UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea

Scienze Biologiche

Creazione di un pesce zebra transgenico per esprimere una proteina heat shock 60 (Hsp60) mutata

Generation of a transgenic zebrafish line to express a mutated heat shock protein 60 (Hsp60)

Tesi di Laurea di:

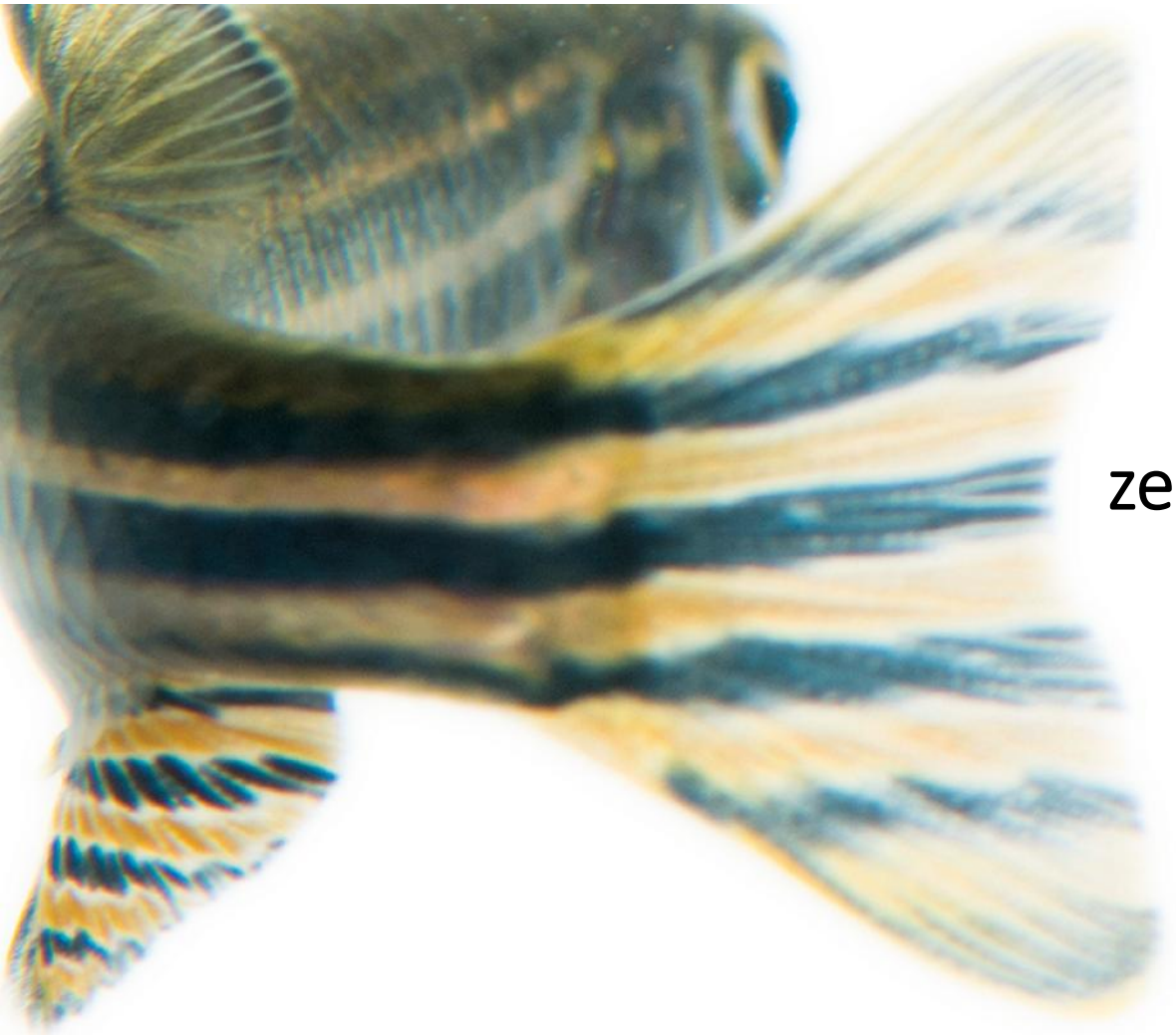
Manuel Scorrano

Docente Referente
Chiar.ma Prof.ssa

Oliana Carnevali

Sessione AUTUNNALE

Anno Accademico 2018/2019



Generation of a transgenic zebrafish line to express a mutated heat shock protein 60 (Hsp60)

