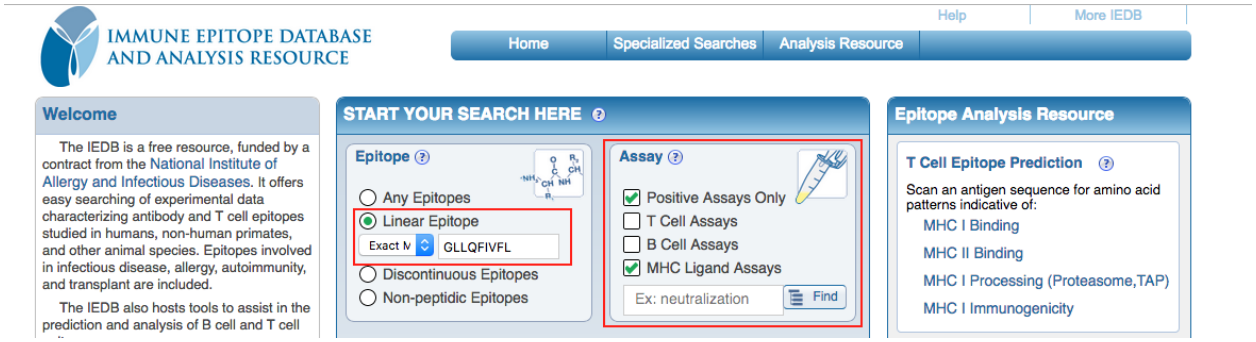
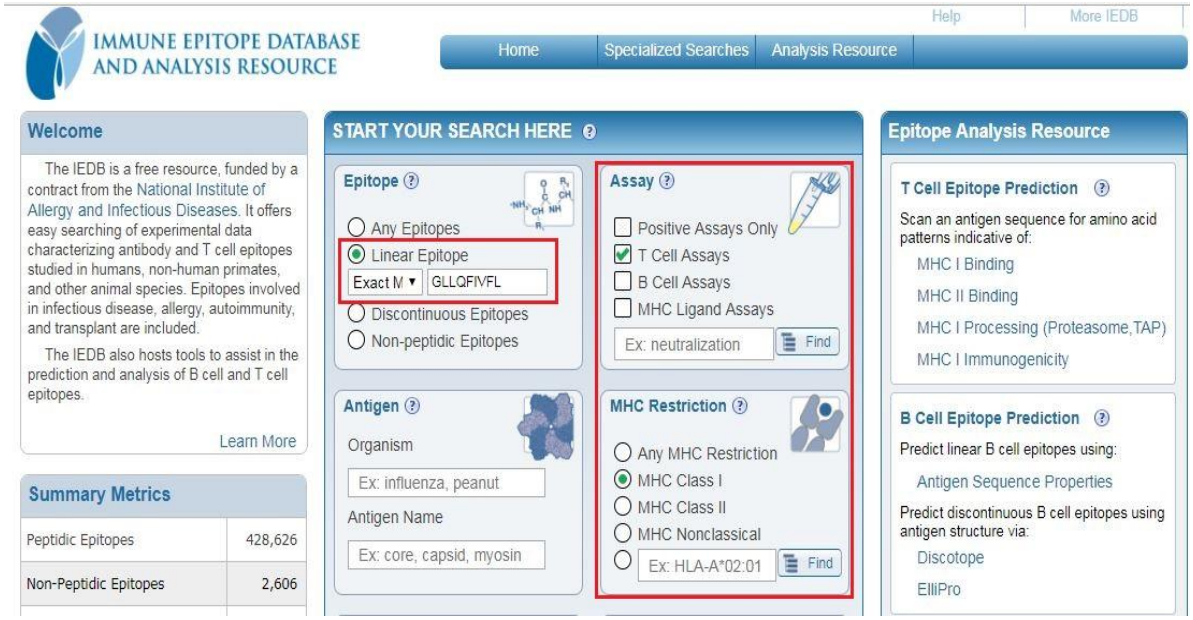
PROTOCOLO DE CURAGEM DE DADOS PARA O CROSSTOPE

Sequência exemplo: GLLQFIVFL
Alelo: HLA-A*0201 ou HLA-A2.1

No IEDB, busque pela sequência alvo.
A única modificação de parâmetros acontece na caixa ao lado da busca.

