Field Flow Fractionation (FFF) or Field Flow Fractionation in combination with Multi Angle Light Scattering (FFF-MALS) or Analytical Ultracentrifugation (AUC).
- **Identity**: checked preferably by intact protein mass or by peptide mass fingerprint or Edman sequencing.

Extended quality control parameters

Depending on the intended use and in addition to the methods listed above:

•Nucleic acid content, checked by UV-Vis spectroscopy. **Mandatory if protein is to be used to study protein-nucleic acid binding.**

•Conformational stability/folding state: Circular Dichroism (CD), Differential Scanning Calorimetry (DSC) or NMR.

•Homogeneity: analytical Ion Exchange Chromatography (IEX), analytical Hydrophobic Interaction Chromatography (HIC) or Isoelectric Focusing (IEF).

•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

•Batch-to-batch consistency: use some of the methods listed above. **Mandatory if more than one batch is used**

tp://goo.gl/forms/aFS8CkH96s
ssword: qcprotein

Implementation of a standardized sample quality control workflow

* Required

General information about the sample

Date of quality control test *

mm/dd/yyyy

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? *

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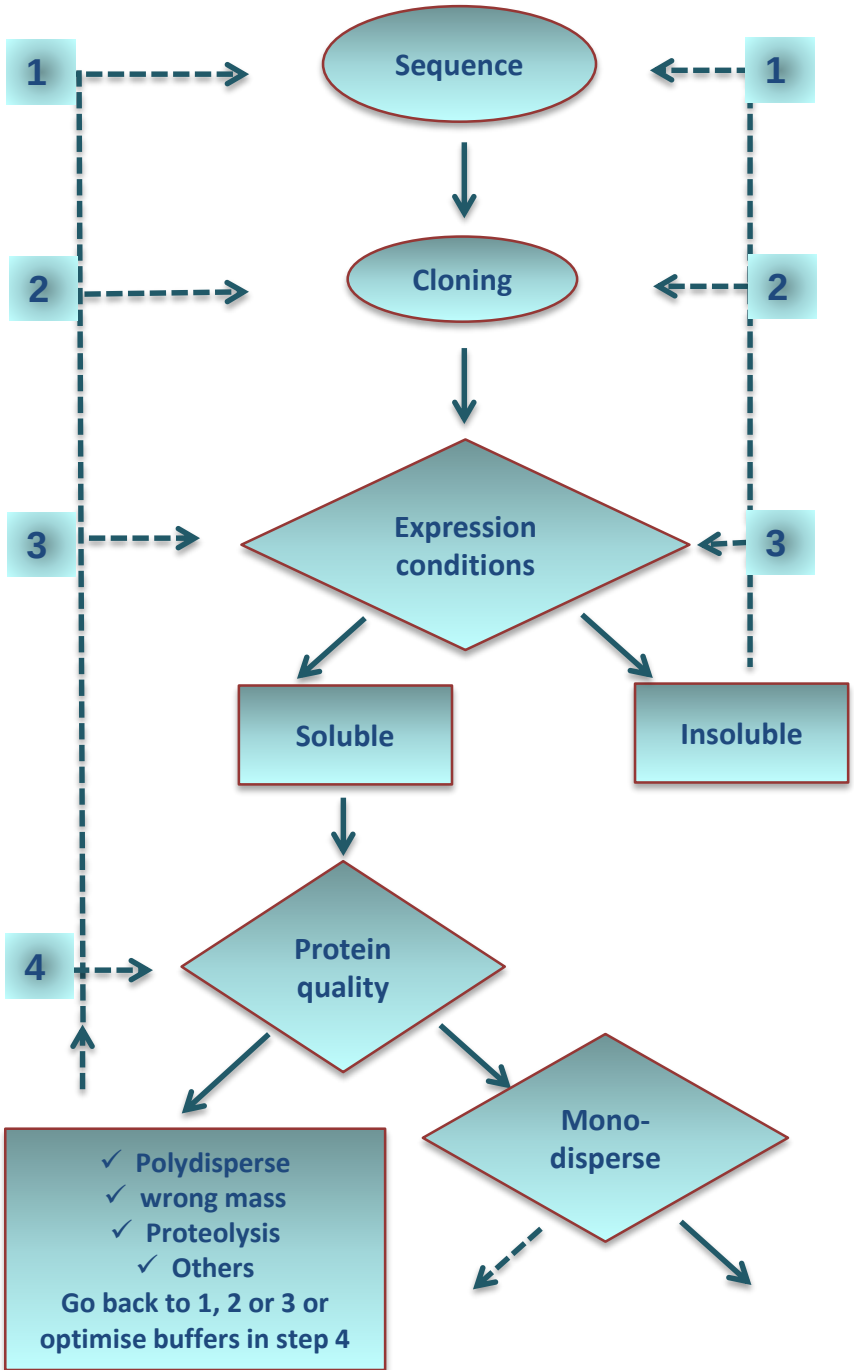
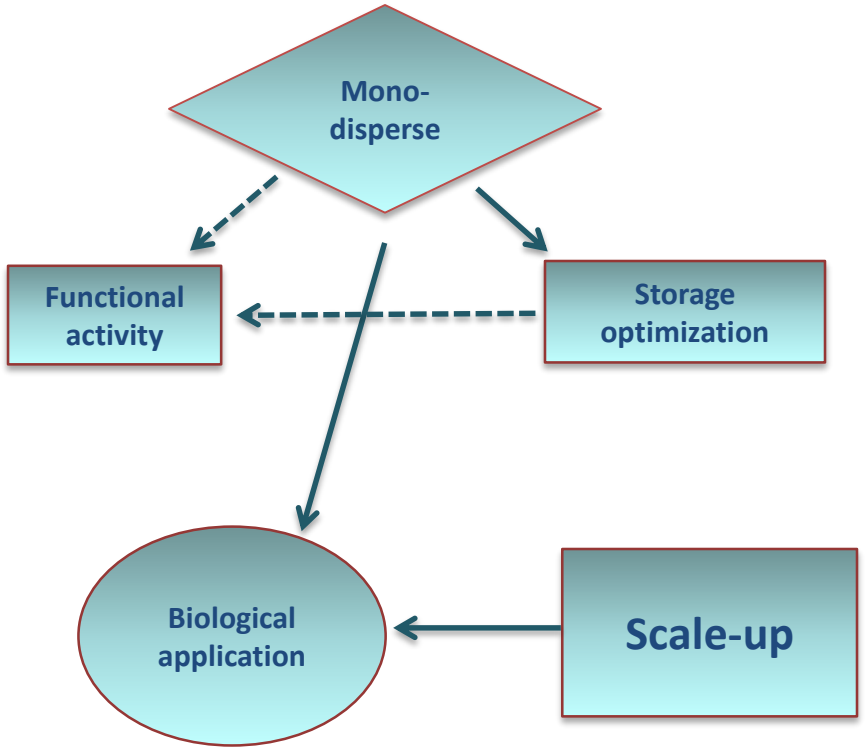
Mario Lebendiker, Tsafi Danieli and Ario de Marco