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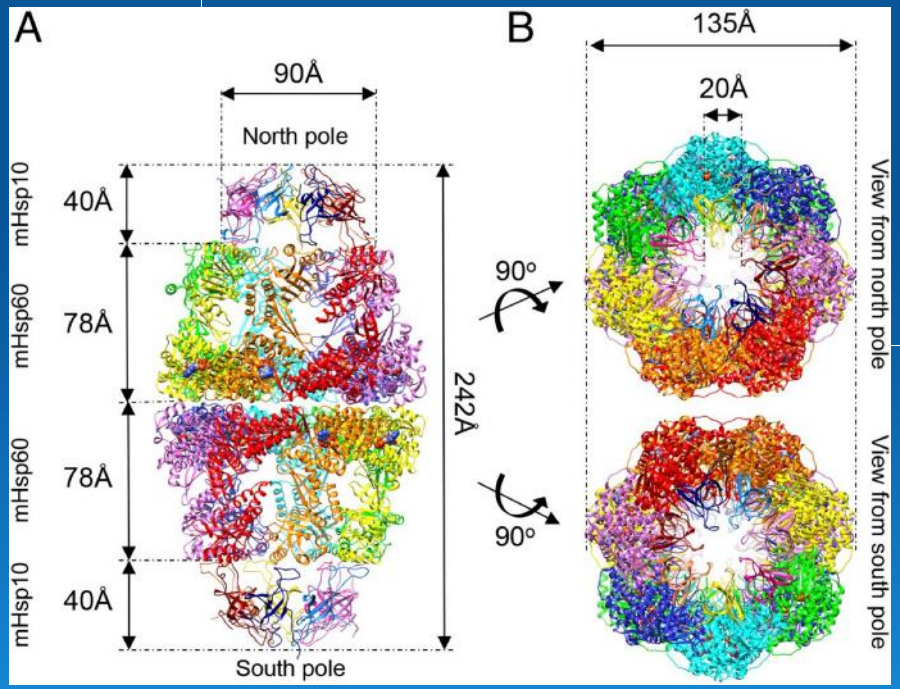
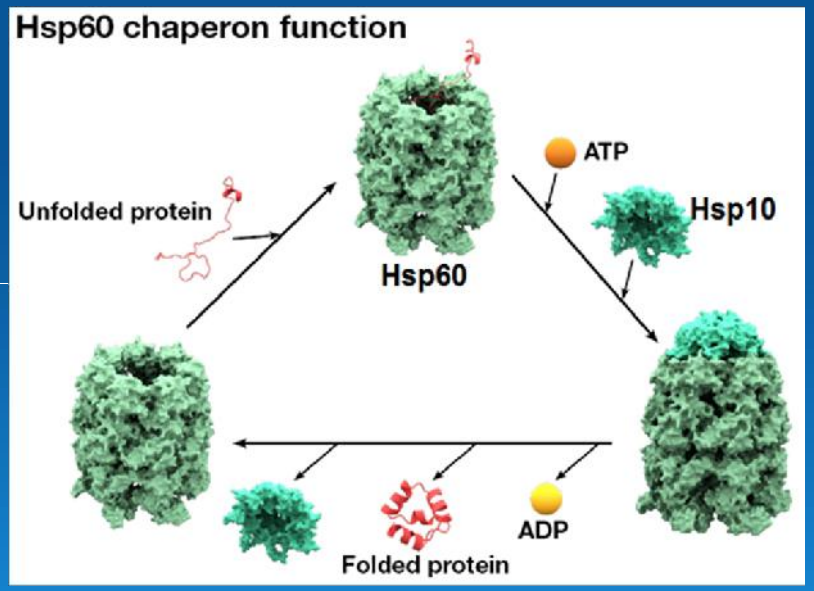
Prof.ssa Oliana Carnevali

Manuel Scorrano
Corso di Laurea in Scienze Biologiche
A.A. 2018/2019

Heat Shock Protein 60 (Hsp60)

Hsp60 is central player in the proteostasis network by assisting nascent and stress-denatured proteins in reaching their native states





Working Hypotesis

Mutations in the gene encoding the human mitochondrial Hsp60 (HSPD1) are linked with the insurgence of neurodegenerative disease

Hereditary Spastic Paraplegia SPG13 Is Associated with a Mutation in the Gene Encoding the Mitochondrial Chaperonin Hsp60

Jens Jacob Hansen,[1] Alexandra Dürr,[2,3,4] Isabelle Cournu-Rebeix,[5] Costa Georgopoulos,[6] Debbie Ang,[6] Marit Nyholm Nielsen,[1] Claire-Sophie Davoine,[5] Alexis Brice,[2,3,4] Bertrand Fontaine,[2,5] Niels Gregersen,[1] and Peter Bross[1]

Mitochondrial Hsp60 Chaperonopathy Causes an Autosomal-Recessive Neurodegenerative Disorder Linked to Brain Hypomyelination and Leukodystrophy

Daniella Magen,[1,2,3] Costa Georgopoulos,[4] Peter Bross,[5] Debbie Ang,[4] Yardena Segev,[2,3] Dorit Goldsher,[6,3] Alexandra Nemirovski,[6] Eli Shahar,[7,3] Sarit Ravid,[7,3] Anthony Luder,[8,3] Bayan Heno,[8,3] Ruth Gershoni-Baruch,[9,3] Karl Skorecki,[2,3] and Hanna Mandel[10,3,*]

A novel mutation in the HSPD1 gene in a patient with hereditary spastic paraplegia.

Hansen J[1], Svenstrup K, Ang D, Nielsen MN, Christensen JH, Gregersen N, Nielsen JE, Georgopoulos C, Bross P.

Author information

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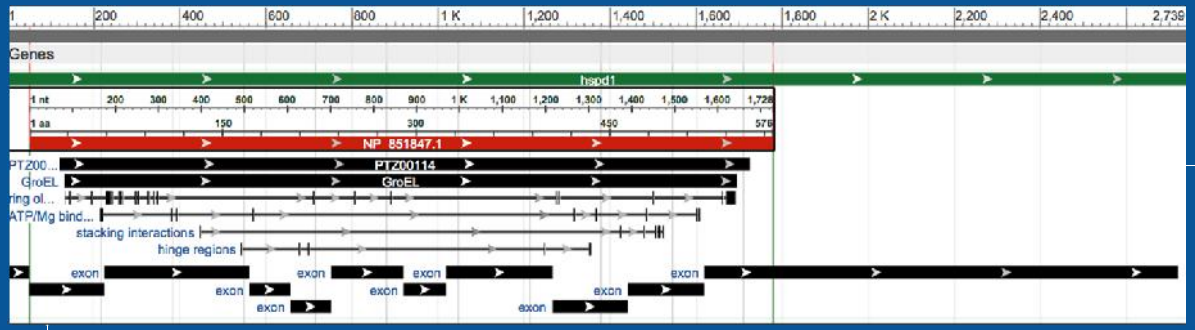
Aim

Generation of a transgenic zebrafish line to express the Hsp60 (V72I) mutation



Coding Sequence: 1728 nt

mRNA Sequence: 2739 nt

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Alignment of Sequence_1:  [Human Hsp60.xprt] with  Sequence_2: [Zebrafish Hsp60.xprt]

Similarity : 534/572 (93,36 %)

Seq_1  1     MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVKFGADARALMLQGVDLLADAVAVTMGPKGR  60
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Seq_2  1     MLRLPSVMRQMRPVCRALAPHLTRAYAKDVKFGADARALMLQGVDLLADAVAVTMGPKGR  60

Seq_1  61    TVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTTTATVLA  120
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Seq_2  61    TVIIEQSWGSPKVTKDGVTVAKSIDLKDRYKNIGAKLVQDVANNTNEEAGDGTTTATVLA  120

Seq_1  121   RSIAKEGFEKISKGANPVEIRRGVMLAVDAVIAELKKQSKPVTTPEEIAQVATISANGDK  180
             | :|||||::|||||||||||||||:||  || ||||:||||||||||||||||||||:
Seq_2  121   RAVAKEGFDTISKGANPVEIRRGVMMAVEEVISELKKNSKPVTTPEEIAQVATISANGDT  180

