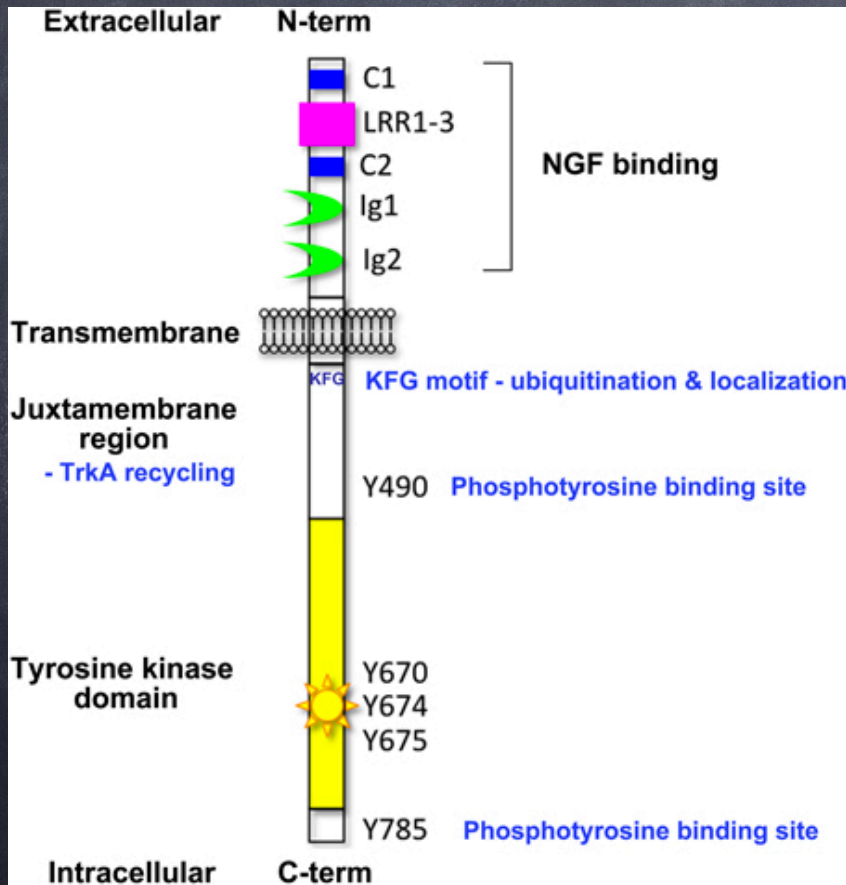


# Case study

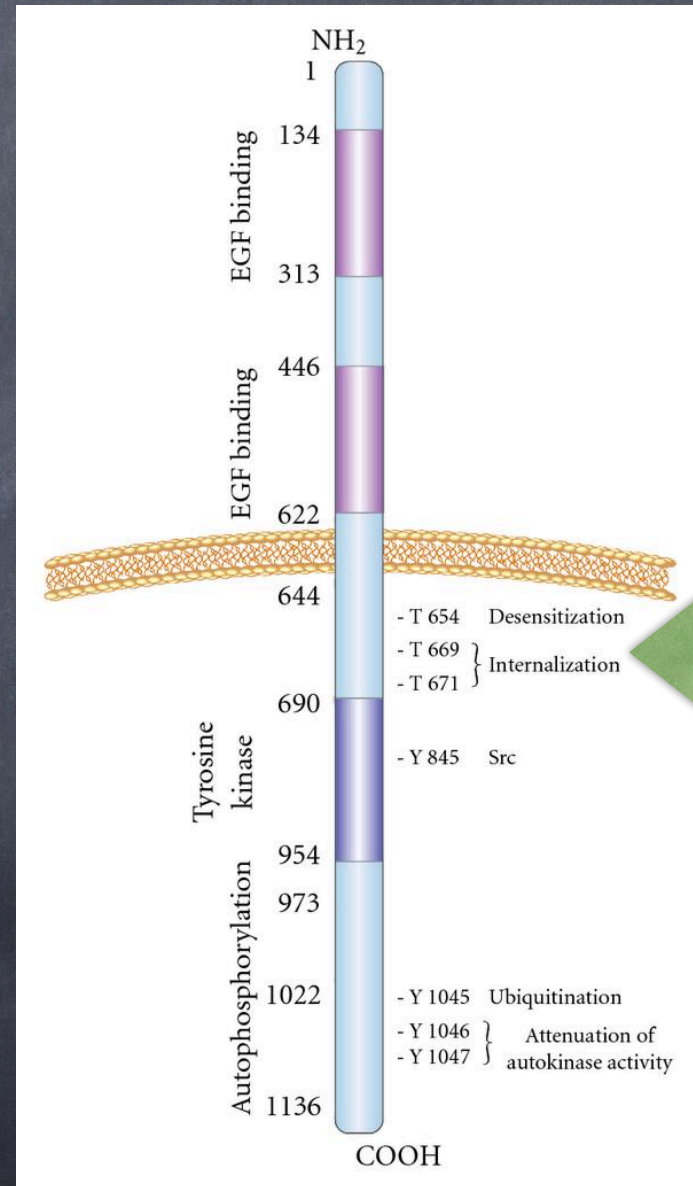


Regulators	Differences in ERK activity	Cellular responses
<p><b>Temporal regulators</b></p> <ul style="list-style-type: none"> <li>PKC</li> <li>Rap1</li> <li>Sprouty</li> <li>⋮</li> </ul>	<p><b>Sustained ERK activation</b></p> <p><b>Transient ERK activation</b></p>	<p>Differentiation</p> <p>PC12 cells</p> <p>Proliferation</p>

