# Arbeiten zur evolutiven Optimierung des HI-Virus:

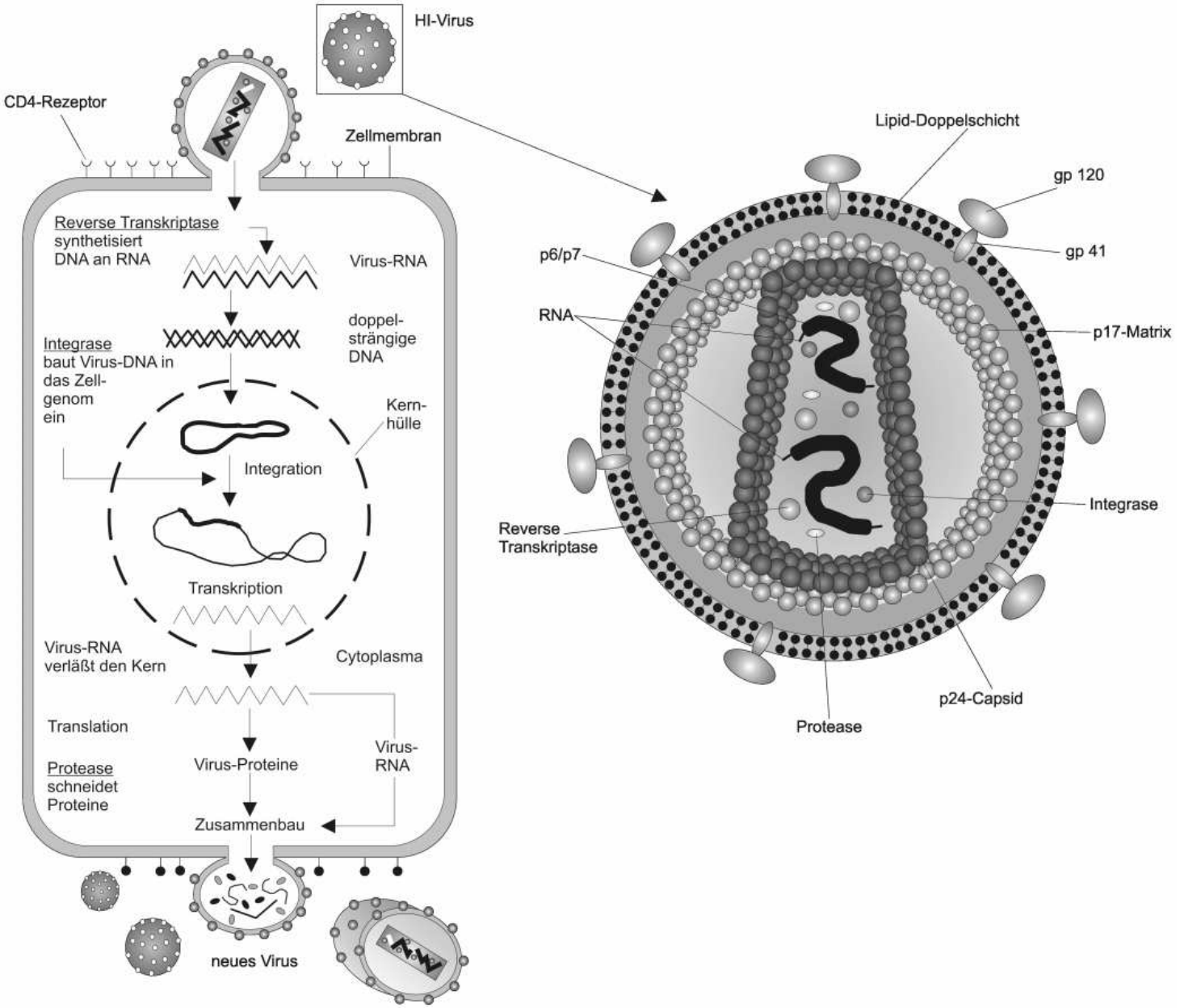
# Erzeugung, funktionale Bewertung und Sequenzierung von Enzymvarianten

# Outline

- background (hiv, starting points, aims)
- construction of *p66*
- construction of english(Mutantenbank)?
- *p66*-polymerase activity
- problems :( und solvings :)