Seq_1  181   EIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQ  240
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Seq_2  181   EVGNIISNAMKKVGRKGVITVKDGKTLHDELEIIEGMKFDRGYISPYFINTAKGQKCEFQ  240

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Seq_2  241   DAYVLLSEKKISSVQSIVPALEIANQHRKPLVIVAEDVDGEALSTLVLNRLKVGLQVVAV  300

Seq_1  301   KAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKDDAMLLKG  360
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Seq_2  301   KAPGFGDNRKNQLQDMAVSTGGTVFGDEAMGLALEDIQAHDFGKVGEVIVTKDDTMLLKG  360

Seq_1  361   KGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEKKDR  420
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Seq_2  361   RGDASAIEKRVNEIAEQLESTNSDYEKEKLNERLAKLSDGVAVIKVGGTSDVEVNEKKDR  420

Seq_1  421   VTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIA  480
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Seq_2  421   VTDALNATRAAVEEGIVPGGGCALLRCIPALDNIKPANADQKIGIDIIRRSLRIPAMTIA  480

Seq_1  481   KNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLT  540
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Seq_2  481   KNAGVEGSLVVEKILQSSTEIGYDAMNGEYVNMVERGIIDPTKVVRTALLDAAGVASLLS  540

Seq_1  541 TAEVVVTEIPKEEKDPGMGAMGGMGGGMGG-G-MF- 573
           ||  :| ::: :| ::       |  ||||| | |:
Seq_2  541 TA-EAVVTEIPKEEKEMPAGGMGGMGGMGGMGGMGF 575
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Procedures

Fish crosses

↓

Collecting embryos

↓

RNA purification

↓

Reverse Transcriptase

↓

Designing Primers

Fragment 1 (F1)

ATGCTGCGTTTACCCAGTGTCATGAGACAGATGAGGCCTGTGTGCAGGGCTCTGGCCCCTCACCTGACCCGTGCATATGCAAAGGATGT
GAAGTTTGGAGCAGATGCCCGGGCCCTCATGCTCCAGGGTGTTGACCTGCTGGCTGATGCAGTGGCCGTCACCATGGGGCCAAAGGG
TCGCACCGTCATCATTGAGCAGAGCTGGGGAAGCCCTAAAGTTACCAAAGATGGGGTCACAGTTGCTAAAAGTATTGATCTAAAGGAC
AGGTATAAGAATATCGGAGCCAAGCTGGTTCAGGATGTGGCCAACAACACTAATGAGGAGGCTGGAGACGGCACCACCACCGCTACA
GTTCTGGCCCGGGCTGTTGCCAAAGAGGGCTTCGACACCATCAGCAAGGGGGCAAACCCAGTTGAGATCCGTAGAGGAGTCATGATG
GCCGTAGAAGAGGTCATCAGT

Fragment 2 (F2)

AAGAGGTCATCAGTGAACTCAAGAAAAACGAACTCAAGAAAAACTCAAAGCCCGTCACGACTCCAGAGGAAATCGCTCAGGTGGCTAC
TATTTCTGCCAATGGAGACACTGAAGTTGGTAACATCATCTCCAATGCTATGAAGAAAGTCGGTCGTAAGGGTGTCATTACAGTGAAGG
ATGGCAAAACCCTTCATGATGAGCTTGAGATCATTGAGGGCATGAAGTTTGACCGTGGTTACATTTCTCCTTACTTCATCAACACCGCTA
AAGGCCAGAAGTGTGAGTTTCAGGATGCATATGTGCTTCTGAGCGAGAAGAAGATCTCCAGTGTTCAGAGTATTGTTCCTGCTCTGGA
AATTGCTAACCAGCATCGCAAACCTCTGGTCATCGTGGCGGAAGATGTGGATGGAGAGGCGCTCAGCACTCTGGTCCTCAACAGGTTG
AAGGTTGGCCTGCAGGT

Fragment 3 (F3)