# NGFR (TRK A)



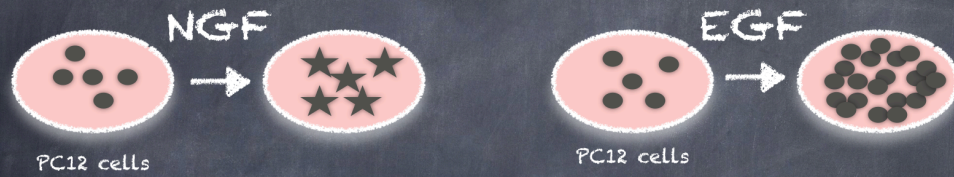
# EGFR



Is the difference in the kinetics due to the rate of internalisation of the receptor?

Is there a correlation between the kinetics in Erk activity and the phenotype?

How would you proceed to answer these questions?

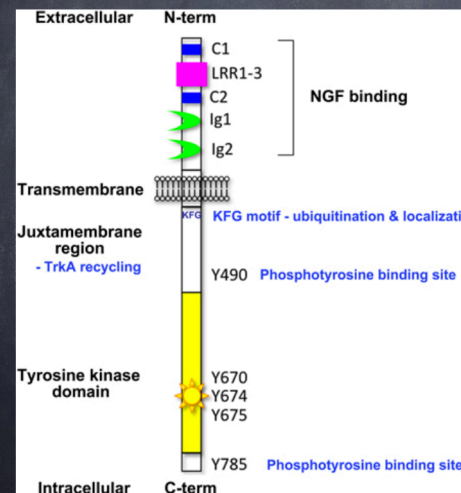


Regulators	Differences in ERK activity	Cellular responses
Temporal regulators PKC Rap1 Sprouty ...		Differentiation PC12 cells Proliferation

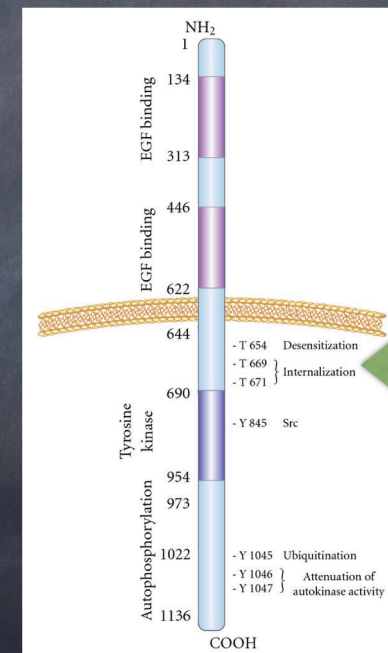
NGF

EGF

### NGFR (TRK A)

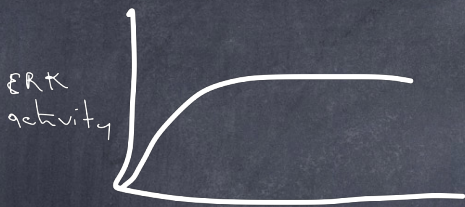


### EGFR



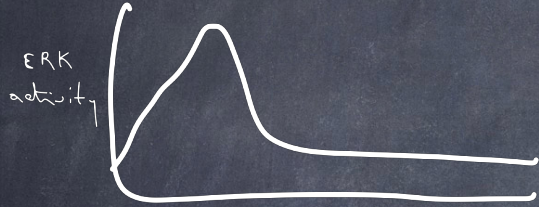
# STATE OF THE ART

NGF



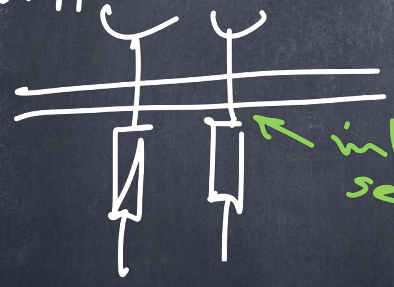
⇒ differentiation

EGF



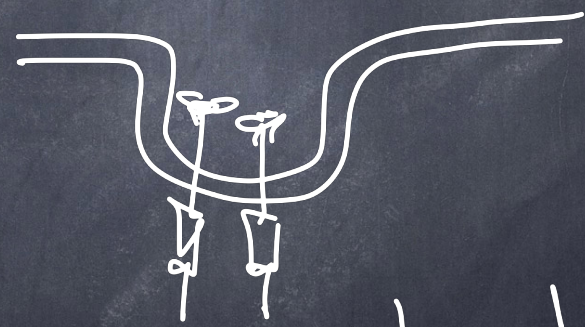
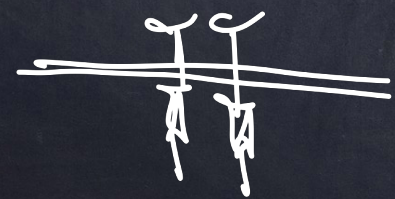
⇒ proliferation

