Generation of RASopathies-associated genetic variants for cell-based assays

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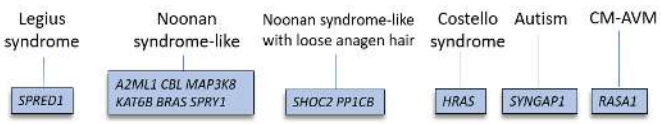
Abstract

The RASopathies are a group of developmental syndromes of diverse genetic origin characterized by germline mutations in genes that encode proteins of the Ras/MAPK pathway. The RASopathies that we study in our lab include neurofibromatosis type 1 (NF1) (MIM ID #162200) and Legius syndrome (LS) (MIM ID #611431), which appear as a result of loss of function mutations in the *NF1* and *SPRED1* genes, respectively.



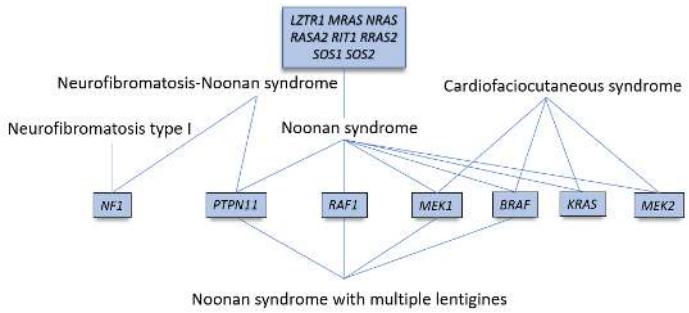
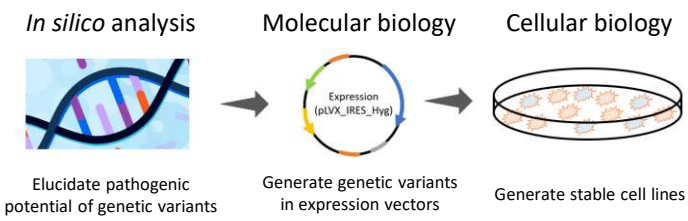


Fig 1. *The 27 RASopahties genes.*

Hypothesis and Objectives

Our study is based on RASopathies-associated genetic variants provided by the Reference Unit for Advanced Diagnosis in Rare Diseases of Castilla y León (DiERCyL). With the provided data, we hypothesize that these genetic variants are the cause of the RASopathies and that it is possible to generate them in stable cell lines that enable to study their impact into the signaling pathways of LS and NF1. The aims of the study were to elucidate the pathogenic potential of these genetic variants, to generate them in expression vectors and to generate stable cell lines expressing the variants.

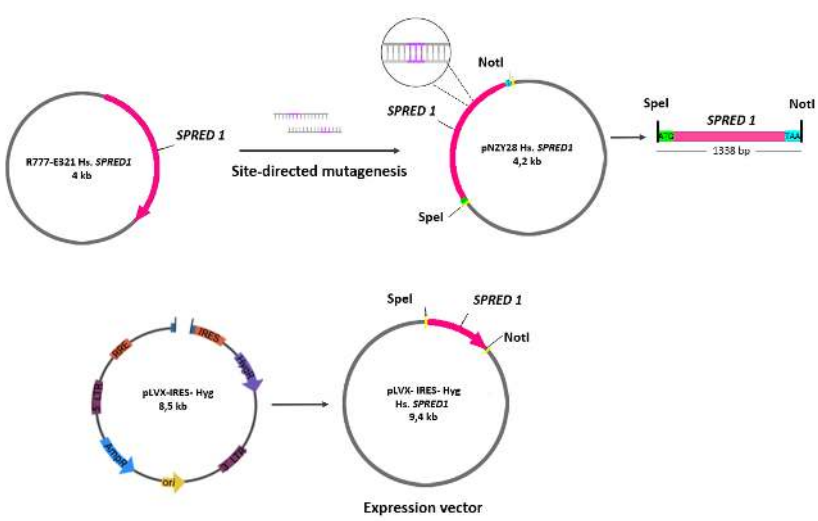


Methods and results

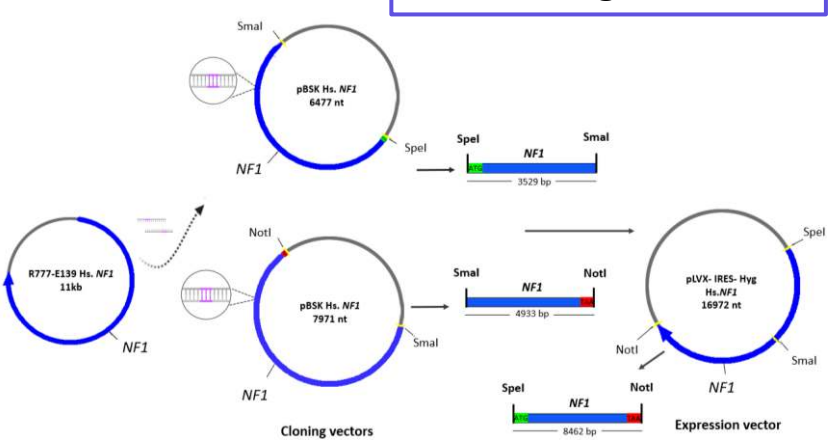
Generation of genetic variants in expression vectors

A total of 6 *NF1* variants and 5 *SPRED1* variants were generated by PCR site directed mutagenesis into the cloning vectors pBSK and pNZY28, respectively, and cloned into the lentiviral expression vector pLVX.

SPRED1 cloning workflow



NF1 cloning workflow



Generation of lentiviruses, transduction and selection of stable cell lines

Once the genetic variants were generated, we proceed to produce lentiviruses that contain them and to the generation of stable cell lines.

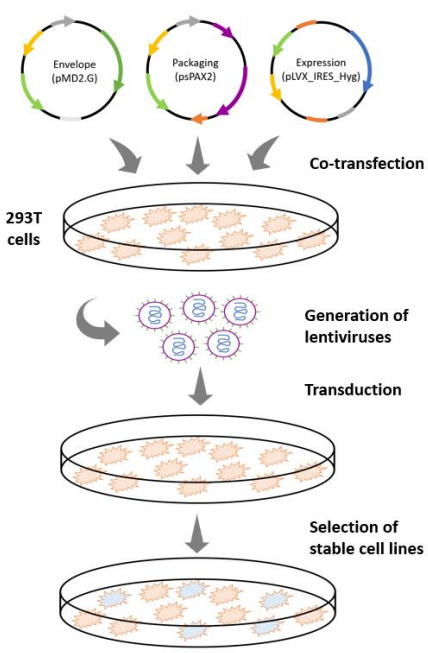


Fig 2. *Generation of lentiviruses, transduction and selection of stable cell lines.*

Discussion

We have successfully generated the different RASopathies-associated genetic variants in cloning vectors and cloned them into vectors that allow their expression in two different mammal cell lines of interest.

This results will enable us to carry out some signaling and expression assays to elucidate the molecular mechanisms underlying NF1 and LS.

In a near future, we aim to study how the Ras/MAPK pathway is involved in tumorgenesis and the role that *NF1* and *SPRED1* would play in it.