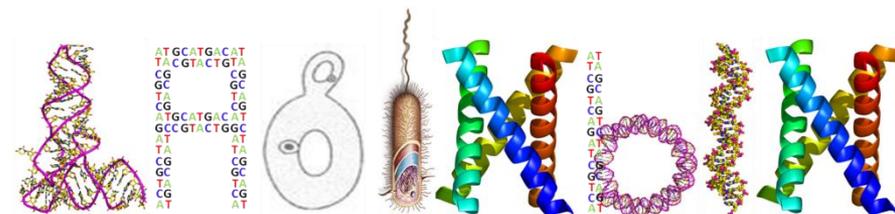
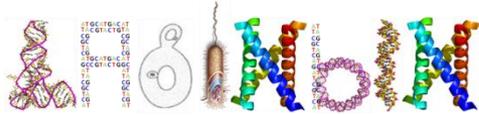




Principais estratégias de biologia molecular para viabilizar o emprego de biomoléculas oriundas de micro-organismos extremófilos como biofármacos, bem como para o aprimoramento dessas biomoléculas

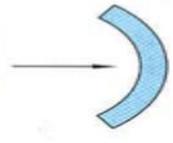
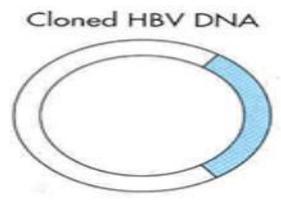
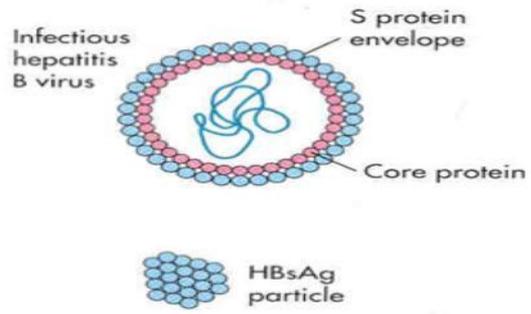
Profa Dra Gisele Monteiro
FBT/FCF/USP



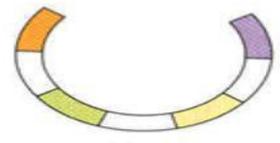


Quando usar organismo geneticamente modificado?

- [MO patogênicos](#)
- Fonte rara ou perigosa
- Rendimento baixo no MO original
- Difícil condição de crescimento e produção
- Produto tóxico para o MO original quando em altas concentrações
- Plantas de produção já estabelecidas
- Potencial de **criar** moléculas que não existiriam na natureza – *biobettters*.

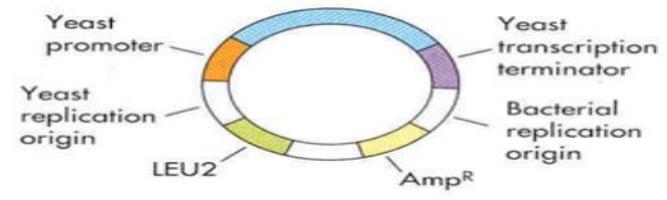


Isolate HBsAg coding sequence

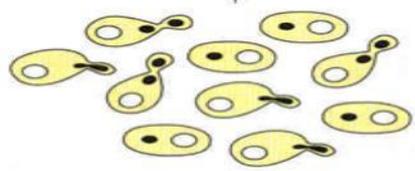


Yeast expression vector

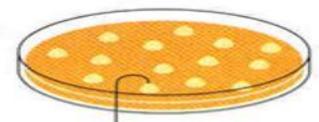
Ligate



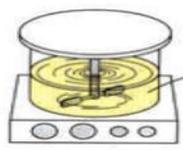
Transform yeast cells



Select cells that contain plasmid by growth on medium lacking leucine



Culture cells in a fermentor



Isolate cells by centrifugation

Break open yeast cells



Purify HBsAg particles

U\$10/dose

U\$0,23/dose

MO patogênicos

Expressão de proteínas antigênicas do vírus da hepatite B - produção de vacina em levedura



Quando usar organismo geneticamente modificado?

MO patogênicos •

Fonte rara ou perigosa •

Rendimento baixo no MO original •

Difícil condição de crescimento e produção •

Produto tóxico para o MO original quando em altas concentrações •

Plantas de produção já estabelecidas •

Potencial de **criar** moléculas que não existiriam na natureza – •

biobetters.



Enzimas e medicamentos

| Enzima | Fonte | Utilização |
|----------------|--------------------------|---|
| Amilase | Pâncreas bovino ou suíno | Auxiliar digestivo |
| Asparaginase | Fúngica Microbiana | Leucemia |
| Bromelina | Abacaxi | Auxiliar digestivo |
| Celulase | Fúngica | Auxiliar digestivo Debridamento de feridas |
| Papaína | Mamão | Hérnia de disco |
| Quimiotripsina | Mamão | Agente dispersante |
| Hialuronidase | Testículos de bovinos | Infecções |
| Lisozima | Clara de ovo | |
| Pancreatina | Pâncreas bovino ou suíno | Insuficiência pancreática |
| Uroquinase | Urina humana | Agente fibrinolítico |
| | Cultura de células | |



Quando usar organismo geneticamente modificado?

MO patogênicos •

Fonte rara ou perigosa •

Rendimento baixo no MO original •

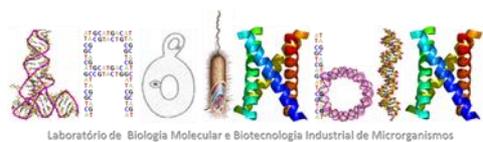
Difícil condição de crescimento e produção •

Produto tóxico para o MO original quando em altas concentrações •

Plantas de produção já estabelecidas •

Potencial de **criar** moléculas que não existiriam na natureza – •

biobettters.