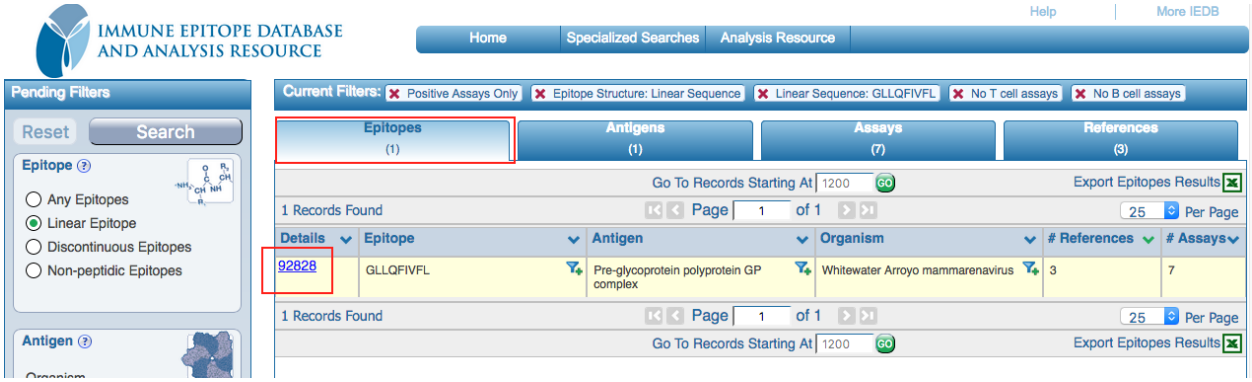


Segunda opção caso o filtro não de saída na busca pelo peptídeo.



Na página de retorno, há duas abas importantes:

Epitopes: de onde pegamos o ID e o link para o ID do epitopo.

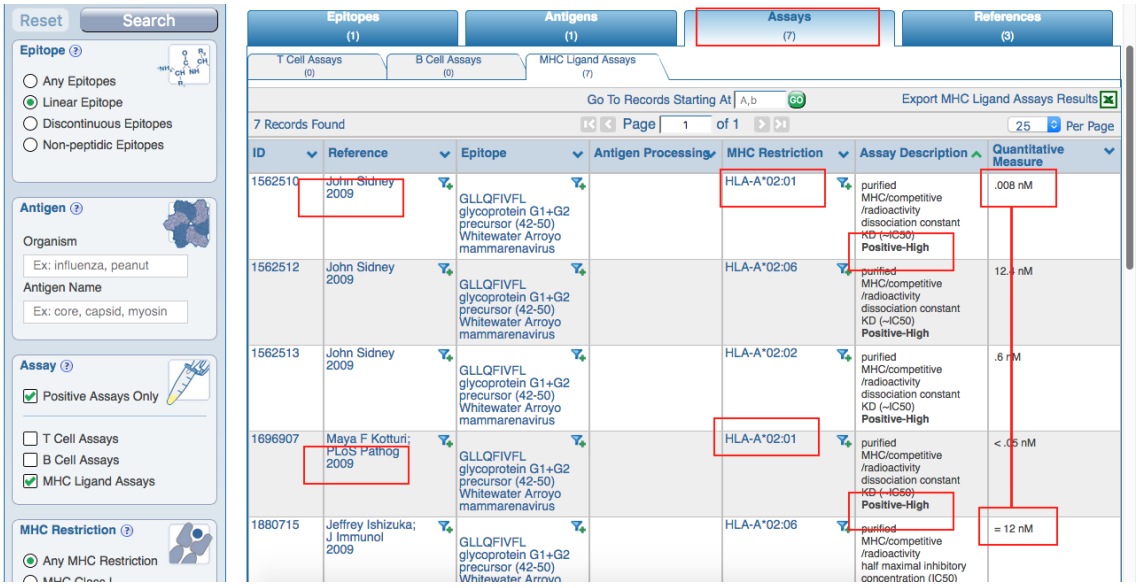


***Assays*:** de onde escolhemos o artigo que servirá como referência para a curagem dos dados.

Ao fazer essa escolha, devemos levar algumas coisas em consideração:

ANO da publicação: a mais atual
MHC restrition: alelo correspondente.
Assay description: positive-high > positive
(low positives podem ser inclusos, mas com observação na tabela de curagem)

Em caso de "empate" no ano das publicações, escolhemos aquela com menor Quantitative measure (última coluna).



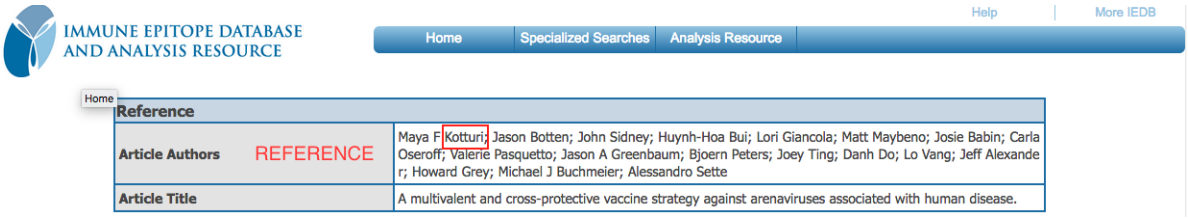
As informações da tabela de curagem são obtidas clicando no ID do ensaio escolhido.
Se for possível salientar o ID que devemos clicar, não causaria confusão nos bragattíneos ex:1696907*

Vamos escolher o artigo de SIDNEY, 2009 como exemplo:

No campo *Reference Type* deve constar a informação: "LITERATURE".

Reference Detail	
Reference ID	1013510
Submission Abstract	Peptides derived from arenavirus strains were selected using various methods, including positional scanning combinatorial library matrices, SMM algorithms, and consensus prediction approaches, as well as simple motif or overlapping peptide scans. Source organisms include the Guanarito, Lassa, LCMV, Machupo, Sabia, Junin and Whitewater Arroyo viruses. Each peptide was tested for its capacity to bind HLA class I specificities in quantitative competition assays based on the competitive inhibition of binding of a radiolabeled standard peptide to purified MHC molecules. Assays were performed essentially as previously described (PMIDs 18432745, 17329346 and 11704282). Briefly, 0.1-1 nM of radiolabeled peptide was incubated at room temperature with 1 nM to 1 µM purified MHC class I molecule in the presence of 1-3 µM human ≤2-microglobulin (Scripps Laboratories, San Diego, CA) and a mixture of protease inhibitors. After a 2-day incubation, binding of the radiolabeled peptide to the given MHC I class molecule was determined by capturing MHC/peptide complexes on Greiner Lumitrac 600 microplates (Greiner Bio-One, Monroe, NC) coated with the W6/32 monoclonal antibody, and measuring bound cpm using the TopCount microscintillation counter (PerkinElmer, Waltham, MA). The concentration of peptide yielding IC50 of the binding of the radiolabeled peptide was calculated. Peptides were typically tested at six different concentrations covering a 100,000-fold dose range, and in three or more independent assays. Under the conditions used, where [label] < [MHC] and IC50 ≥ [MHC], the measured IC50 values are reasonable approximations of the true Kd values. This work has been supported by funds provided through NIH NIAID contract N01-AI-40023.
Submission Affiliations	Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037, USA
Submission Date	2009
Reference Type	Submission
Submission ID	1000410
Submitter Name	John Sidney

Como não é o caso desse ensaio, vamos descartá-lo, voltar à página anterior e escolher o próximo artigo: KOTTURI, 2009.