EGFR



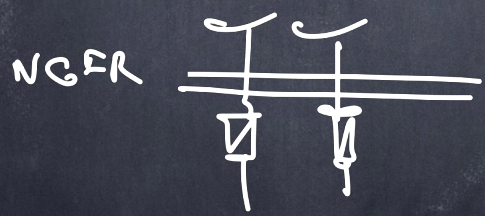
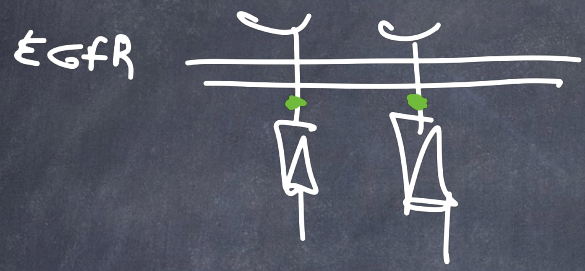
interuolization sequence

NGFR

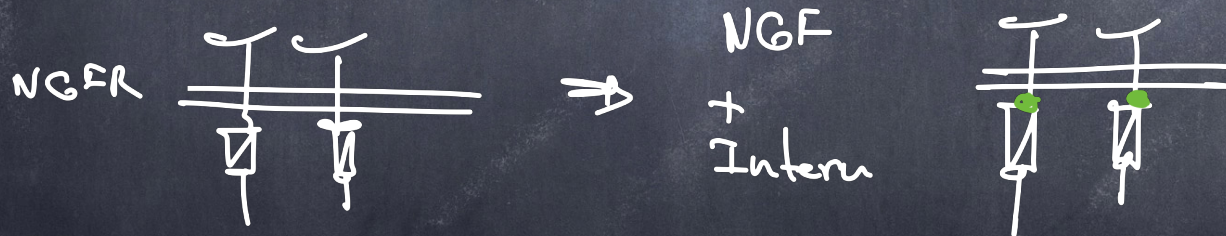
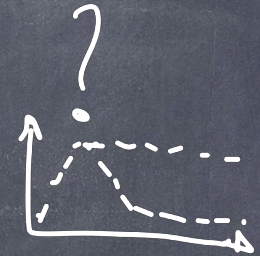
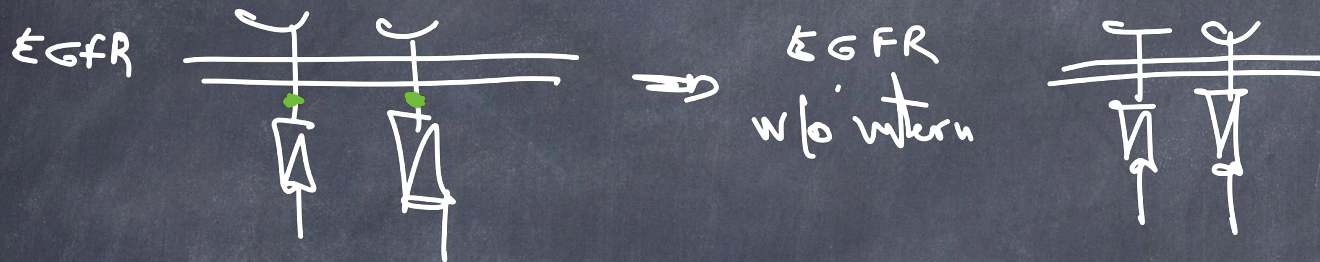


↳ interuoliz.

# in kinetics due to  
Internalisation sequence?



$\neq$  in kinetics due to internalisation sequence?



# EXPERIMENTAL APPROACH



## EXPERIMENTAL APPROACH

⇒ recombinant  
molecule  
production

{ in vitro mutagenesis  
deletion/insertion  
⇒ techniques 2 or 3

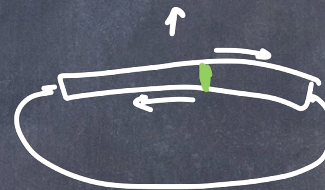
# EXPERIMENTAL APPROACH

⇒ recombinant molecule production

Prasmita  
EGFR cDNA

in vitro mutagenesis  
deletion/insertion  
⇒ techniques 2 or 3

N  C



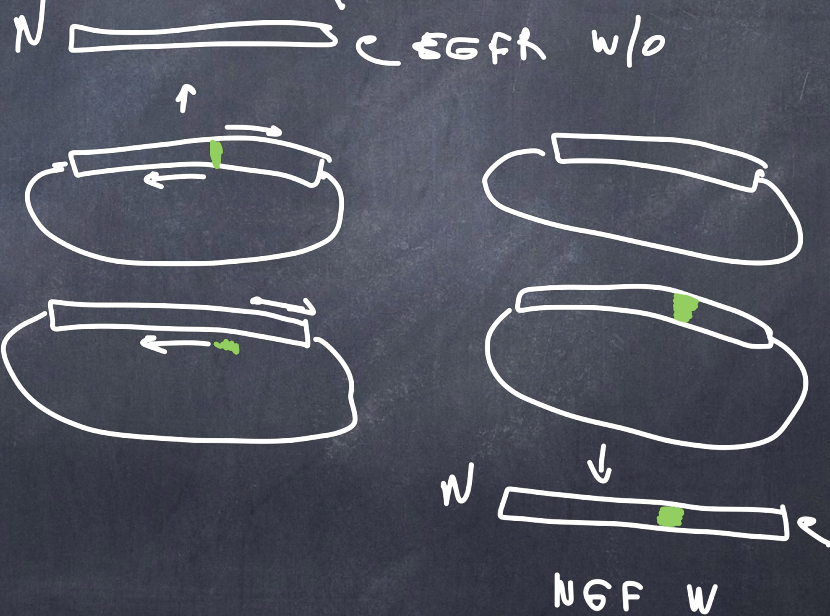
# EXPERIMENTAL APPROACH

⇒ Recombinant molecule production

in vitro mutagenesis  
deletion/insertion  
⇒ techniques 2 or 3

Plasmide  
EGFR cDNA

Plasmide  
NGFR cDNA



# EXPERIMENTAL APPROACH

Transfection  $\left\{ \begin{array}{l} \text{COS ?} \\ \text{PE12 ?} \end{array} \right.$