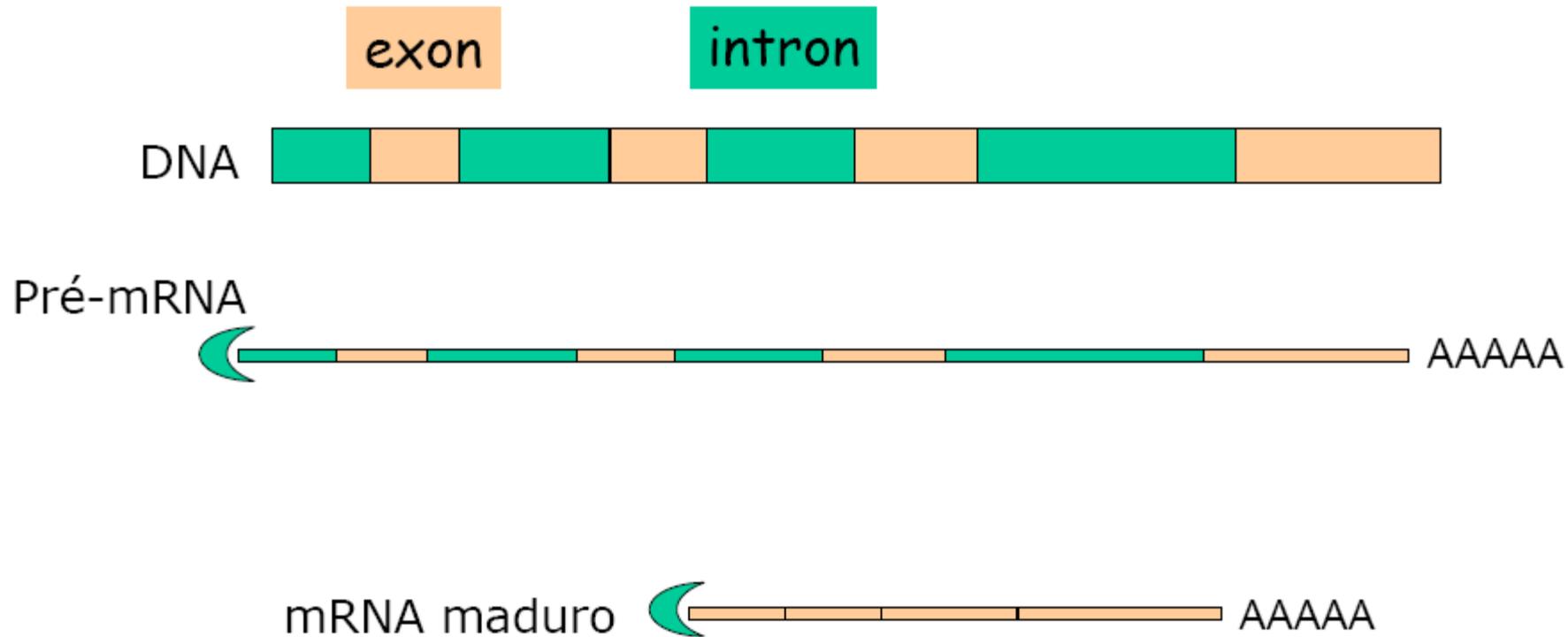


Preciso usar OGMs - estratégias

- Escolher a fonte do material genético
- Escolher a célula hospedeira
- Escolher o tipo de transformação
 - Seleção x estabilidade x quantidade