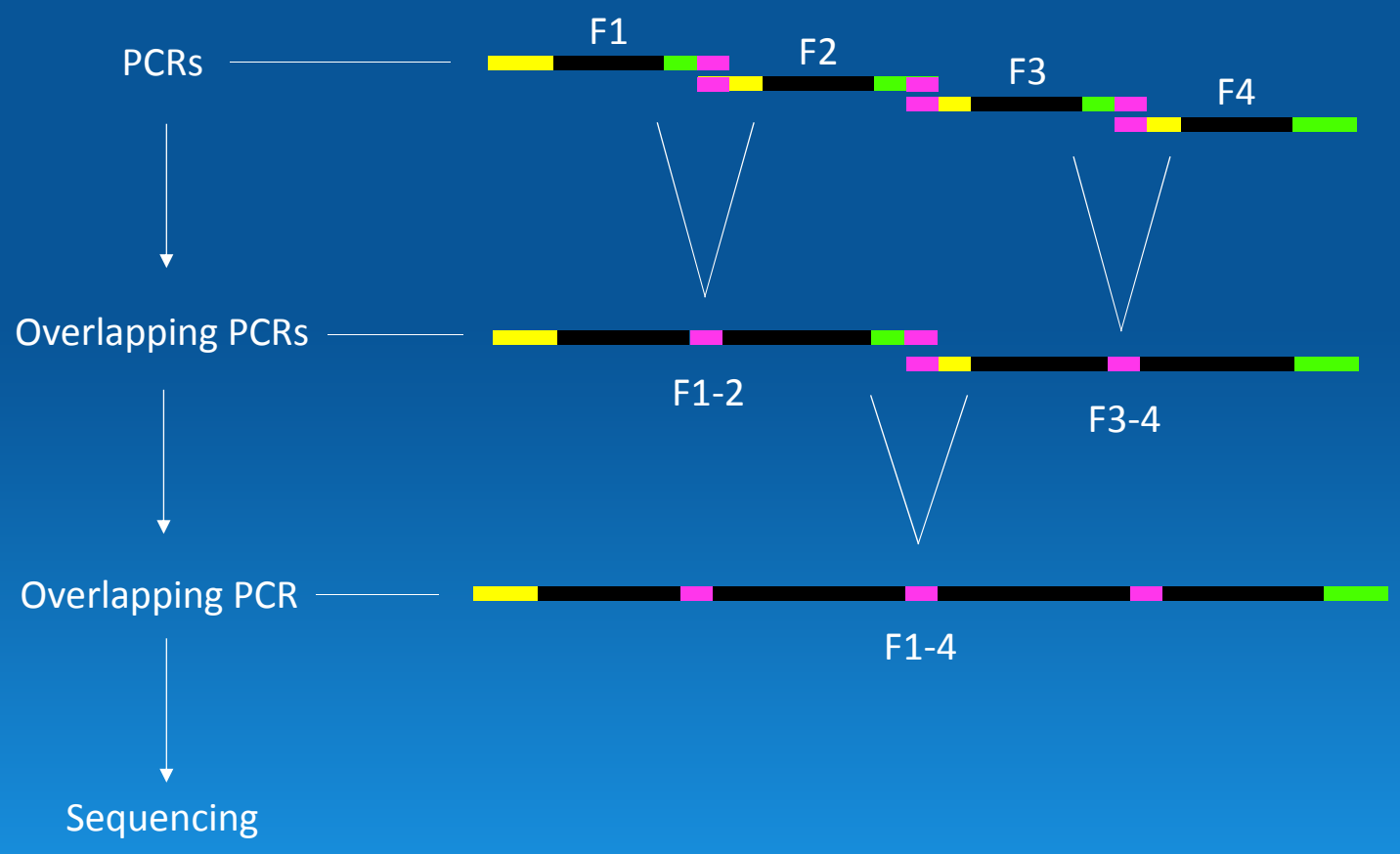
TTGGCCTGCAGGTCGTGGCGGTGAAGGCTCCAGGATTTGGGGACAACAGGAAAAACCAACTGCAGGACATGGCAGTTTCCACTGGAG
GAACCGTGTTTGGTGATGAGGCTATGGGTCTTGCTCTTGAGGATATTCAAGCTCATGACTTTGGCAAGGTCGGCGAGGTCATTGTTACT
AAAGACGACACCATGCTCCTGAAAGGCCGCGGTGATGCATCTGCCATTGAGAAGCGCGTCAATGAGATTGCAGAGCAGCTGGAGTCC
ACAAACAGTGATTATGAGAAGGAGAAACTCAACGAACGACTGGCCAAGCTCTCAGATGGAGTGGCCGTCATTAAGGTCGGAGGAACA
AGTGACGTTGAGGTGAATGAGAAGAAAGATCGTGTCACTGATGCCCTGAACGCCACTCGTGCCGCTGTGGAGGAAGGAATTGTGCCC
GGAGGAGGA

Fragment 4 (F4)

TGCCCGGAGGAGGATGTGCTTTACTGCGCTGCATCCCTGCCCTCGACAACATCAAGCCAGCCAATGCTGACCAGAAGATCGGTATCGAC
ATCATTCGCAGATCTCTTCGTATTCCTGCAATGACCATTGCTAAGAATGCTGGAGTTGAAGGCTCTCTGGTGGTGGAGAAGATCCTGCA
GAGCTCCACAGAGATTGGATATGATGCTATGAACGGAGAATATGTCAATATGGTTGAAAGAGGCATCATTGATCCCACAAAGGTGGTG
AGGACGGCACTGCTAGATGCCGCAGGTGTTGCATCTCTCTTGTCCACCGCTGAAGCTGTCGTCACCGAGATCCCCAAGGAGGAGAAGG
AAATGCCAGCTGGAGGAATGGGAGGCATGGGTGGAATGGGAGGAATGGGCGGCATGGGCTTCTAA

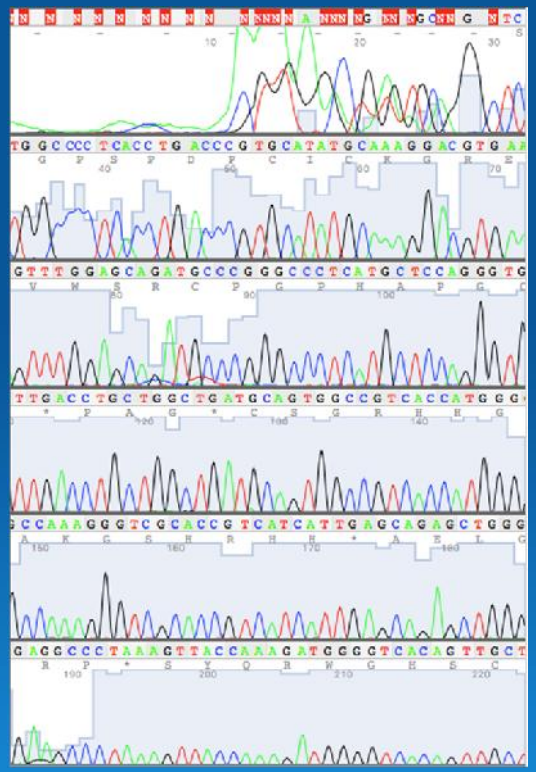
Forward Primer Sequence Reverse Primer Sequence Overlap