# EXPERIMENTAL APPROACH

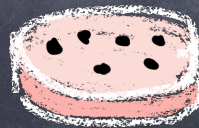
Transfection  $\left\{ \begin{array}{l} \text{COS ?} \\ \text{PE12 ?} \end{array} \right.$

$\Rightarrow$  COS



ERK activity

$\Rightarrow$  PE12



phenotype

# EXPERIMENTAL APPROACH

Transfection  $\left\{ \begin{array}{l} \text{COS ?} \\ \text{PE12 ?} \end{array} \right.$

$\Rightarrow$  PE12  $\rightarrow$  Endogenous receptors  
 $\left\{ \begin{array}{l} \text{Transfection} \\ \rightarrow \text{Recombinant molecules} \end{array} \right.$

How to study only the effect of recombinant molecules?

# EXPERIMENTAL APPROACH

Transfection  $\left\{ \begin{array}{l} \text{COS ?} \\ \text{PE12 ?} \end{array} \right.$

$\Rightarrow$  PE12  $\rightarrow$  Endogenous receptors  
           $\downarrow$  Transfection  $\rightarrow$  Recombinant molecules

How to study  
only the effect  
of recombinant  
molecules?

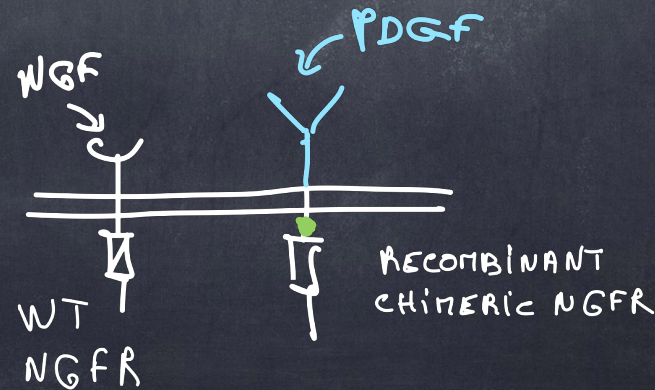
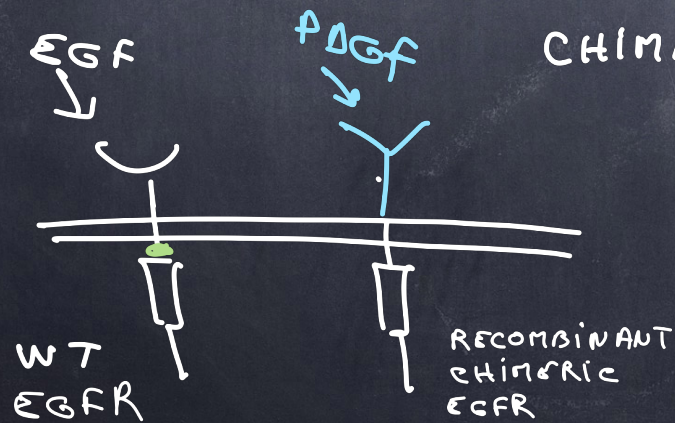
## CHIMERIC RECEPTORS

# EXPERIMENTAL APPROACH

Transfection  $\left\{ \begin{array}{l} \text{COS ?} \\ \text{PE12 ?} \end{array} \right.$

$\Rightarrow$  PE12  $\rightarrow$  Endogenous receptors  
 $\quad \quad \quad \rightarrow$  Recombinant molecules  
 $\quad \quad \quad \text{Transfection}$

How to study only the effect of recombinant molecules?

