

Quality controls of productions with APS-One/Mastercalve system

To validate Petri dishes prepared, the Mediaprep service has implemented a series of quality controls. The quality controls consist of:

- Validating the appropriate selection conditions by taking randomly two plates per run and place them for overnight at 37°C (in the incubator located at the CEBGS), one closed and one opened.
- 2) Confirming the presence of the appropriate antibiotic by transforming BL21(DE3) cells with 4 plasmids, each one harboring a specific resistance gene and encoding a specific colored protein, and spread cells, as well as non-transformed cells, on a plate. The plasmids used are:

(A) pHGWA-avEYFP for ampicillin resistance and encoding yellow protein (K) pHGWK-DsRed for kanamycin resistance and encoding red protein

(C) pCoGWC-avECFP for chloramphenicol resistance and encoding cyan protein

(S) pCoGWS-avGFP for spectinomycin resistance and encoding green protein

- (Ø) Non-transformed cells
- 3) Confirming appropriate selection by replicating the grown cells on plate added with IPTG (1mM) to induce protein expression to validate that the appropriate cells grew.

Control of expression for each transformed plasmid in BL21(DE3) after spreading on a plate containing the appropriate antibiotic.

pHGWA-EYFP



Ampicillin plate



Kanamycin plate

pCoGWC-avECFP



Chloramphenicol plate



pCoGWS-avGFP

Spectinomycin plate



Med2012/109C

Date: 12 September 2012 LB-Agar (Lot LB-Agar/00021) Demineralized water: 2 L Antibiotic: Chloramphenicol (Chlor34/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 102



Conclusion:



Med2012/108A

Date: 11 September 2012 LB-Agar (Lot LB-Agar/00020) Demineralized water: 2 L Antibiotic: Ampicilin (Lot Ampi100/00007) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 106

37°C37°C - OpenCell growthExpression

Conclusion:

Order for Seraphin team, production OK



Med2012/107A

Date: 10 September 2012 LB-Agar (Lot LB-Agar/00020) Demineralized water: 8 L Antibiotic: Ampicilin (Lot Ampi100/00007) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 427

37°C

37°C - Open

Cell growth

Expression

Conclusion:



Med2012/106C

Date: 10 September 2012 LB-Agar (Lot LB-Agar/00020) Demineralized water: 1 L Antibiotic: Chloramphenicol (Chlor34/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 40

37°C

37°C - Open

Cell growth

Expression

Conclusion:



Date: 03 September 2012 LB-Agar (Lot LB-Agar/00020) Demineralized water: 4 L Antibiotic: Kanamycin (Lot Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 217



Conclusion:



Med2012/100A

Date: 28 August 2012 LB-Agar (Lot LB-Agar/00019) Demineralized water: 8 L Antibiotic: Ampicilin (Lot Ampi100/00006 + Ampi100/00007) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 434

37°C

37°C - Open

Cell growth

Expression

Conclusion:



Date: 13 August 2012 LB-Agar (Lot LB-Agar/00019) Demineralized water: 1 L Antibiotic: Gentamycin (Genta30/00002) – Concentration: 15µg/mL Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 46

37°C37°C - OpenCell growthExpression

N.A.

Conclusion:



Date: 10 August 2012 LB-Agar (Lot LB-Agar/00019) Demineralized water: 1 L Antibiotic: Spectinomycin (Spec30/00001) – appropriate volume to have a final concentration of 50μg/ml Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 48

37°C

37°C - Open

Cell growth

Expression

Conclusion:



Med2012/094K

Date: 08 August 2012 LB-Agar (Lot LB-Agar/00018 + LB-Agar/00019) Demineralized water: 2 L Antibiotic: Kanamycin (Lot Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 100

37°C37°C - OpenCell growthExpression

Conclusion:

Order for Seraphin team, production OK



Date: 8 August 2012 LB-Agar (Lot LB-Agar/00018) Demineralized water: 8 L Antibiotic: Ampicilin (Lot Ampi100/00005 + Ampi100/00006) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 423

37°C

37°C - Open

Cell growth

Expression

Conclusion:



Date: 26 July 2012 LB-Agar (Lot LB-Agar/00017 + LB-Agar/00018) Demineralized water: 8 L Antibiotic: Ampicilin (Lot Ampi100/00005) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 424

37°C

37°C - Open

Cell growth

Expression

Conclusion:

Problems during transformation but production OK



Med2012/090K

Date: 18 July 2012 LB-Agar (Lot LB-Agar/00016 + LB-Agar/00017) Demineralized water: 8 L Antibiotic: Kanamycin (Lot Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 425



Conclusion:



Date: 17 July 2012 LB-Agar (Lot LB-Agar/00016) Demineralized water: 8 L Antibiotic: Ampicilin (Lot Ampi100/00004 + Ampi100/00005) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 439