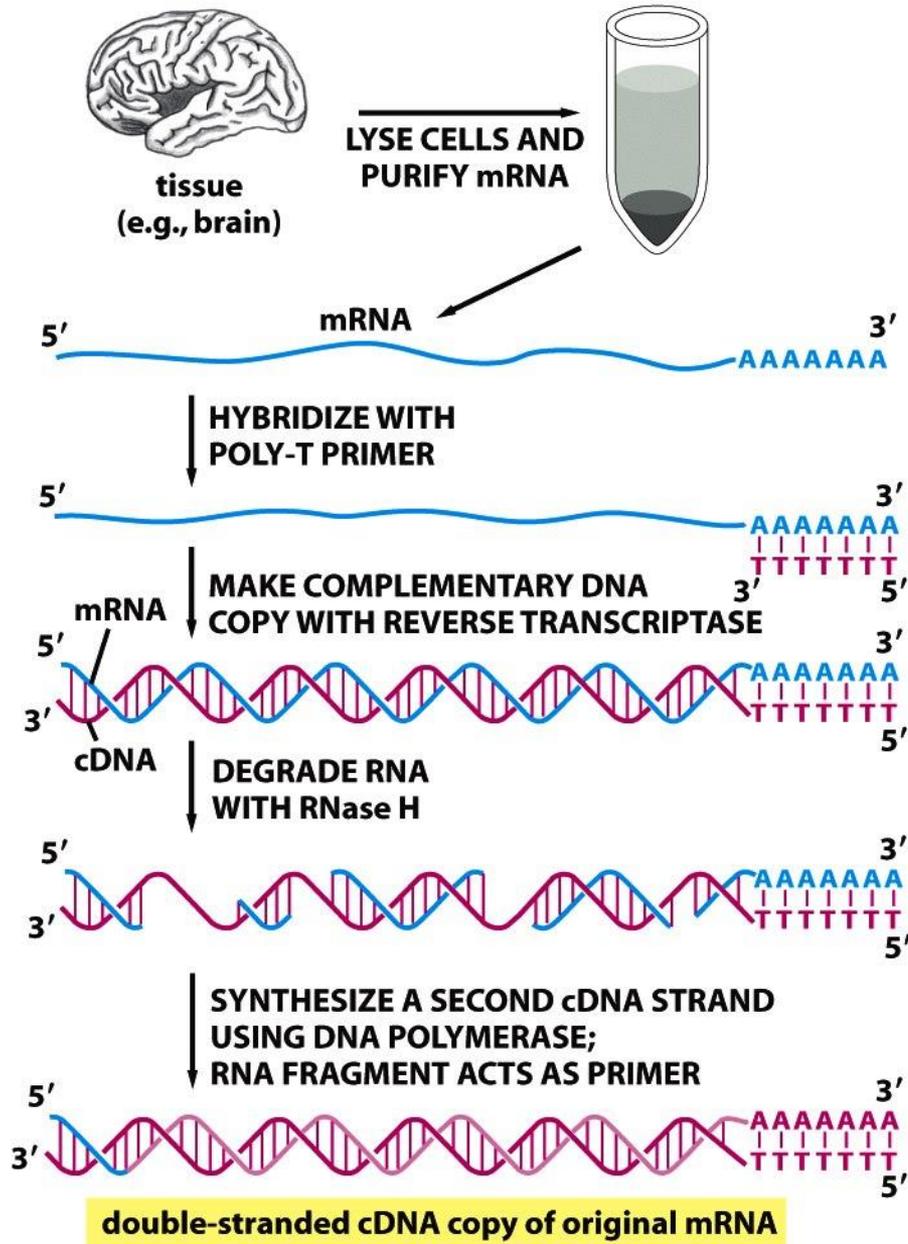


GENES DE EUCARIOTOS SÃO MAIS COMPLEXOS QUE OS DE PROCARIOTOS



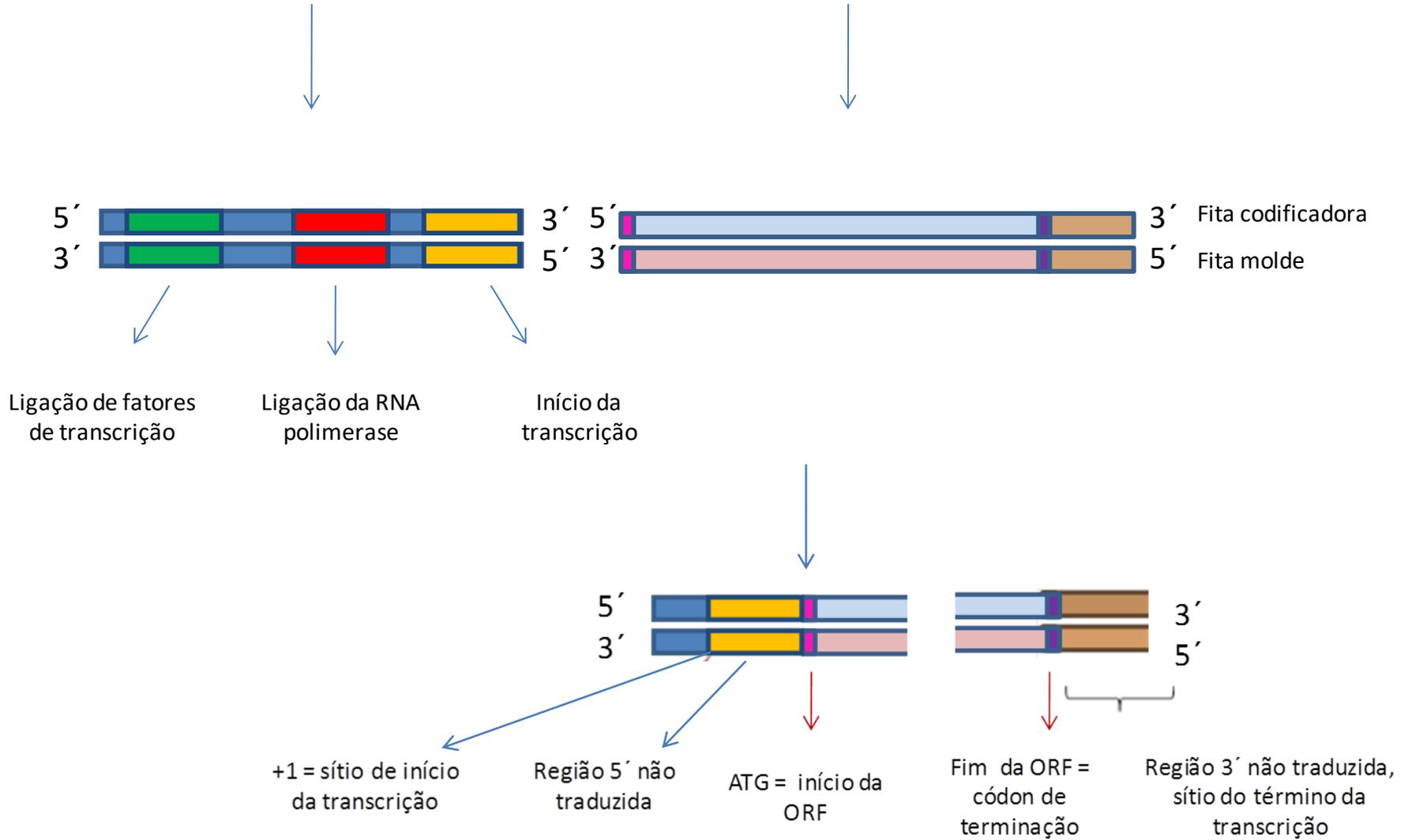
O RNA MENSAGEIRO MADURO É MENOR QUE O DNA QUE DEU ORIGEM A ELE

Transcriptase reversa



Região Promotora ou regulatória

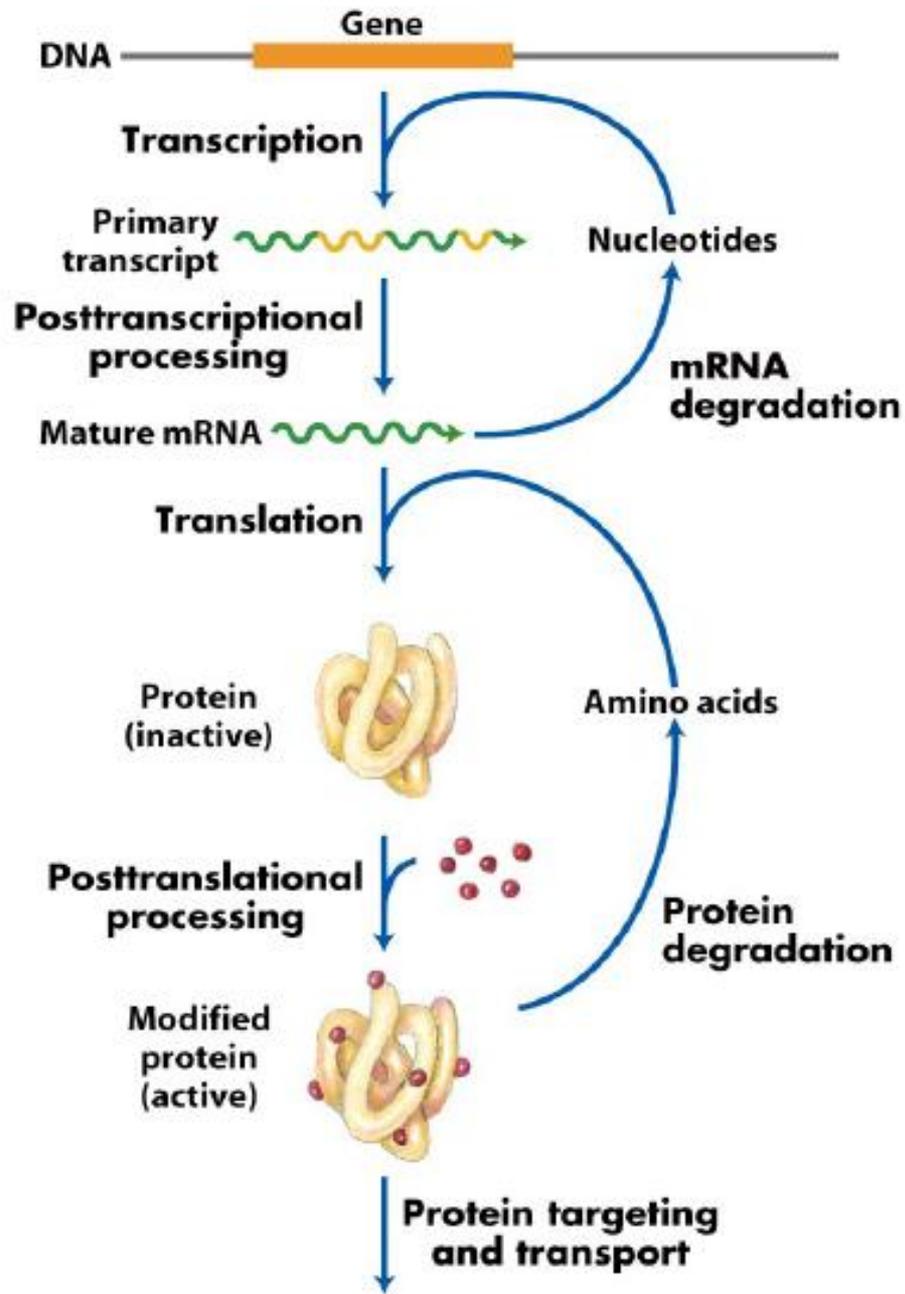
Região Estrutural ou codificadora



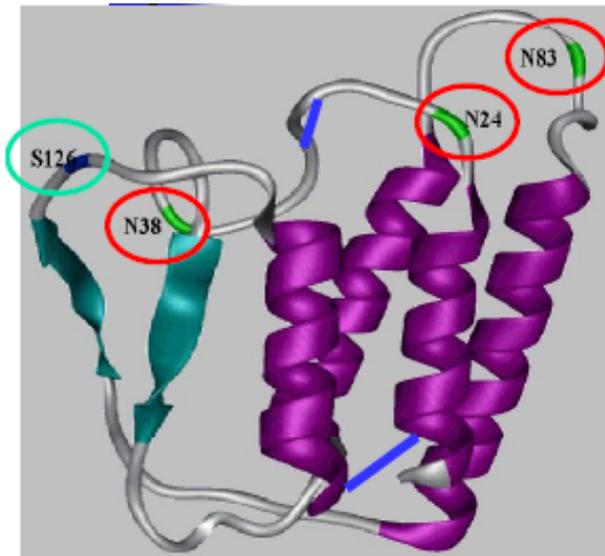


Preciso usar OGMs - estratégias

- Escolher a fonte do material genético
- Escolher a célula hospedeira
- Escolher o tipo de transformação
 - Seleção x estabilidade x quantidade