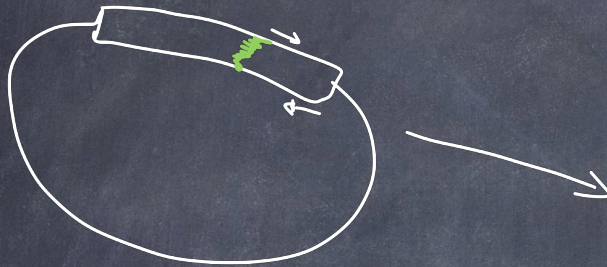




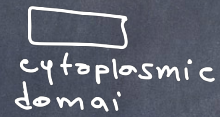
# ALTERNATIVE RECOMBINANT STRATEGY

# CHimeric EGFR w/o INTERNALISATION SEQUENCE

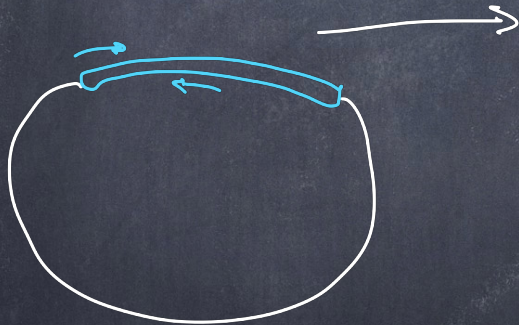
WT  
EGFR



c-term  
EGFR



WT  
PDGFR

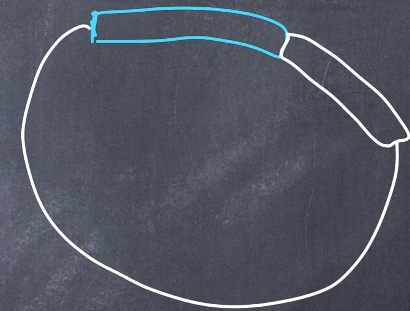


N-term  
PDGFR  
Extra Cy  
domain

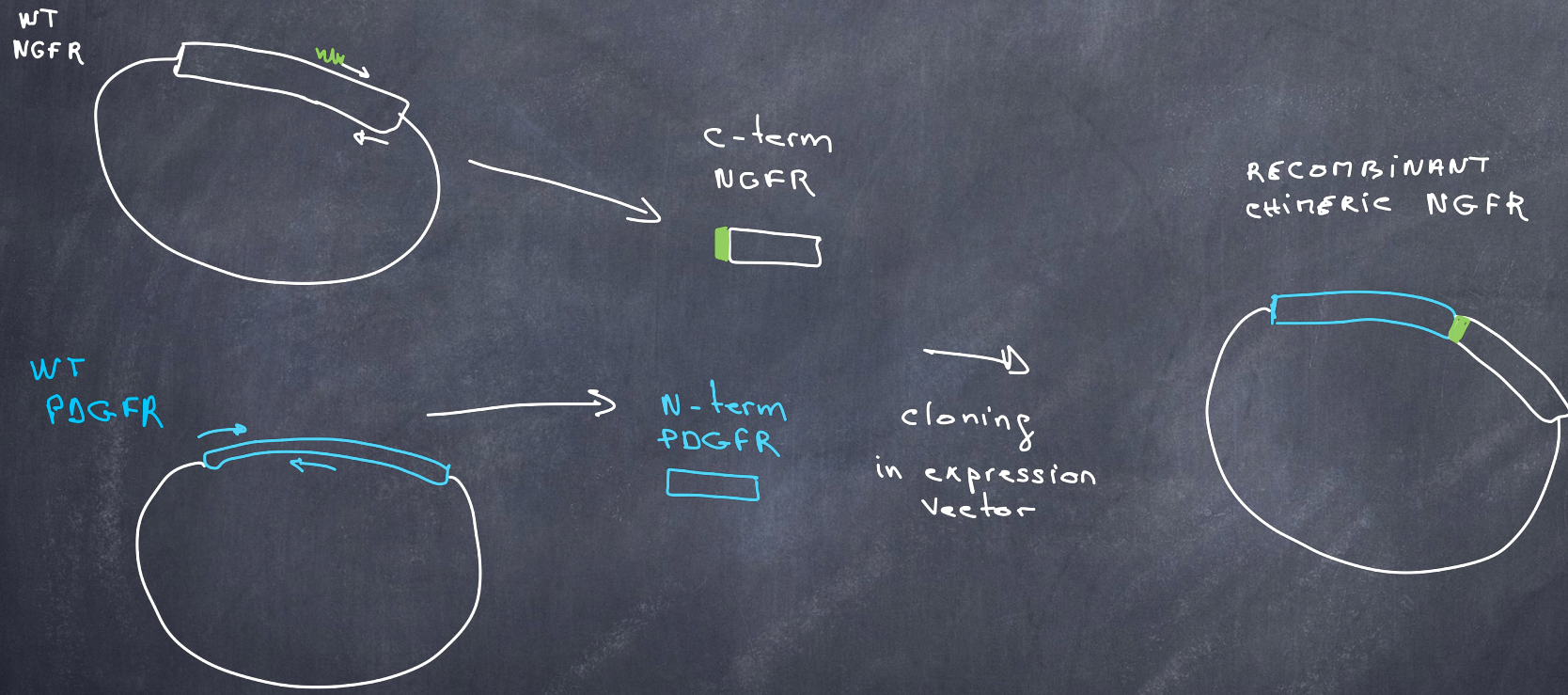