Conclusion:



Date: 13 July 2012 LB-Agar (Lot LB-Agar/00016) Demineralized water: 2 L Antibiotic: Ampicilin (Ampi100/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 100



Conclusion:

Production OK totally provided to Séraphin team



Date: 11 July 2012 LB-Agar (Lot LB-Agar/00016) Demineralized water: 1 L Antibiotic: Gentamycin (Genta30/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 41



Conclusion:

igbmc Med2012/083K

Date: 10 July 2012 LB-Agar (LB-Agar/00015 + LB-Agar/00016) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 217





Cell growth

Expression



Conclusion:



Med2012/081A

Date: 09 July 2012 LB-Agar (Lot LB-Agar/00015) Demineralized water: 8 L Antibiotic: Ampicilin (Ampi100/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 439



Conclusion:



Med2012/080C

Date: 02 July 2012 LB-Agar (Lot LB-Agar/00014+ LB-Agar/00015) Demineralized water: 1 L Antibiotic: Chloramphenicol (Chlor34/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 46



Conclusion:



Date: 28 June 2012 LB-Agar (LB-Agar/00014) Demineralized water: 2 L Antibiotic: Kanamycin (Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 102



Conclusion:

Production OK totally provided to Séraphin team



Date: 26June 2012 LB-Agar (Lot LB-Agar/00014) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 435



Conclusion:

ighter Med2012/073K

Date: 18 June 2012 LB-Agar (LB-Agar/00013) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 208



Conclusion:

Production OK. Colonies observed on the « S » sector come from a flow of the near sector during plate handling after spreading. This is confirmed by the red color that is characteristic of cells bearing a plasmid conferring resistance to kanamycin.



Date: 14 June 2012 LB-Agar (Lot LB-Agar/00013) Demineralized water: 2 L Antibiotic: Ampicillin (Ampi100/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 103

37°C

37°C - Open







Expression

Not performed

Conclusion:

Production OK totally provided to Séraphin team



Date: 13 June 2012 LB-Agar (Lot LB-Agar/00013) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 439



Conclusion:

Production OK. Colonies observed on the « Ø » sector come from a flow of the near sector during plate handling after spreading. This is confirmed by the yellow color that is characteristic of cells bearing a plasmid conferring resistance to ampicillin.



Date: 04 June 2012 LB-Agar (Lot LB-Agar/00012) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00003 + Ampi100/00004) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 430



Conclusion:

Production OK. Colonies observed on the « Ø » sector come from a flow of the near sector during plate handling after spreading. This is confirmed by the yellow color that is characteristic of cells bearing a plasmid conferring resistance to ampicillin.



Date: 30 May 2012 LB-Agar (LB-Agar/00012) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 207

37°C

37°C - Open





Cell growth

Not performed



Expression

Conclusion:



Date: 25 May 2012 LB-Agar (LB-Agar/00012) Demineralized water: 1 L Antibiotic: Chloramphenicol (Chlor34/00003) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 45



Conclusion: Production OK



Date: 23 May 2012 LB-Agar (Lot LB-Agar/00011) Demineralized water: 2 L Antibiotic: Ampicillin (Ampi100/00003) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 99



Conclusion:

Production OK totally provided to Séraphin team



Date: 22 May 2012 LB-Agar (Lot LB-Agar/00011) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00003) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 430



Conclusion:



Date: 14 May 2012 LB-Agar (Lot LB-Agar/00010) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00003) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 439



Conclusion:



Date: 04 May 2012 LB-Agar (Lot LB-Agar/00010) Demineralized water: 2 L Antibiotic: Ampicillin (Ampi100/00003) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 106



Conclusion:

Growth on opened plate at room temperature only (maybe due to contaminated environment).

Production OK totally provided to Séraphin team



Date: 03 May 2012 LB-Agar (Lot LB-Agar/00010) Demineralized water: 1 L Antibiotic: Spectinomycin (Spec30/00001) – appropriate volume to have a final concentration of $50\mu g/ml$ Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 50



Conclusion:

Few colonies are visible on other sectors of the plate but do not allow colored protein expression. The selection is specifically marked for plasmid bearing the spectinomycin resistance gene.