Procedures

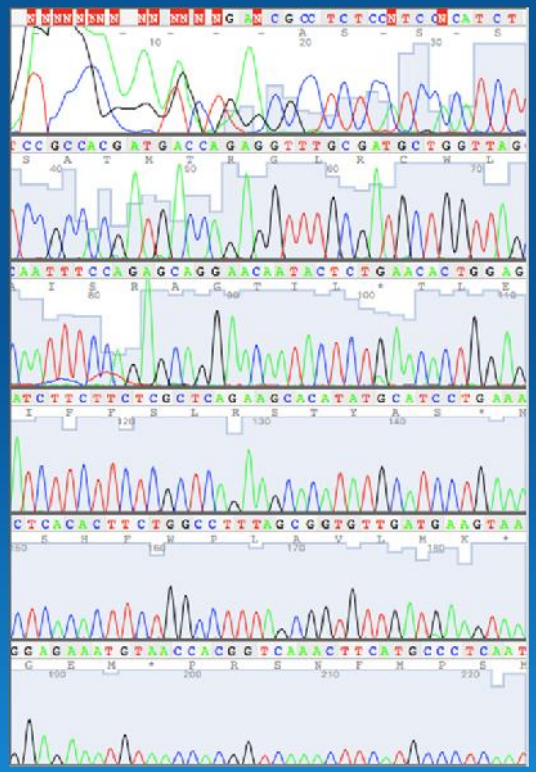

PCRs
F1
F2
F3
F4
Overlapping PCRs
F1-2
F3-4
Overlapping PCR
F1-4
Sequencing

Results

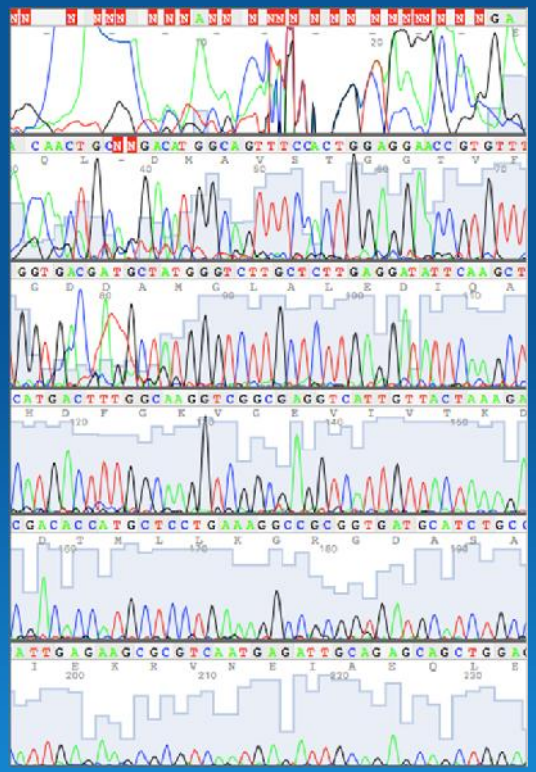
F1-4 + 1Fhsp60-FRW Primer



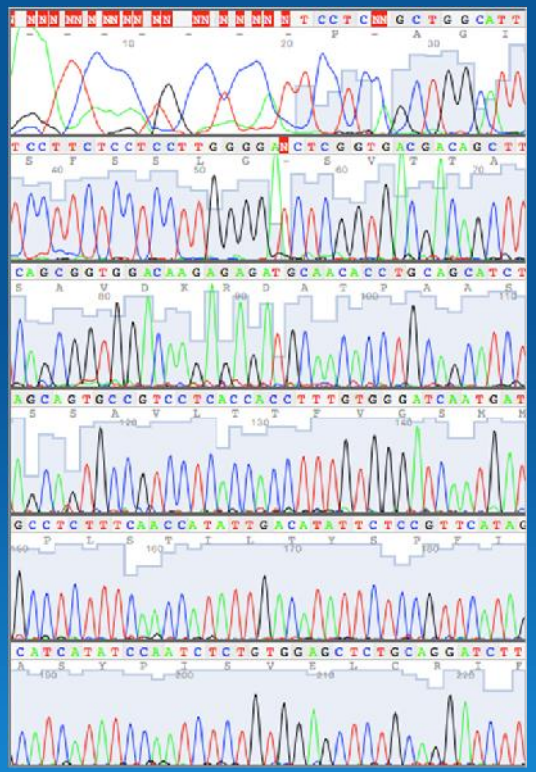
F1-4 + 2Fhsp60-REV Primer



F1-4 + 3Fhsp60-FRW Primer



F1-4 + 4Fhsp60-REV Primer



Results

Alignment of Seq_1: [Fragment 1-4] with Seq_2: [Hsp60 CS]

Similarity: 1426/1728 (82,52 %)

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Seq_1  1     ---------------------------------------------------TCTGGCCCCT  10
                                                                 ||||||||||
Seq_2  1     ATGCTGCGTTTACCCAGTGTCATGAGACAGATGAGGCCTGTGTGCAGGGCTCTGGCCCCT  60

Seq_1  11    CACCTGACCCGTGCATATGCAAAGGACGTGAAGTTTGGAGCAGATGCCCGGGCCCTCATG  70
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Seq_2  61    CACCTGACCCGTGCATATGCAAAGGATGTGAAGTTTGGAGCAGATGCCCGGGCCCTCATG  120

Seq_1  71    CTCCAGGGTGTTGACCTGCTGGCTGATGCAGTGGCCGTCACCATGGGGCCAAAGGGTCGC  130
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Seq_2  121   CTCCAGGGTGTTGACCTGCTGGCTGATGCAGTGGCCGTCACCATGGGGCCAAAGGGTCGC  180

Seq_1  131   ACCGTCATCATTGAGCAGAGCTGGGGAGGCCCTAAAGTTACCAAAGATGGGGTCACAGTT  190
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Seq_2  181   ACCGTCATCATTGAGCAGAGCTGGGGAAGCCCTAAAGTTACCAAAGATGGGGTCACAGTT  240

Seq_1  191   GCTAAAAGTATTGATCTGAAGGACAGGTATAAGAATATCGGAGCCAAGCTGGTTCAGGAT  250
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Seq_2  241   GCTAAAAGTATTGATCTAAAGGACAGGTATAAGAATATCGGAGCCAAGCTGGTTCAGGAT  300

Seq_1  251   GTGGCCAACAACACTAATGAGGAGGCTGGAGACGGCACCACCACCGCTACAGTTCTGGCC  310
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Seq_1  311   CGGGCTGTCGCCAAAGAGGGCTTCGACACCATCAACAAGGGGGCAAACCCAGTTGAGATC  370
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Seq_2  361   CGGGCTGTTGCCAAAGAGGGCTTCGACACCATCAGCAAGGGGGCAAACCCAGTTGAGATC  420

Seq_1  371   CGTAGAGGAGTCATGATGGCCGTAGAA---------------CTCAAGAAAAACTCAAAG  415
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Seq_2  421   CGTAGAGGAGTCATGATGGCCGTAGAAGAGGTCATCAGTGAACTCAAGAAAAACTCAAAG  480

Seq_1  416   CCCGTCACGACTCCAGAGGAAATCGCTCAGGTGGCTACTATTTCTGCCAATGGAGACACT  475
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Seq_2  481   CCCGTCACGACTCCAGAGGAAATCGCTCAGGTGGCTACTATTTCTGCCAATGGAGACACT  540