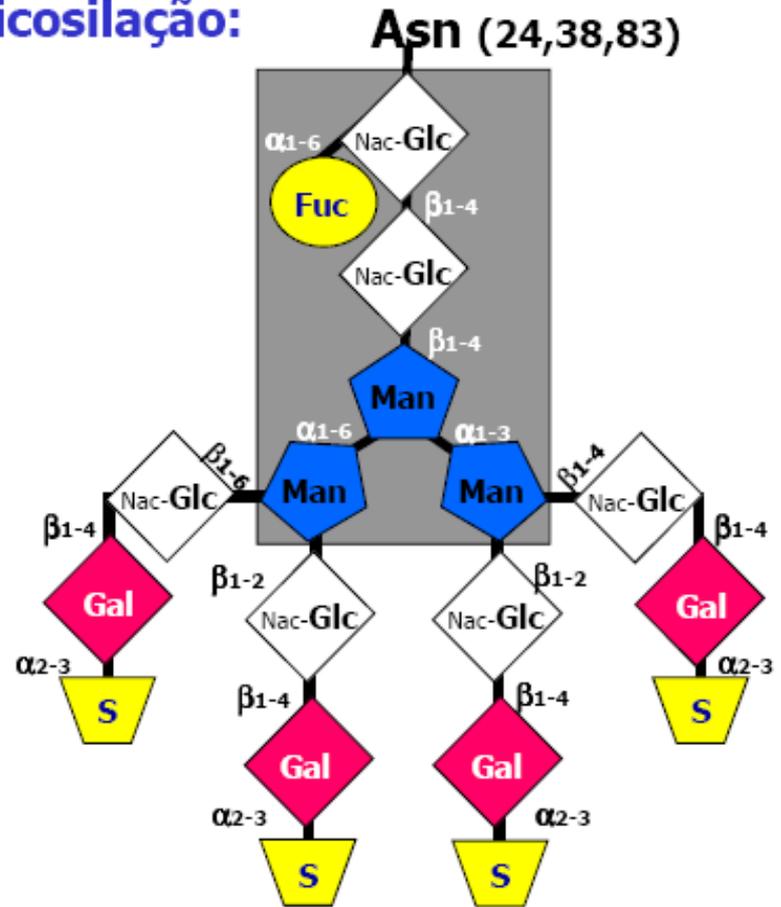


Eritropoietina (EPO)

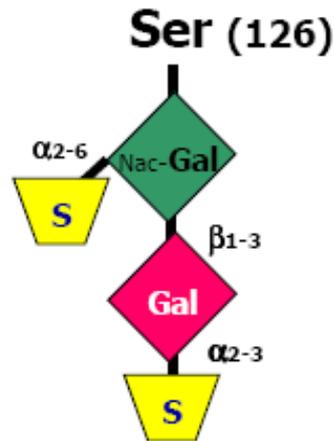


Eritropoietina

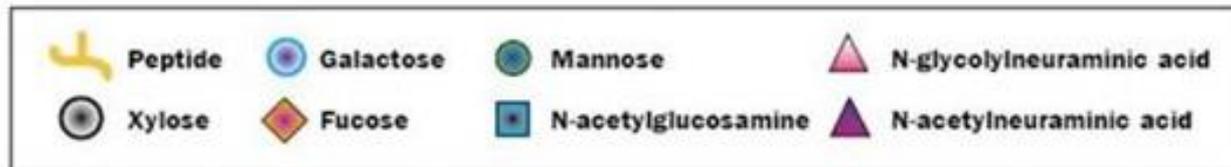
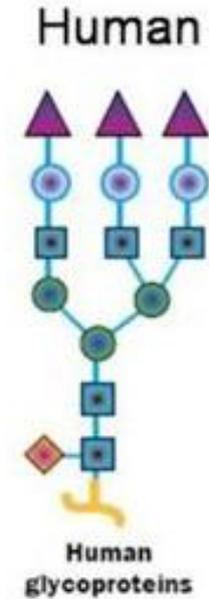
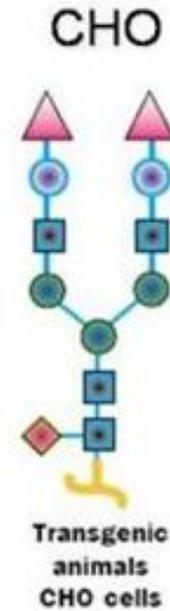
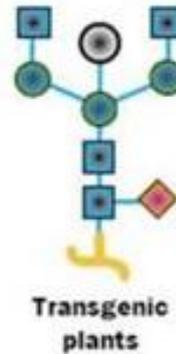
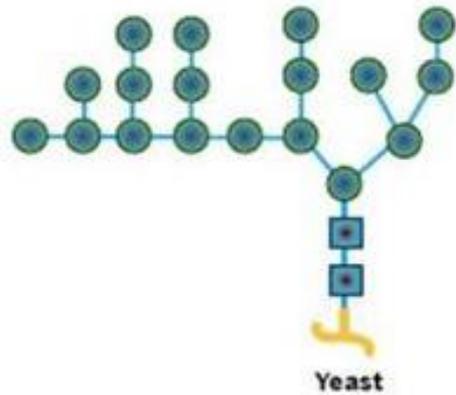
N-Glicosilação:



O-Glicosilação:



(Adapted from Pharmaceutical visions)



Leveduras geneticamente modificadas para produzir padrão de glicosilação humanizado.



Preciso usar OGMs - estratégias

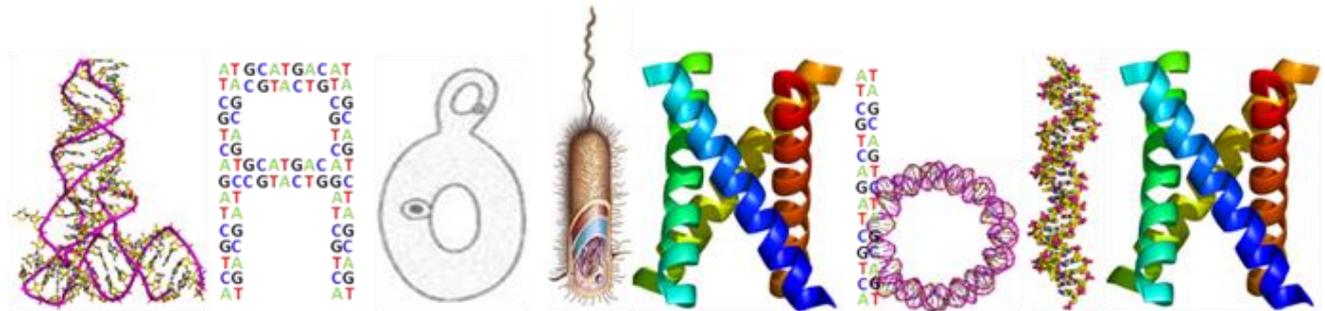
- Escolher a fonte do material genético
- Escolher a célula hospedeira
- Escolher o tipo de transformação
 - Seleção x estabilidade x quantidade



Transformação

- Plasmídeo epissomal
 - Muitas cópias por célula
 - Exige seleção constante
 - Marcas de seleção – antibióticos (aspectos regulatórios)
- Integrativo no DNA genômico
 - Estável
 - Uma ou poucas cópias
 - Seleção apenas 1 vez
 - Integrar em locais que diminuem a produtividade

Principais estratégias de biologia molecular para o desenvolvimento de *biobetters*



Qual objetivo de bioprospecções?