Date: 30 April 2012 LB-Agar (Lot LB-Agar/00009) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00003) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 429



Conclusion:



Date: 26 April 2012 LB-Agar (Lot LB-Agar/00009) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 212



Conclusion:

ighter Med2012/048K

Date: 26 April 2012 LB-Agar (Lot LB-Agar/00008 + LB-Agar/00009) Demineralized water: 2 L Antibiotic: Kanamycin (Kana50/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 106



Conclusion:

Production OK totally provided to Séraphin team



Date: 18 April 2012 LB-Agar (Lot LB-Agar/00008) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00003) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 436



Conclusion:

igbmc Med2012/044A

Date: 11 April 2012 LB-Agar (Lot LB-Agar/00007 + LB-Agar/00008) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 438



Conclusion:



Date: 10 April 2012 LB-Agar (LB-Agar/00007) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 217



Conclusion:



Date: 10 April 2012 LB-Agar (Lot LB-Agar/00007) Demineralized water: 2 L Antibiotic: Ampicillin (Ampi100/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 103



Conclusion:

Production OK totally provided to Séraphin team



Date: 04 April 2012 LB-Agar (Lot LB-Agar/00007) Demineralized water: 1 L Antibiotic: Gentamycin (Genta30/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 50



Conclusion: Production OK



Date: 04 April 2012 LB-Agar (Lot LB-Agar/00007) Demineralized water: 1 L Antibiotic: Gentamycin (Genta30/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 50

37°C

37°C - Open

Cell growth

Expression

Conclusion:

Production aborted due to technical problem with the automate



Date: 03 April 2012 LB-Agar (LB-Agar/00007) Demineralized water: 2 L Antibiotic: Kanamycin (Kana50/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 106



Conclusion:

Production OK totally provided to Séraphin team

Med2012/034A iqbmc

Date: 29 March 2012 LB-Agar (Lot LB-Agar/00006+ LB-Agar/00007) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 433





Cell growth

OK – Picture not taken



Expression

Conclusion:



Date: 22 March 2012 LB-Agar (LB-Agar/00006) Demineralized water: 1 L Antibiotic: Chloramphenicol (Chlor34/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 50



Conclusion: Production OK



Date: 22 March 2012 LB-Agar (LB-Agar/00006) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 217



Conclusion:



Date: 20 March 2012 LB-Agar (Lot LB-Agar/00005) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00001 + Ampi100/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 439



Conclusion:

Production OK

On the first test, cells grew whatever the plasmid and even non-transformed cells grew. A test will be made on the Molecular Biology service to check if the problem came from the antibiotic => No.

The lot has been placed in stand-by.

Finally, the problem came from the 2xLB used that was contaminated => layer on LB+Amp plate.



Spreading tests have been made from à 450 μ l transformation with a hoese, 10 μ l and 40 μ l => 40 μ l is ok.





Date: 15 March 2012 LB-Agar (LB-Agar/00005) Demineralized water: 1 L Antibiotic: Chloramphenicol (Chlor34/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 47



Conclusion: Production OK



Date: 12 March 2012 LB-Agar (Lot LB-Agar/00004) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 436



Conclusion:



Date: 08 March 2012 LB-Agar (Lot LB-Agar/00004) Demineralized water: 1 L Antibiotic: Gentamycin (Genta30/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 47



Conclusion: Production OK



Date: 05 March 2012 LB-Agar (Lot LB-Agar/00004) Demineralized water: 1 L Antibiotic: Spectinomycin (Spec30/00001) – appropriate volume to have a final concentration of 50µg/ml Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 47



Conclusion:

Few very small colonies are visible on other sectors of the plate but do not allow colored protein expression. The selection is specifically marked for plasmid bearing the spectinomycin resistance gene.



Med2012/020S

Date: 01 March 2012 LB-Agar (Lot LB-Agar/00004) Demineralized water: 1 L Antibiotic: Spectinomycin (Spec30/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 46



Conclusion:

Production discarded. Non-transformed as well as transformed cells, whatever the plasmid used, were able to grow. The 1 ml of antibiotic did not reach the medium. To be redone.

igbmc Med2012/017A

Date: 28 Feb. 2012 LB-Agar (Lot LB-Agar/00003 + LB-Agar/00004) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 436



Conclusion:



Med2012/016K

Date: 27 Feb. 2012 LB-Agar (LB-Agar/00003) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 214



Conclusion:



Med2012/012A

Date: 15 Feb. 2012 LB-Agar (Lot LB-Agar/00002) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 436



Conclusion:

Production ok.



Med2012/010C

Date: 02 Feb. 2012 LB-Agar (LB-Agar/00002) Demineralized water: 2 L Antibiotic: Chloramphenicol (Chlor34/00002) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 100



Conclusion: Production ok.



Date: 01 Feb. 2012 LB-Agar (LB-Agar/00002) Demineralized water: 4 L Antibiotic: Kanamycin (Kana50/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 206

37°C



Cell growth



Expression

Conclusion: Production ok.



Date: 31 Jan. 2012 LB-Agar (Lot LB-Agar/00001 + LB-Agar/00002) Demineralized water: 8 L Antibiotic: Ampicillin (Ampi100/00001) Sterilization: 15 minutes at 121°C Pouring temperature: 47°C APS-One program: 18 ml of medium per plate Number of plates: 428

37°C

37°C - Open







Conclusion: Production ok.