Seq_1  476   GAAGTTGGTAACATCATCTCCAATGCTATGAAGAAAGTCGGTCGTAAGGGTGTCATTACA  535
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Seq_2  541   GAAGTTGGTAACATCATCTCCAATGCTATGAAGAAAGTCGGTCGTAAGGGTGTCATTACA  600

Seq_1  536   GTGAAGGATGGCAAAACCCTTCATGATGAGCTTGAGATCATTGAGGGCATGAAGTTTGAC  595
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Seq_2  601   GTGAAGGATGGCAAAACCCTTCATGATGAGCTTGAGATCATTGAGGGCATGAAGTTTGAC  660

Seq_1  596   CGTGGTTACATTTCTCCTTACTTCATCAACACCGCTAAAGGCCAGAAGTGTGAGTTTCAG  655
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Seq_2  661   CGTGGTTACATTTCTCCTTACTTCATCAACACCGCTAAAGGCCAGAAGTGTGAGTTTCAG  720

Seq_1  656   GATGCATATGTGCTTCTGAGCGAGAAGAAGATCTCCAGTGTTCAGAGTATTGTTCCTGCT  715
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Seq_2  721   GATGCATATGTGCTTCTGAGCGAGAAGAAGATCTCCAGTGTTCAGAGTATTGTTCCTGCT  780

Seq_1  716   CTGGAAATTGCTAACCAGCATCGCAAACCTCTGGTCATCGTGGCGGAAGATG--------  767
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Seq_2  781   CTGGAAATTGCTAACCAGCATCGCAAACCTCTGGTCATCGTGGCGGAAGATGTGGATGGA  840

Seq_1  768   ------------------------------------------------------------  767

Seq_2  841   GAGGCGCTCAGCACTCTGGTCCTCAACAGGTTGAAGGTTGGCCTGCAGGTCGTGGCGGTG  900

Seq_1  768   ----------------------------------------GACATGGCAGTTTCCACT  785
                                                     ||||||||||||||||||
Seq_2  901   AAGGCTCCAGGATTTGGGGACAACAGGAAAAACCAACTGCAGGACATGGCAGTTTCCACT  960

Seq_1  786   GGAGGAACCGTGTTTGGTGACGATGCTATGGGTCTTGCTCTTGAGGATATTCAAGCTCAT  845
             |||||||||||||||||||| || |||||||||||||||||||||||||||||||||||||
Seq_2  961   GGAGGAACCGTGTTTGGTGATGAGGCTATGGGTCTTGCTCTTGAGGATATTCAAGCTCAT  1020

Seq_1  846   GACTTTGGCAAGGTCGGCGAGGTCATTGTTACTAAAGACGACACCATGCTCCTGAAAGGC  905
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Seq_2  1021  GACTTTGGCAAGGTCGGCGAGGTCATTGTTACTAAAGACGACACCATGCTCCTGAAAGGC  1080

Seq_1  906   CGCGGTGATGCATCTGCCATTGAGAAGCGCGTCAATGAGATTGCAGAGCAGCTGGAGTCC  965
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Seq_2  1081  CGCGGTGATGCATCTGCCATTGAGAAGCGCGTCAATGAGATTGCAGAGCAGCTGGAGTCC  1140

Seq_1  966   ACAAACAGTGATTATGAGAAGGAGAAACTCAACAAACGACTGGCCAAGCTCTCAGATGGA  1025
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Seq_2  1141  ACAAACAGTGATTATGAGAAGGAGAAACTCAACGAACGACTGGCCAAGCTCTCAGATGGA  1200

Seq_1  1026  GTGGCCGTCATTAAGGTCGGAGGAACAAATGAC-TTGAGGTGAATGA-------------  1071
             |||||||||||||||||||||||||||||| |||| |||||||||||||
Seq_2  1201  GTGGCCGTCATTAAGGTCGGAGGAACAAGTGACGTTGAGGTGAATGAGAAGAAAGATCGT  1260

Seq_1  1072  ---------------------------------------------------TGCCCGGAGGA  1082
                                                               |||||||||||
Seq_2  1261  GTCACTGATGCCCTGAACGCCACTCGTGCCGCTGTGGAGGAAGGAATTGTGCCCGGAGGA  1320

Seq_1  1083  GGATGTGCTTTACTGCGCTGCATCCCTGCCCTCGACAACATCAAGCCAGCCAATGCTGAC  1142
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Seq_2  1321  GGATGTGCTTTACTGCGCTGCATCCCTGCCCTCGACAACATCAAGCCAGCCAATGCTGAC  1380

Seq_1  1143  CAGAAGATCGGTATCGACATCATTCGCAGATCTCTTCGTATTCCTGCAATGACCATTGCT  1202
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Seq_2  1381  CAGAAGATCGGTATCGACATCATTCGCAGATCTCTTCGTATTCCTGCAATGACCATTGCT  1440

Seq_1  1203  AAGAATGCTGGAGTTGAAGGCTCGCTGGTGGTGGAGAAGATCCTGCAGAGCTCCACAGAG  1262
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Seq_2  1441  AAGAATGCTGGAGTTGAAGGCTCTCTGGTGGTGGAGAAGATCCTGCAGAGCTCCACAGAG  1500

Seq_1  1263  ATTGGATATGATGCTATGAACGGAGAATATGTCAATATGGTTGAAAGAGGCATCATTGAT  1322
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Seq_2  1501  ATTGGATATGATGCTATGAACGGAGAATATGTCAATATGGTTGAAAGAGGCATCATTGAT  1560

Seq_1  1323  CCCACAAAGGTGGTGAGGACGGCACTGCTAGATGCTGCAGGTGTTGCATCTCTCTTGTCC  1382
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Seq_2  1561  CCCACAAAGGTGGTGAGGACGGCACTGCTAGATGCCGCAGGTGTTGCATCTCTCTTGTCC  1620

Seq_1  1383  ACCGCTGAAGCTGTCGTCACCGAG-TCCCCAAGGAGGAGAAGGAAATGCCAGC--GAGGA  1439
             |||||||||||||||||||||||| |||||||||||||||||||||||||||||  |||||
Seq_2  1621  ACCGCTGAAGCTGTCGTCACCGAGATCCCCAAGGAGGAGAAGGAAATGCCAGCTGGAGGA  1680