cloning  
in expression  
vector

RECOMBINANT  
CHimeric EGFR



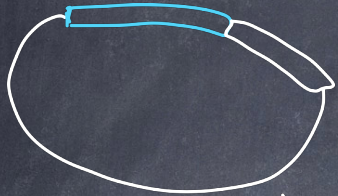
# CHIMERIC NGFR WITH INTERNALISATION SEQUENCE



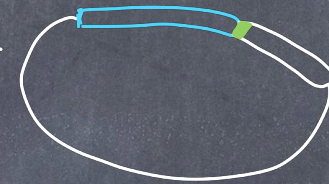
NEXT STEP ?  
o

# NEXT STEP ?

RECOMBINANT  
CHIMERIC EGFR



RECOMBINANT  
CHIMERIC NGFR



TRANSFECTION

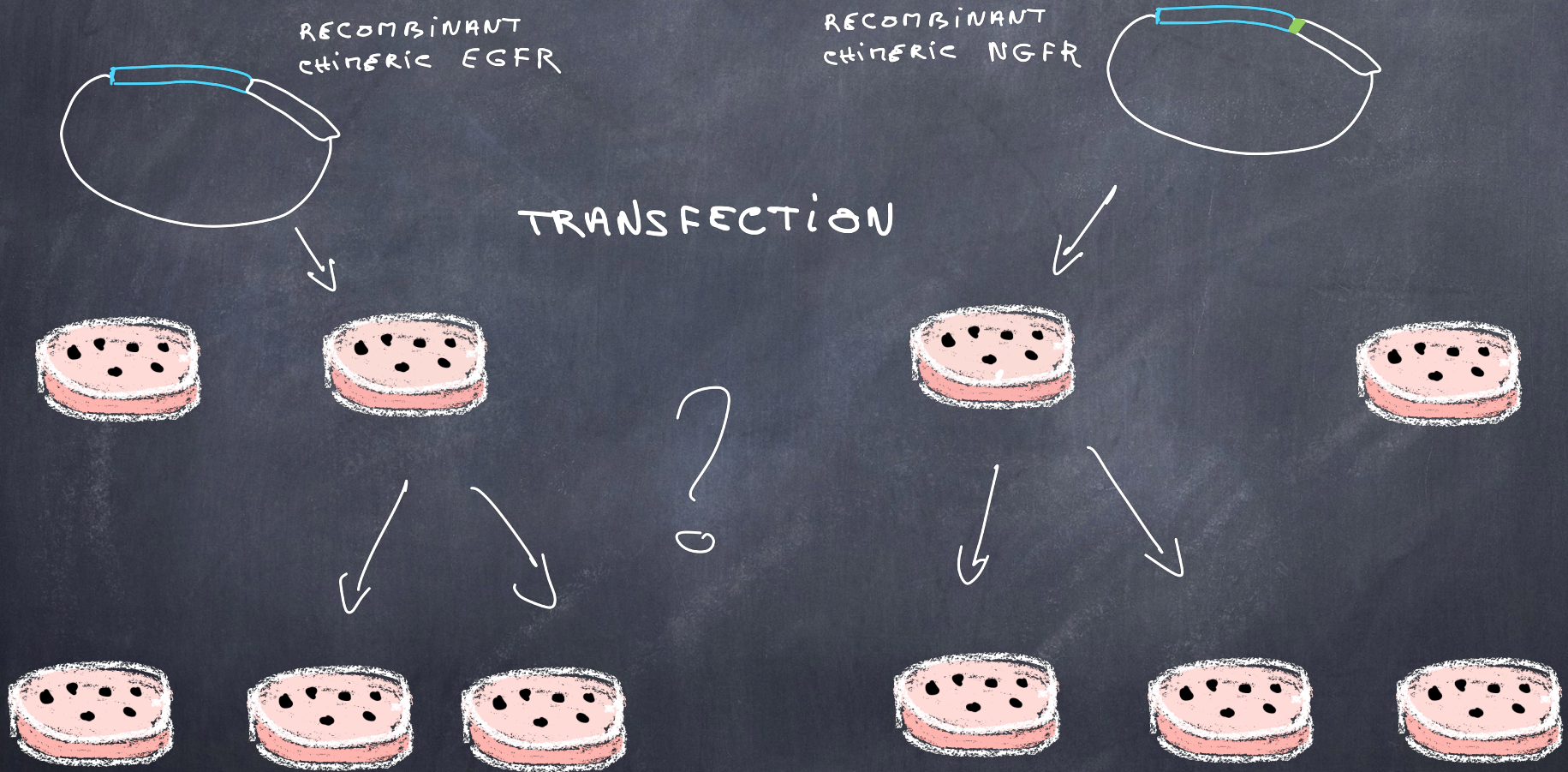
?