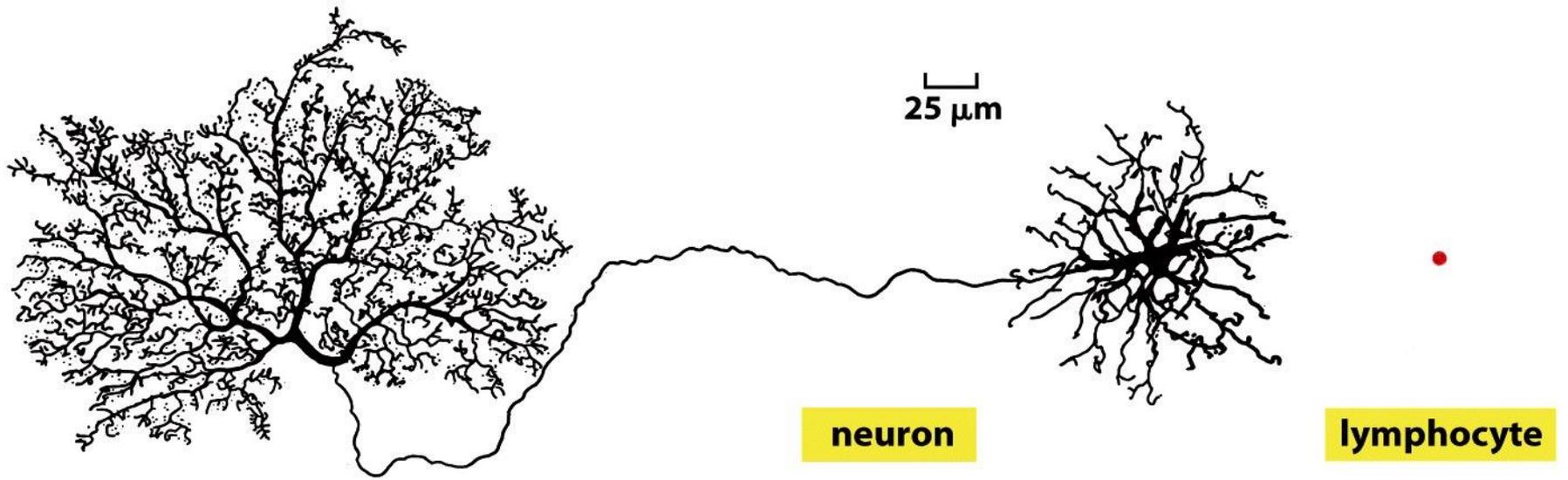


Figure 7-1 *Molecular Biology of the Cell* (© Garland Science 2008)



Ex. Asparaginases

UniProtKB Advanced

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Other organisms

Search terms

Filter "asparaginase" as:
gene name (3)

gene ontology (21,154)

protein and gene name (26,468)

| <input type="checkbox"/> | Entry | Entry name | Protein names | Gene names | Organism | Length |
|--------------------------|--------|-------------|---|-------------------------|---|--------|
| <input type="checkbox"/> | Q7L266 | ASGL1_HUMAN | Isoaspartyl peptidase/L-asparaginase... | ASRGL1 ALP, CRASH | Homo sapiens (Human) | 308 |
| <input type="checkbox"/> | Q8VI04 | ASGL1_RAT | Isoaspartyl peptidase/L-asparaginase... | Asrgl1 Alp, Gliap, Hiob | Rattus norvegicus (Rat) | 333 |
| <input type="checkbox"/> | P00805 | ASPG2_ECOLI | L-asparaginase 2 | ansB b2957, JW2924 | Escherichia coli (strain K12) | 348 |
| <input type="checkbox"/> | P0CX77 | ASP22_YEAST | L-asparaginase 2-2 | ASP-2 YLR157C, L9632.7 | Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast) | 362 |
| <input type="checkbox"/> | P0CX79 | ASP24_YEAST | L-asparaginase 2-4 | ASP3-4 YLR160C, L9632.9 | Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast) | 362 |
| <input type="checkbox"/> | P0CZ17 | ASP21_YEAST | L-asparaginase 2-1 | ASP3-1 YLR155C, L9632.6 | Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast) | 362 |
| <input type="checkbox"/> | P0CX78 | ASP23_YEAST | L-asparaginase 2-3 | ASP3-3 YLR158C, L9632.8 | Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast) | 362 |
| <input type="checkbox"/> | P0A962 | ASPG1_ECOLI | L-asparaginase 1 | ansA b1767, JW1756 | Escherichia coli (strain K12) | 338 |
| <input type="checkbox"/> | Q8YQB1 | ASGX_NOSSI | Isoaspartyl peptidase/L-asparaginase... | all3922 | Nostoc sp. (strain PCC 7120 / SAG 25.82 / UTEX 2576) | 318 |
| <input type="checkbox"/> | P74383 | ASGX_SYNY3 | Isoaspartyl peptidase/L-asparaginase... | sl0422 | Synechocystis sp. (strain PCC 6803 / Kazusa) | 329 |
| <input type="checkbox"/> | Q9ZSD6 | ASPG_LUPLU | Isoaspartyl peptidase/L-asparaginase... | | Lupinus luteus (European yellow lupine) | 325 |
| <input type="checkbox"/> | O88202 | LPP60_RAT | 60 kDa lysophospholipase | Aspg | Rattus norvegicus (Rat) | 564 |