Seq_1  1440  ----------------------------------------------  1439

Seq_2  1681  ATGGGAGGCATGGGTGGAATGGGAGGAATGGGCGGCATGGGCTTCTAA  1728
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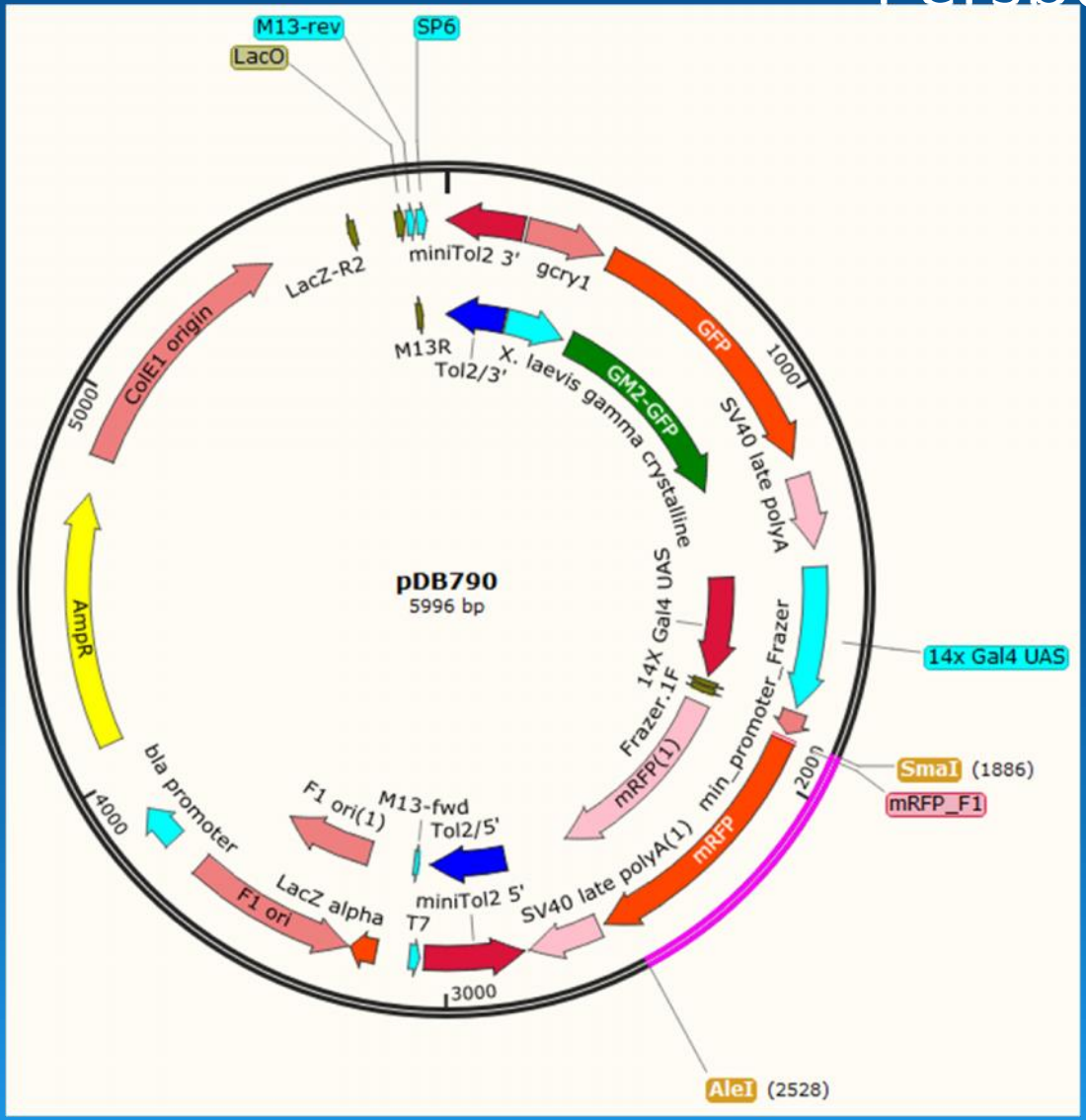
Future Perspectives



