1-Sequence

2-Cloning

3-Expression conditions, and
4-Small-scale production

Soluble

Insoluble

5-Protein quality

Mono-dispersed

Polydispersed, wrong mass, proteolysis: go back to 1, 2 or 3 or optimize buffers in step 4

Functional activity

6-Storage optimization

6-Scale-up

Biological application
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 sequence, cellular localization, specific protease sites, etc.)
- d) Fusion tags
- e) Protease sites for tag removal
- f) Full translated region, DNA and AA sequences

Cloning

- a) Vector name either commercial or from known database (such as link to AddGene - vector sequence, 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 producer, genotype, cat. Number, or database)

Expression conditions

- a) Expression system (Host specification such as: bacterial strain, cell line, cat. Number, link to database)
- b) Growth and induction conditions: temperature, media and supplements, rpm, oxygenation, inducer, time, quantity
- c) Instrumentation used (Bioreactors, shakers, flasks, etc.)

Small scale production: Soluble / Insoluble

- a) Cell lysis: buffers and lysis procedure
- b) Purification conditions: resin, buffers, yield
- c) PAGE-SDS analysis: method (sup vs. pellet, and/or affinity binding: analyzed on Coomasie and/or Western blot)
Protein quality

Basic requirements for evaluating monodispersity and degradation

a) PAGE-SDS (coomassie and/or Western blot)

b) Analytical size exclusion chromatography (SEC)

Additional information for evaluating protein quality

c) Functional activity: short description of the assay and results

d) Others: Circular Dicroism (CD), Optical Density (OD) spectrum, Mass Spectrum (MS), analytical ion exchange (IEX), Reverse phase chromatography (RPC), Dynamic and Static Light Scattering (DLS and SLS), Size Exclusion Chromatography - Multi Angle (Laser) Light Scattering Method (SEC-MALS), etc

Scale-up and storage conditions

a) Initial growth volume (OD, cell mass etc.)

b) Lysis conditions: buffers, additives, lysis methodology, clarification procedure

c) Chromatography: Resin supplier, column volume, buffers, loading, washing, and elution conditions. Final yield

d) Description of successive chromatographic steps as before

e) Storage conditions: protein concentration, storage buffer, temperature

f) Quality control: PAGE-SDS analysis, analytical SEC, protein quantification method, etc