Ex. Asparaginases: asnA x asnB

Alignments

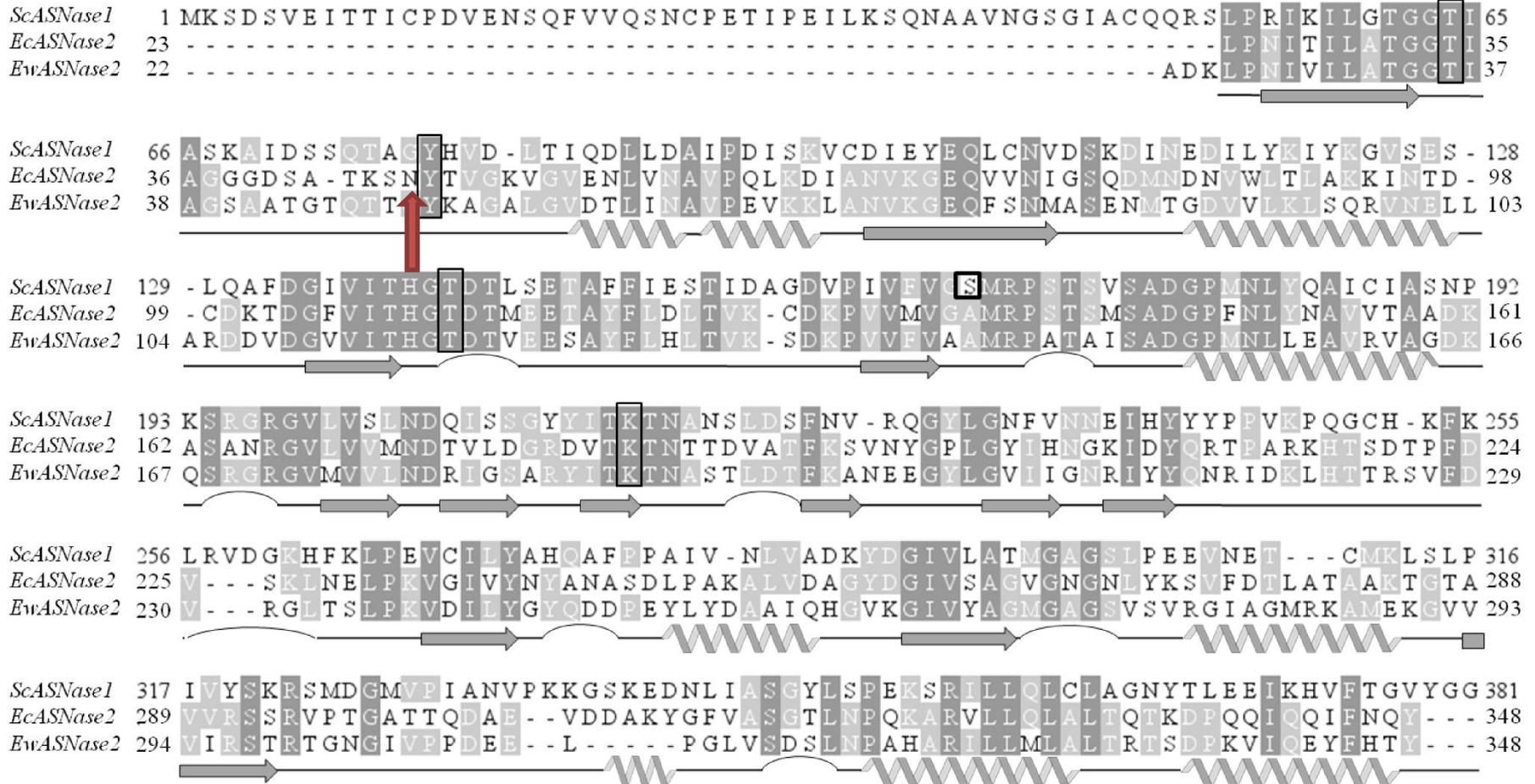
Download Graphics

sp|P0A962|ASPG1_ECOLI L-asparaginase 1 OS=Escherichia coli (strain K12) OX=83333 GN=ansA PE=1 SV=1
 Sequence ID: Query_42753 Length: 338 Number of Matches: 1

Range 1: 60 to 328 Graphics Next Match Previous Match

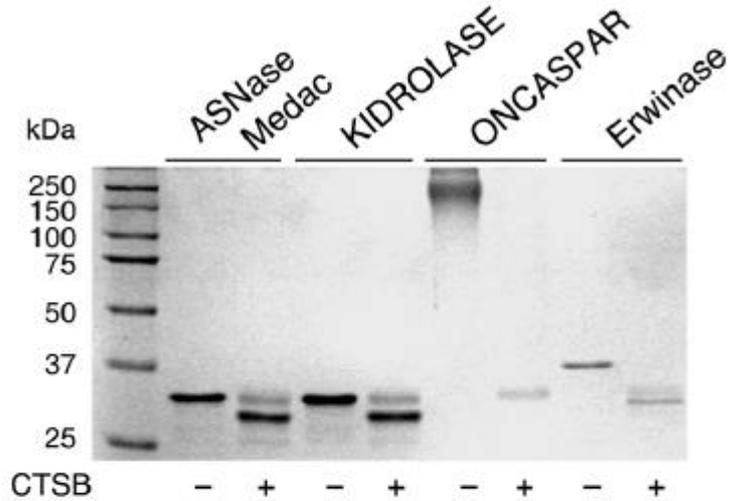
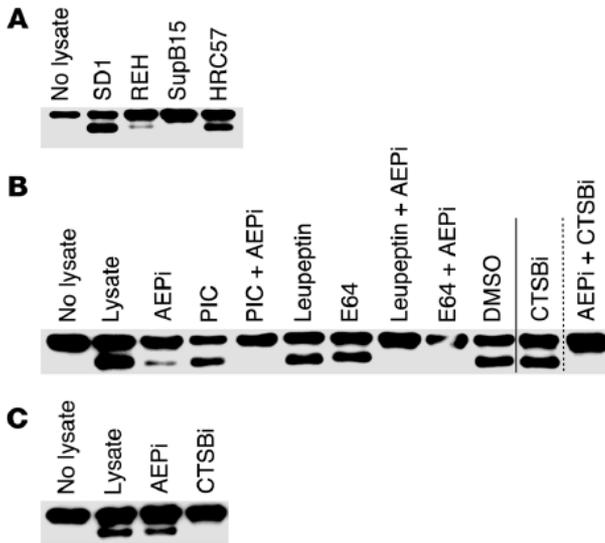
| Score | Expect | Method | Identities | Positives | Gaps |
|----------------|--|--|-------------|--------------|------------|
| 81.6 bits(200) | 6e-22 | Compositional matrix adjust. | 79/282(28%) | 116/282(41%) | 27/282(9%) |
| Query 80 | SQDMNDNVWLT | LAKKINTDCDKTIDGFVITHGIDTMEETAYFLDLTVK-CDKPVVMVGAMR | 138 | | |
| Sbjct 60 | SSDMPEDWQHIAEDIKAHYDDYDGFVILHGTDMAYTASALSFMLENLGKPVIVIGSQI | 119 | | | |
| Query 139 | PSTSMSADGPFNLYNAVVTAAADKASANRGLVVMNDTVLDGRDVTKTNTTDVATFKSVNY | 198 | | | |
| Sbjct 120 | PLAELRSDGQINLLNALYVAANYPIKE--VTLFFNNRLYRGNRTTKAHADGFDAFASPNL | 177 | | | |
| Query 199 | GPL--GYIHNGKIDYQRTIPARKHTSDTPFDVSKLNLKPKVGVYNYANAS-----DL | 248 | | | |
| Sbjct 178 | PPLLEAGIHIRRLN---TPPH-GEDELIVHPITPQP-IGVVTIYPGISADVVRNFLRQ | 232 | | | |
| Query 249 | PAKALVDAGYDGIVSAGVGNGLYKSVFDTLATAAKTGTAVVRSRVPITGATTQDAEVDD | 308 | | | |
| Sbjct 233 | PVKALI-----LRSYGVGNAPQNKAFLELQEQEASDRGIVVVNLTQCMGKVNMGGYATG | 286 | | | |
| Query 309 | ---AKYGFVASGTLNPPQKARVLLQLALTQTKDPQQIQQIFNQ | 347 | | | |
| Sbjct 287 | NALAHAGVIGGADMTVEATLTKLHYLLSQELDTETIRKAMSQ | 328 | | | |