BMC Research Notes 2014, 7:585 doi:10.1186/1756-0500-7-585





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1. Sequence:

A. Gene ID from a known database (PubMed tools, etc.)

B. Domain determination of the specific gene ID, (i.e. aa 48-330 from Id xxx)

C. Specific elements (Signal peptide, Trans-membrane, cellular localization, specific protease sites, etc.)

D. Fusion tags (Full seq. + Ref.)

E. Protease sites for tag removal (Full seq. +ref. of)

F. Full translated region, DNA and AA seq.

2. Cloning

A. Vector name either commercial or from known database (such as link to AddGene). Should include: Full seq of the vector, annotated map with resistance, ori, cloning methodology, etc

B. Cloning method (ligation, recombination, etc.)

C. DNA region into which the sequence described is inserted

D. Bacterial strain used for storage and propagation (commercial maker, genotype, cat. Number, or database

3. Expression conditions

A. Expression system (Host specification such as: bacterial genotype, cell line, cat. Number, and link to database)

B. Growth & induction conditions: temperature, media & supplements [maker & cat numbers], if shaking : rpm, oxygenation (?),inducer, time, quantity

C. Instrumentation used (Bioreactors, shakers, flasks, etc)

4. Soluble / Insoluble

A. Cell lysis :buffers & lysis procedure

B. Low scale purification (resin, buffers, yield)

C. PAGE-SDS analysis: method (sup vs. pellet,and/ or affinity binding: analyzed on Coomasie and/or Western)

5. Scale-up and storage conditions

A. Initial growth volume + (OD, cell mass etc.)

B. Lysis conditions: buffers, additives, lysis methodology, clarification procedure used

C. Chromatography: Resin supplier, column volume, buffers use, loading, wash and elution conditions. Which criteria was used to select fractions

D. Description of next chromatographic steps as before

E. Final storage: protein concentration method use, storage buffer , storage conditions. Final yield

F. Quality control: PAGE-SDS analysis , analytical SEC , protein quantification method, etc

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5. Protein quality:

Basic requirements for evaluating protein quality

A. PAGE-SDS (coomassie and/or Western blot) provides multiple information regarding the quality of the protein such as the presence of degradation products as well as the absence of protein contamination

B. Analytical size exclusion chromatography (SEC) provides information regarding the correct oligomeric structure of the protein and the absence of soluble aggregates that can cause non-specific results in downstream experiments.

C. Identity (checked by mass finger print, intact protein mass, Edman). *For laboratories without accessibility for these procedures, nucleic acid sequence during cloning plus PAGE-SDS western blot after purification could be a possible alternative.*

Additional information for evaluating protein quality

C. Protein quantification: procedure used

D. Functional activity: short description of the assay and results

E. Others: CD, OD spectrum, MS, analytical ion exchange (IEX) , Reverse phase chromatography (RPC), DLS, SLS, SEC-MALS, etc