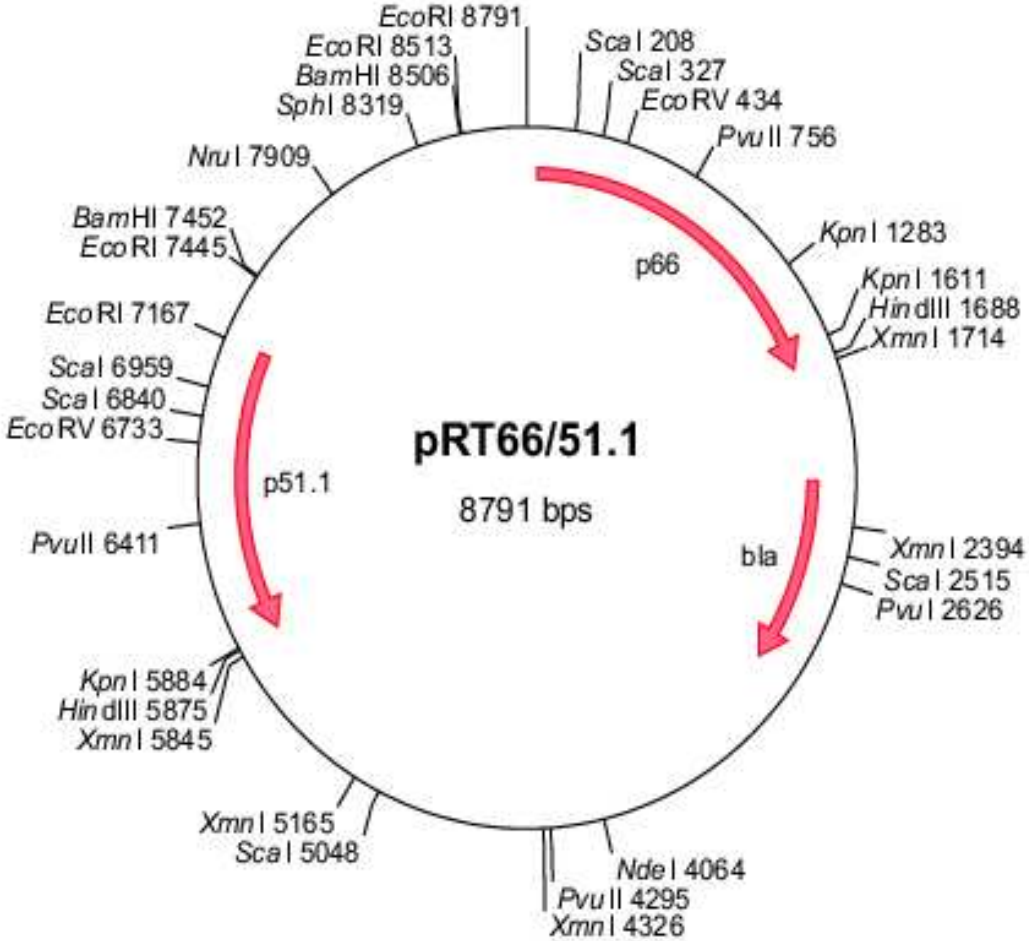
## HIV

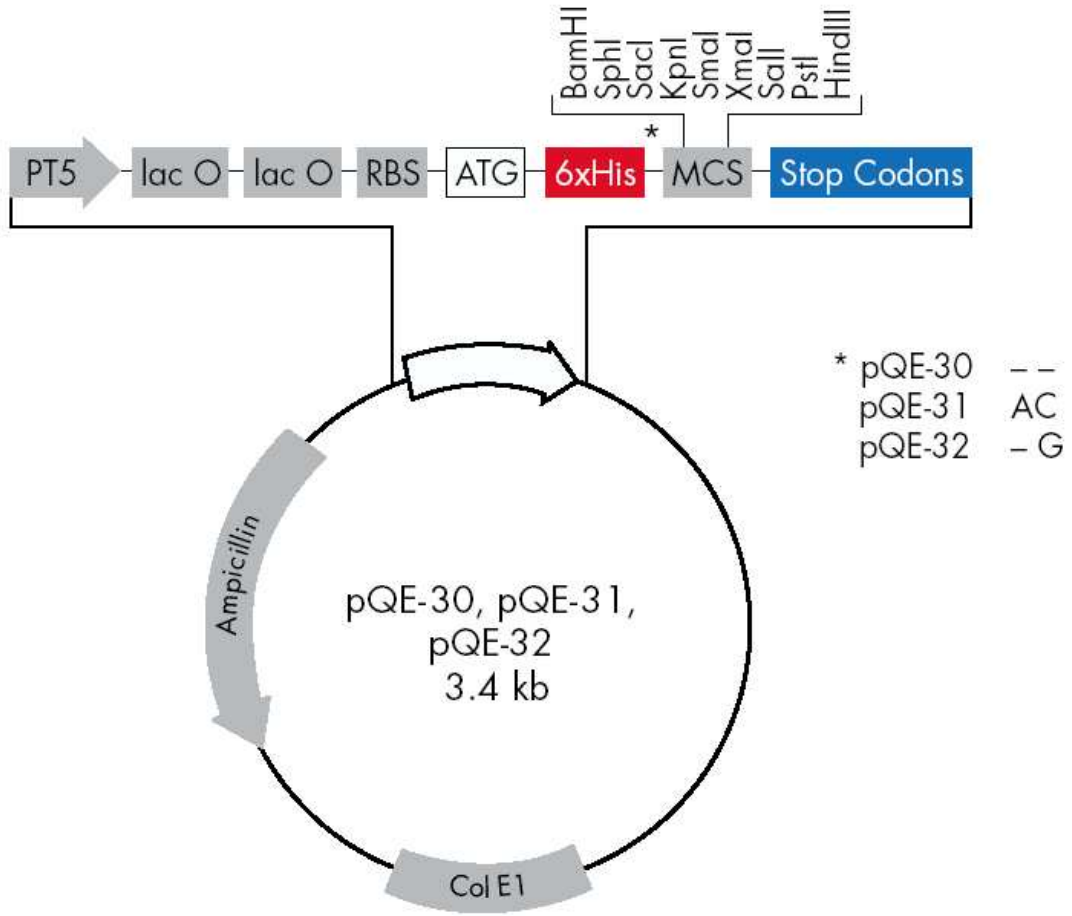
- retrovirus
- medicin: nukleosidanaloga, NNRTI, protease-inhibitors fusionsinhibitors, HAART
- HIV-RT: error rate is $3 \cdot 10^{-}4$