Asparaginases tipo II

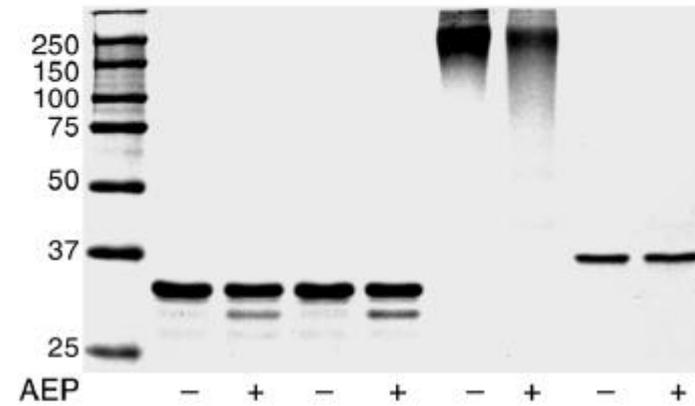


Mutação racional

research article



| | | | |
|----------|-----|-----------------|-----|
| ASNase | 17 | GDSATK-SNYTVGK | 29 |
| | | :::T: :.Y..G | |
| Erwinase | 17 | AATGTQTTGYKAGA | 30 |
| ASNase | 119 | TSMSADGPFNLYNA | 132 |
| | | T::SADGP.NL :A | |
| Erwinase | 122 | TAISADGPMNLLEA | 135 |
| ASNase | 136 | AADKASANRGVLLVV | 149 |
| | | A:DK.S .RGV:VV | |
| Erwinase | 139 | ASDKQSRGRGVMVV | 152 |



N24*

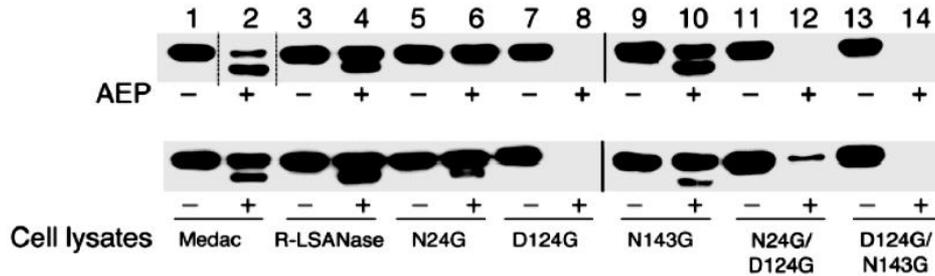
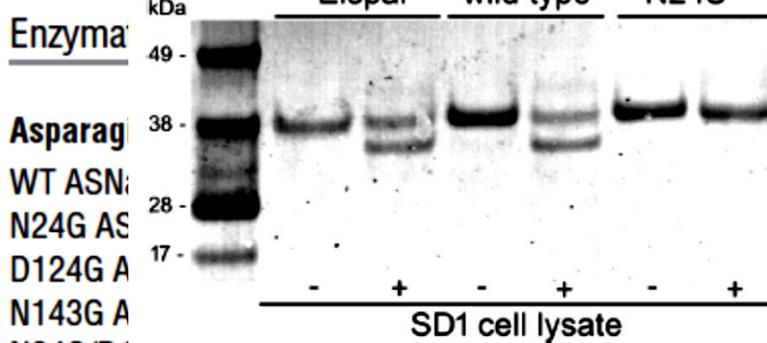


Figure 6

An N24G mutant resists AEP cleavage. Immunoblots of commercial (Medac) and recombinant WT (R-LASNase) and mutant ASNase proteins incubated with (+) and without (-) recombinant AEP (0.4 μg) or whole-cell lysates (HRC57). N24G is resistant to AEP cleavage. D124 mutants are degraded completely by AEP and cellular lysates. Vertical lines demarcate different gels; dashed vertical lines denote spliced noncontiguous lanes within gels.

Table

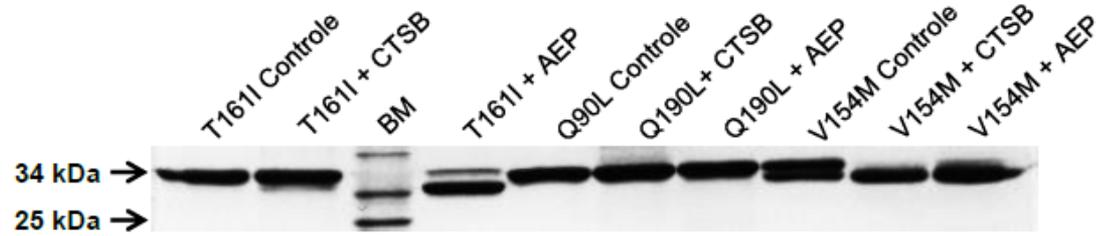
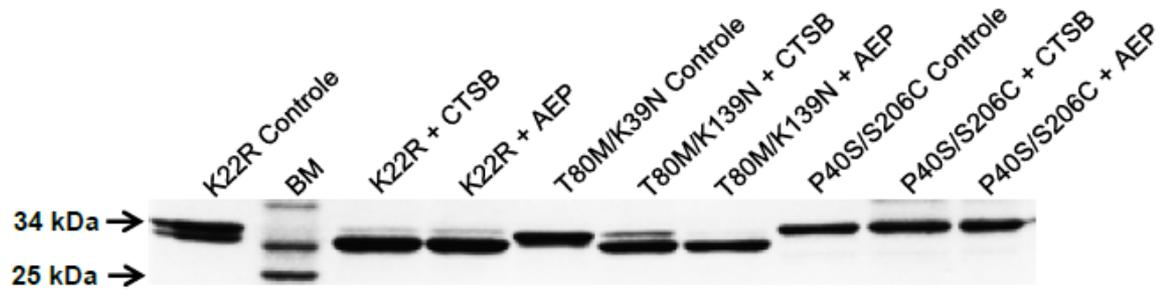
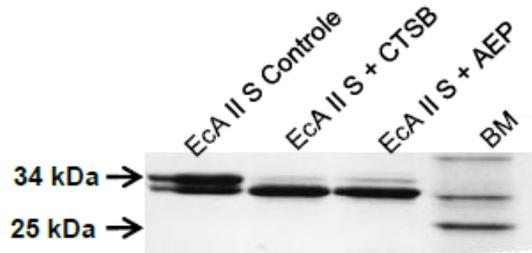


| Variant | Specific Activity (U/mg) | |
|-----------|--------------------------|-------------|
| | L-Asn | L-Gln |
| Wild-type | 105.20 ± 8.14 | 0.89 ± 0.22 |
| N24S | 103.50 ± 10.23 | 0.93 ± 0.20 |

Figure 4. Elspar, EcAII WT and N24S degradation by SD1 cell lysate (Ponceau Red staining). +/- indicates the presence or absence of cell lysate.