# NEXT STEP ?

RECOMBINANT  
CHimeric EGFR

RECOMBINANT  
CHimeric NGFR

TRANSFECTION

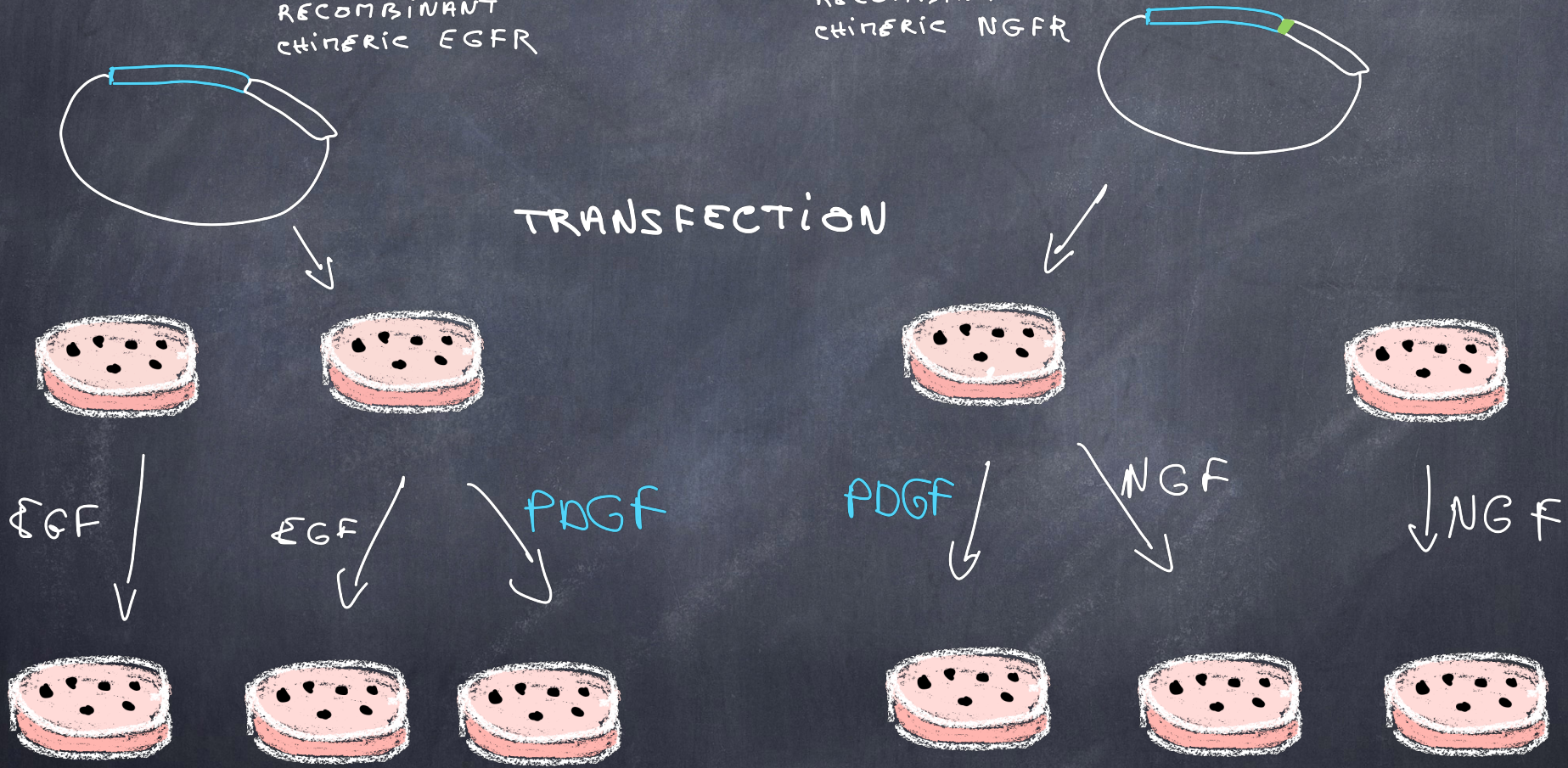


# GF treatment

RECOMBINANT  
CHIMERIC EGFR

RECOMBINANT  
CHIMERIC NGFR

TRANSFECTION

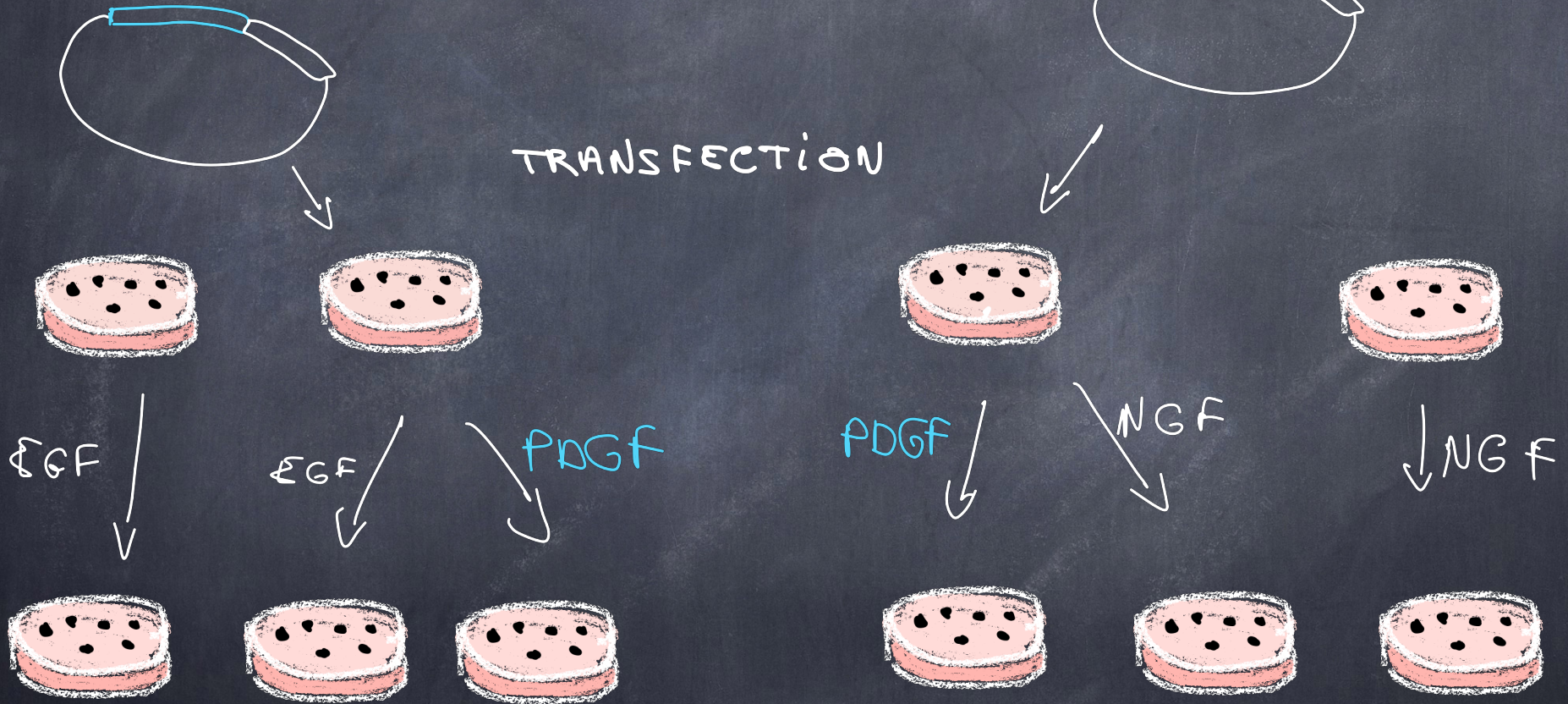