Reference Detail	
Reference ID	1017786 LINK PARA REFERENCE
Abstract	Arenaviruses are the causative pathogens of severe hemorrhagic fever and aseptic meningitis in humans, for which no licensed vaccines are currently available. Pathogen heterogeneity within the Arenaviridae family poses a significant challenge for vaccine development. The main hypothesis we tested in the present study was whether it is possible to design a universal vaccine strategy capable of inducing simultaneous HLA-restricted CD8+ T cell responses against 7 pathogenic arenaviruses (including the lymphocytic choriomeningitis, Lassa, Guanarito, Junin, Machupo, Sabia, and Whitewater Arroyo viruses), either through the identification of widely conserved epitopes, or by the identification of a collection of epitopes derived from multiple arenavirus species. By inoculating HLA transgenic mice with a panel of recombinant vaccinia viruses (rVACVs) expressing the different arenavirus proteins, we identified 10 HLA-A02 and 10 HLA-A03-restricted epitopes that are naturally processed in human antigen-presenting cells. For some of these epitopes we were able to demonstrate cross-reactive CD8+ T cell responses, further increasing the coverage afforded by the epitope set against each different arenavirus species. Importantly, we showed that immunization of HLA transgenic mice with an epitope cocktail generated simultaneous CD8+ T cell responses against all 7 arenaviruses, and protected mice against challenge with rVACVs expressing either Old or New World arenavirus glycoproteins. In conclusion, the set of identified epitopes allows broad, non-ethnically biased coverage of all 7 viral species targeted by our studies.
Affiliations	Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA.
Date	2009 ANO DA PUBLICAÇÃO
Reference Type	Literature tá ok
PubMed ID	20019801
Journal	PLoS Pathog
Journal Volume	5
Article Pages	e1000695
Journal ISSN	1553-7374
Article Chemical List	Antigens, Viral;Epitopes;HLA-A Antigens;Viral Vaccines
Article MeSH List	Animals; Antigens, Viral(therapeutic use); Arenaviridae(immunology); Arenaviridae Infections(prevention & control; therapy); CD8-Positive T-Lymphocytes(immunology); Cross Reactions(immunology); Epitopes(therapeutic use); HLA-A Antigens(therapeutic use); Hemorrhagic Fevers, Viral(prevention & control; therapy); Humans; Immunization; Mice; Mice, Transgenic; Treatment Outcome; Viral Vaccines(immunology)

O link para a referência é o PubMed ID - alterar imagem

Reference: Sobrenome do primeiro autor, et al. + Ano da publicação do ensaio.
Kotturi et al. 2009

Retirando as demais informações do IEDB para completar a TABELA de curagem:

* **ID do ensaio escolhido**

Epitope	
Epitope ID	92828 ID do epitopo e LINK
Chemical Type	Linear peptide
Linear Sequence	GLLQFIVFL
Starting Position	42 EPITOPE
Ending Position	50 POSITION
Source Molecule Name	glycoprotein G1+G2 precursor Source Protein
Source Accession	AAK60497.1 Link para Source Protein
Source Organism ID	46919 Link para Source Organism
Source Organism	Whitewater Arroyo mammarenavirus Source Organism

O Link para Source Protein deve direcionar para a proteína no NCBI, por exemplo:
https://www.ncbi.nlm.nih.gov/protein/AAK60497.1

OBS.: Caso não exista um link para o NCBI inserimos o link do UNIPROT.

Enquanto o Link para o Source Organism, retorna um link do próprio IEDB, por exemplo:
http://www.iedb.org/source/46919

OBS: Tanto na página inicial, logo após o input da sequência de aminoácidos em EPITOPES → ANTIGEN. Quanto em SOURCE MOLECULE NAME localizado como mostrado acima, pode

tanto mostrar a mesma informação quanto informações conflitantes ou inespecíficas (ex: SOURCE MOLECULE NAME → UNCHARACTERIZED PROTEIN xxxx). Escolher a mais completa nesses casos de grande discrepância.