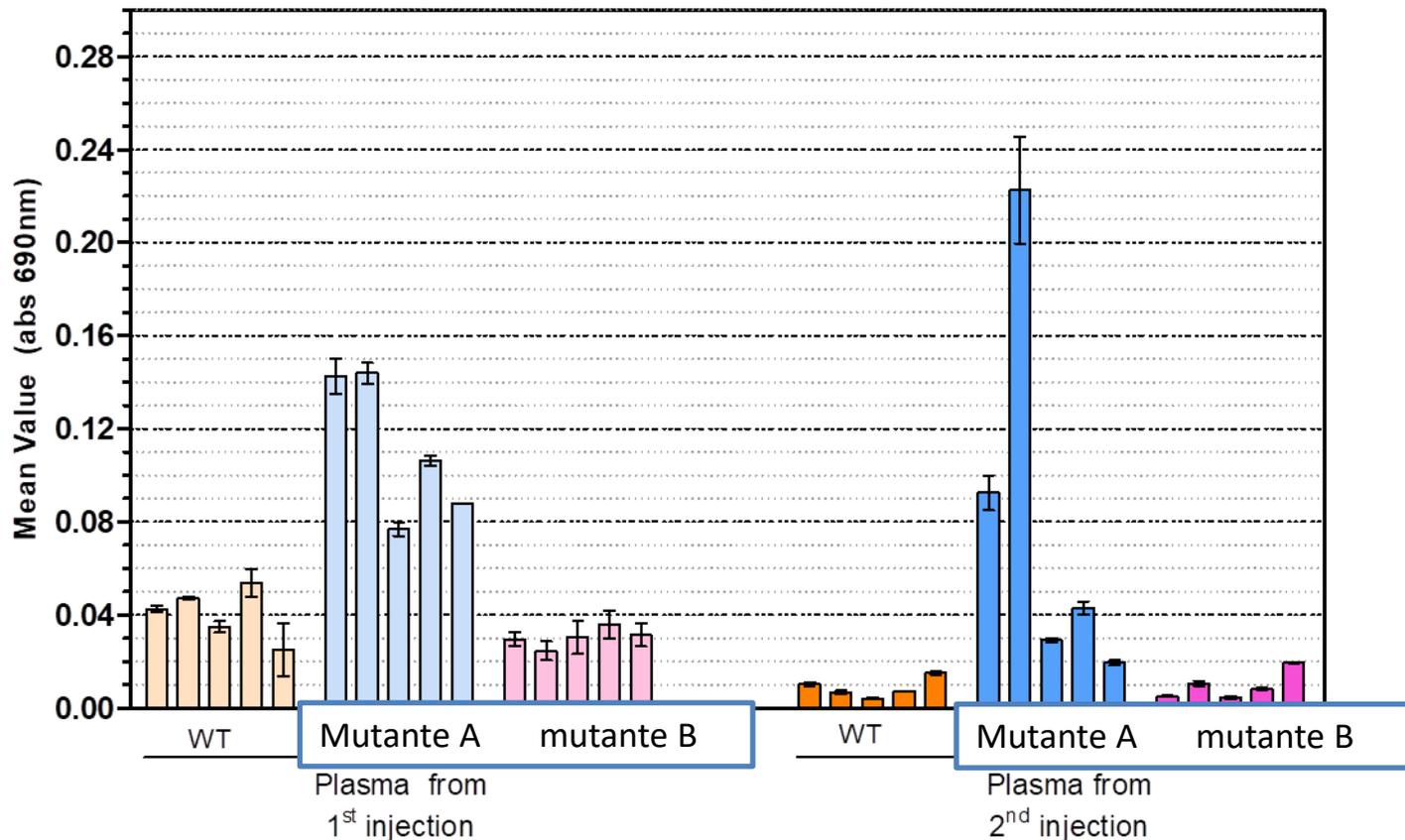
Maggiore et al., 2017. *SRP714479* | DOI:10.1038/s41598-017-15075-4
 products using the Medac Asparaginase-Activity-Test (MAAT) assay kit (Medac). ^ARecombinant ASNase activity/WT ASNase activity.

Mutações randômicas - epPCR



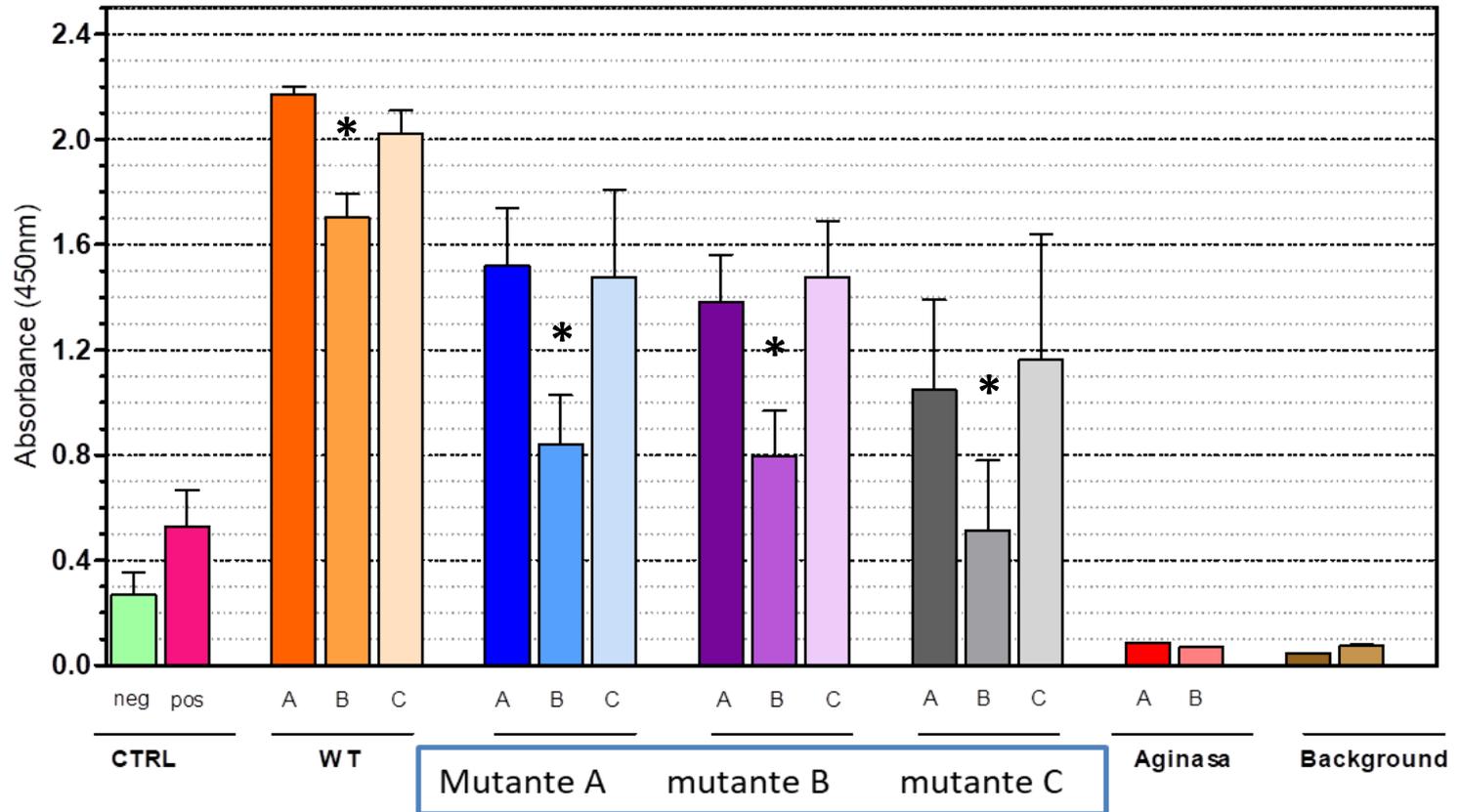
Atividade no soro - teste *in vivo*

Activity analysis 1st and 2nd weeks - new group (after 12h; 1050IU/kg)



Formação de anticorpos anti-ASNase

ELISA to detect anti-asparaginase antibodies in plasma of Balb/c mice after 4 weeks IP injections (1050 IU/Kg)



A) 1:100
 B) 1:1000
 C) 1:100 against Aginasa

Obrigada!!!