# EXPECTED RESULTS ?

RECOMBINANT CHIMERIC EGFR

RECOMBINANT CHIMERIC NGFR

TRANSFECTION



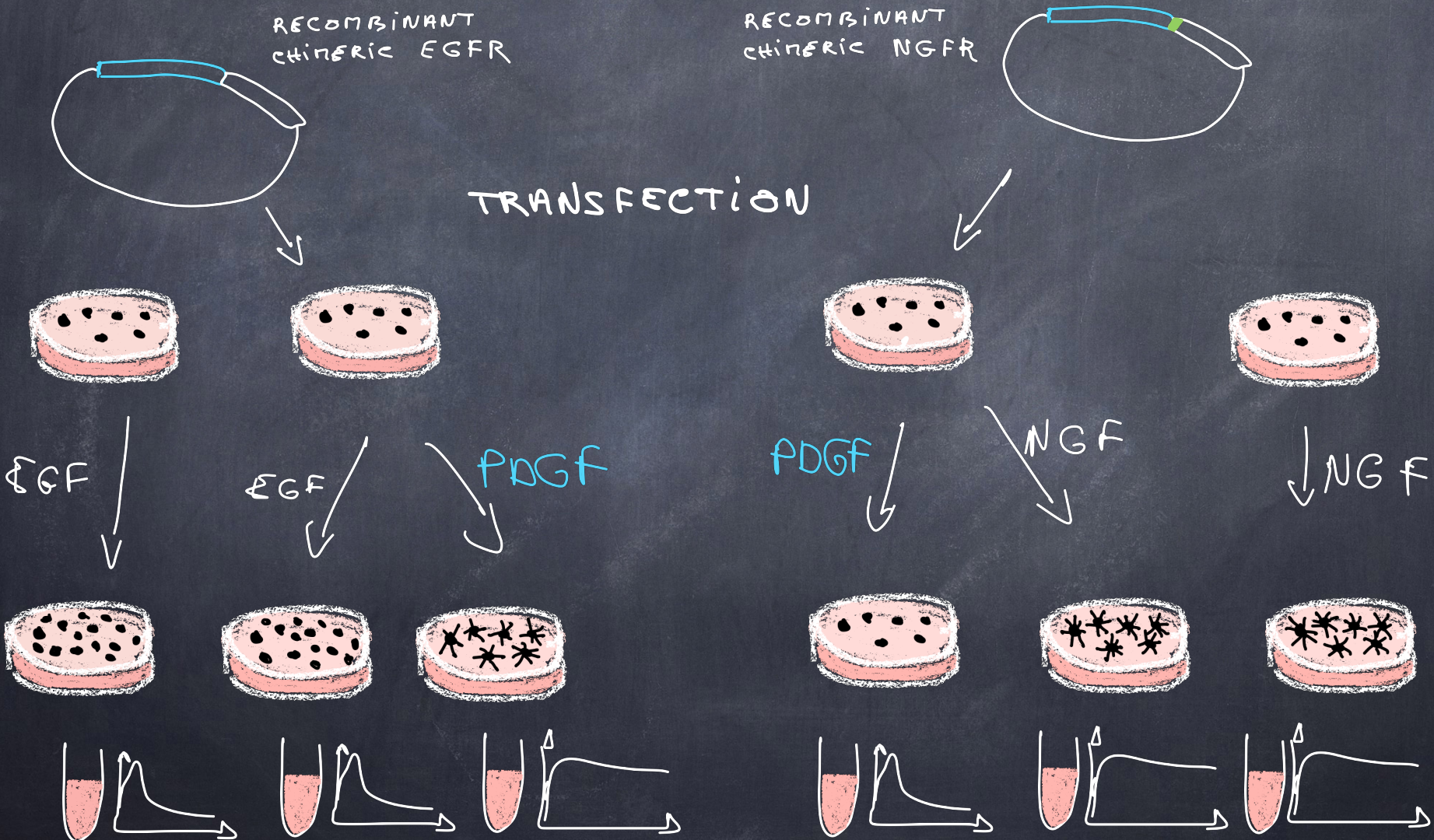


# EXPECTED RESULTS

RECOMBINANT CHimeric EGFR

RECOMBINANT CHimeric NGFR

TRANSFECTION



CONCLUSION?