Hsp60 CS Fragment

pDB790 Plasmid

Digestion
with Sma I and Ale I
Restriction Enzymes

Digested Hsp60 Fragment + Digested pDB790 Plasmid

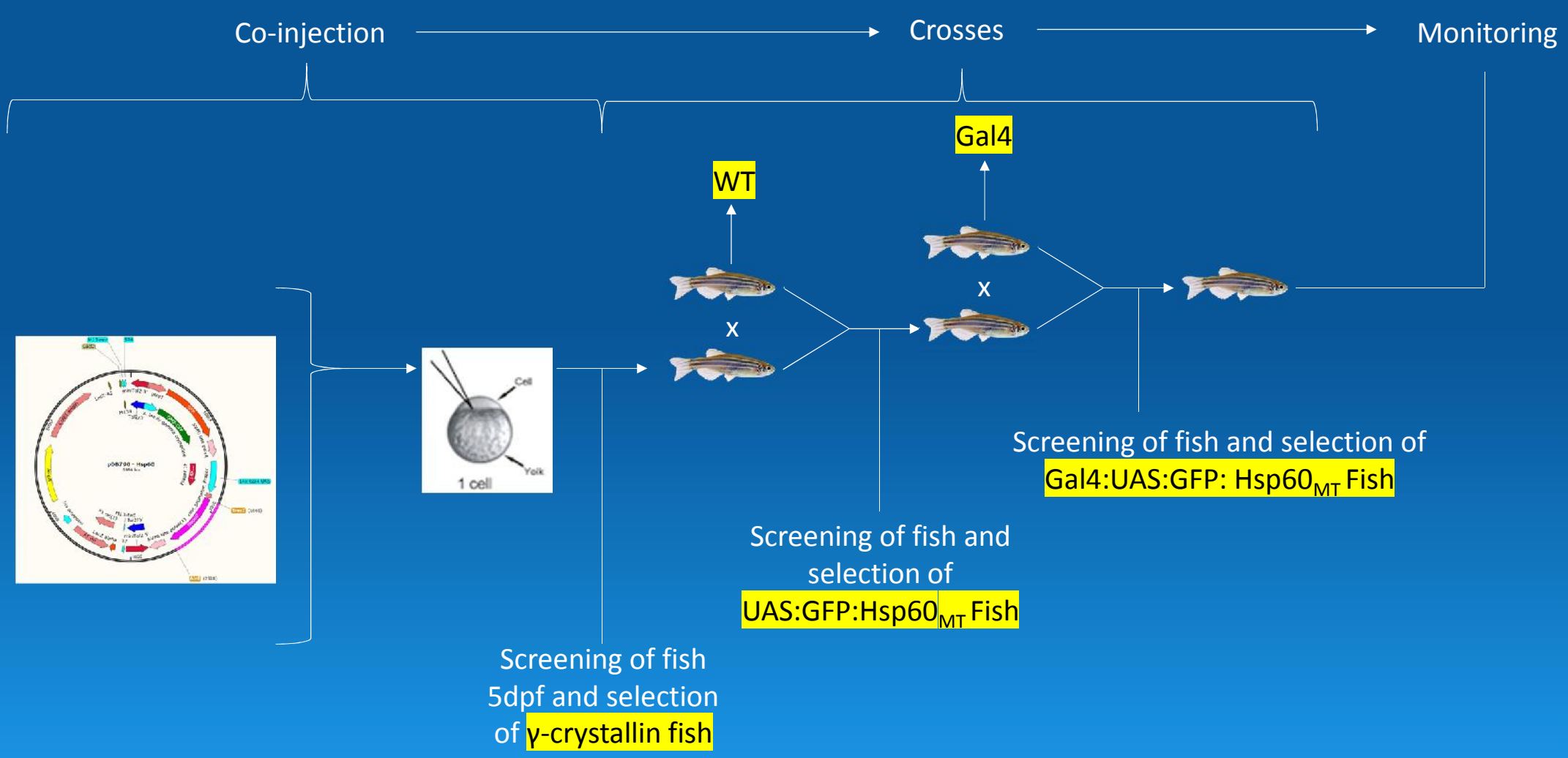
Ligation

Recombinant DNA Molecule

Bacterium

Transformation

Future Perspectives



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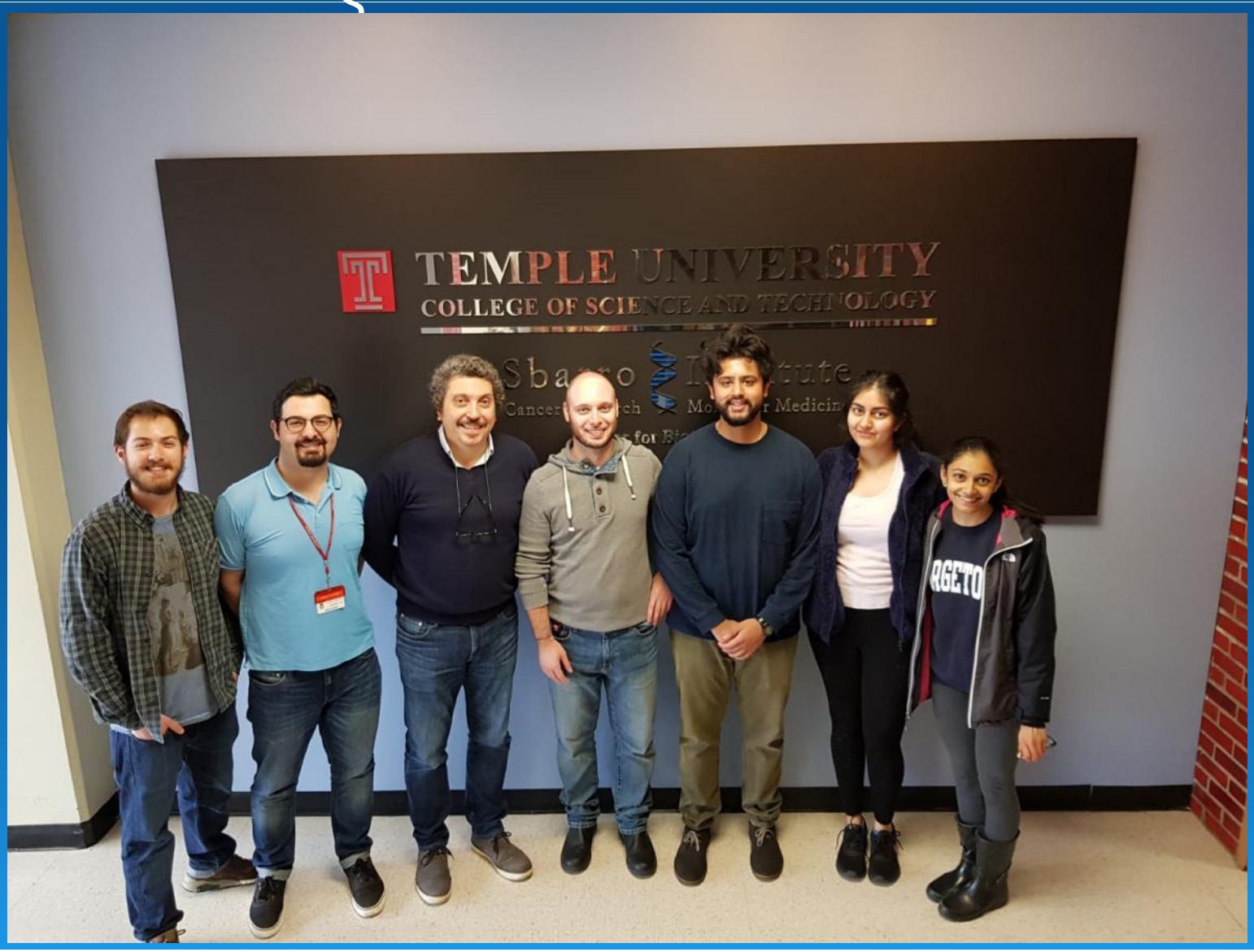
- Marco Pelosi
- Mandeep Shergill
- Pooja Kaneria
- Pooja Parikh



References

- Hansen JJ, Dürr A, Cournu-Rebeix I, Georgopoulos C, Ang D, Nielsen MN, Davoine CS, Brice A, Fontaine B, Gregersen N, Bross P., 2002. Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60.

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