HI-Virus
CD4-Rezeptor
Zellmembran
Reverse Transkriptase
synthetisiert
DNA an RNA
Virus-RNA
doppel-
strängige
DNA
Integrase
baut Virus-DNA in
das Zell-
genom
ein
Kern-
hülle
Integration
Transkription
Virus-RNA
verläßt den Kern
Cytoplasma
Translation
Protease
schneidet
Proteine
Virus-Proteine
Virus-
RNA
Zusammenbau
neues Virus
Lipid-Doppelschicht
gp 120
gp 41
p6/p7
RNA
p17-Matrix
Integrase
Reverse
Transkriptase
Protease
p24-Capsid

## starting point

- eigens theory
- molecular evolution: mutation, rekombination und selection/screening
- selection: thermosensitive properties
- materials: vectors, bacterias
- workings up to now

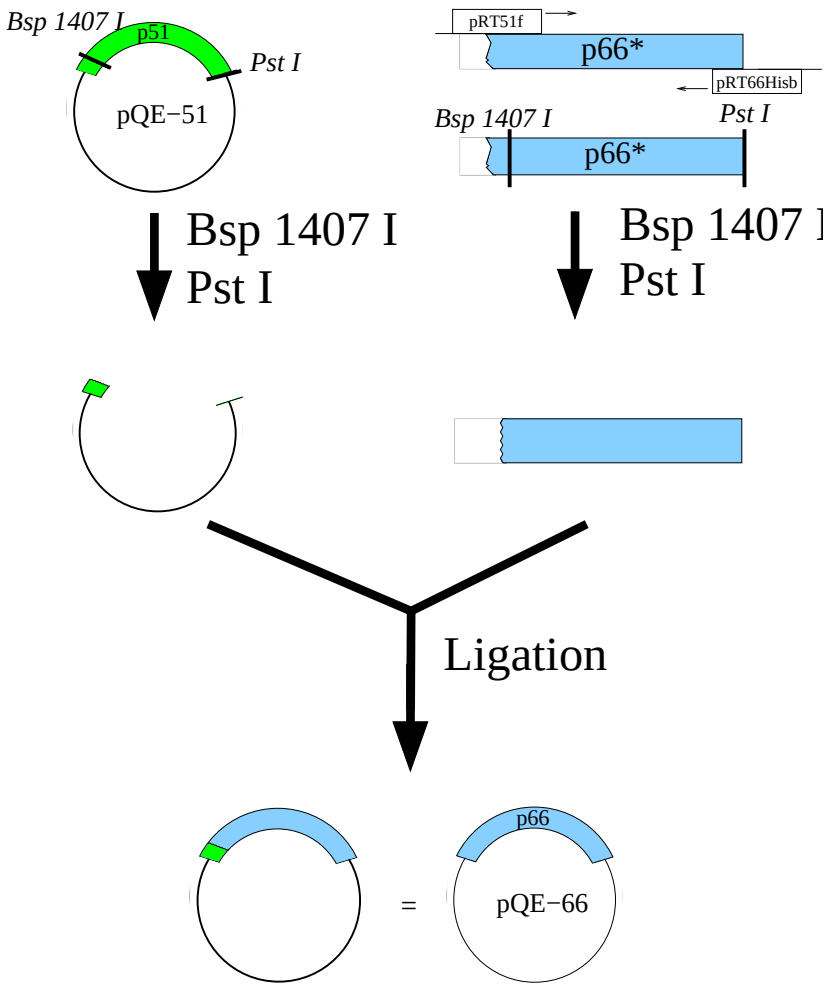

pRT66/51.1
8791 bps
p66
p51.1
bla
EcoRI 8791
EcoRI 8513
BamHI 8506
SphI 8319
NruI 7909
BamHI 7452
EcoRI 7445
EcoRI 7167
ScaI 6959
ScaI 6840
EcoRV 6733
PvuII 6411
KpnI 5884
HindIII 5875
XmnI 5845
XmnI 5165
ScaI 5048
NdeI 4064
PvuII 4295
XmnI 4326
XmnI 2394
ScaI 2515
PvuI 2626
KpnI 1611
HindIII 1688
XmnI 1714
KpnI 1283
PvuII 756
EcoRV 434
ScaI 327
ScaI 208

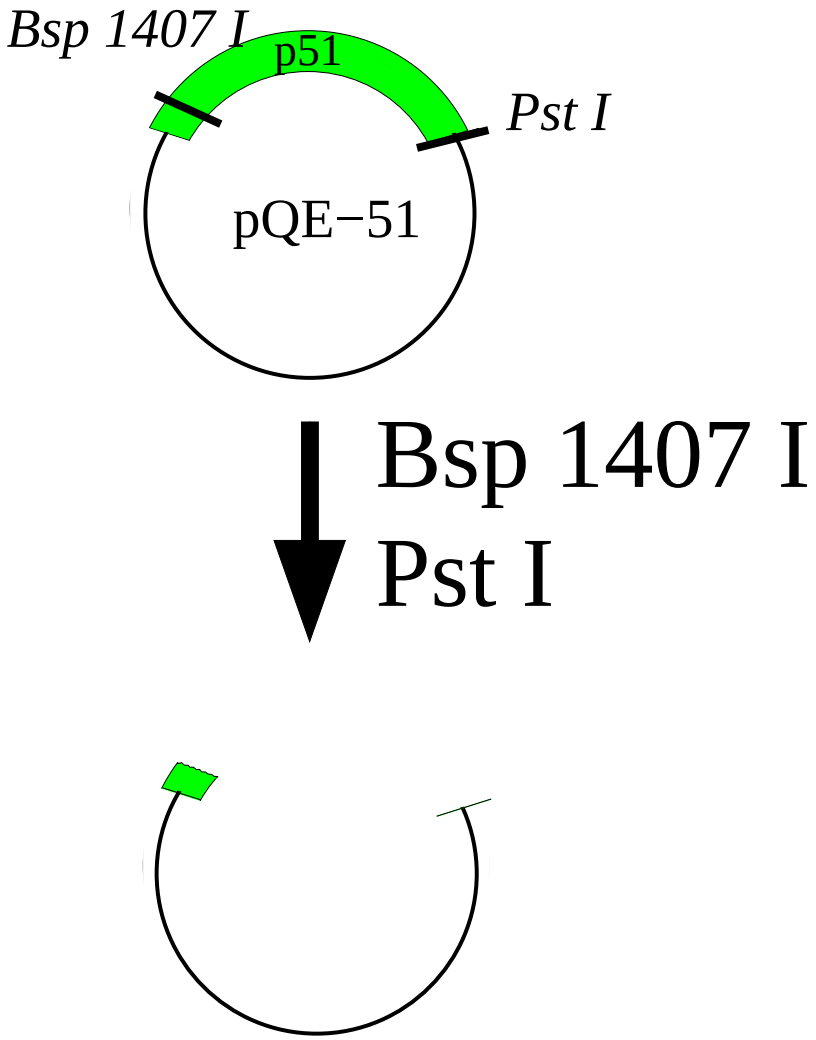

BamHI
SphI
SacI
KpnI
SmaI
XmaI
SalI
PstI
HindIII
*
PT5
lac O
lac O
RBS
ATG
6xHis
MCS
Stop Codons
* pQE-30 – –
pQE-31 AC
pQE-32 – G
Ampicillin
pQE-30, pQE-31,
pQE-32
3.4 kb
Col E1

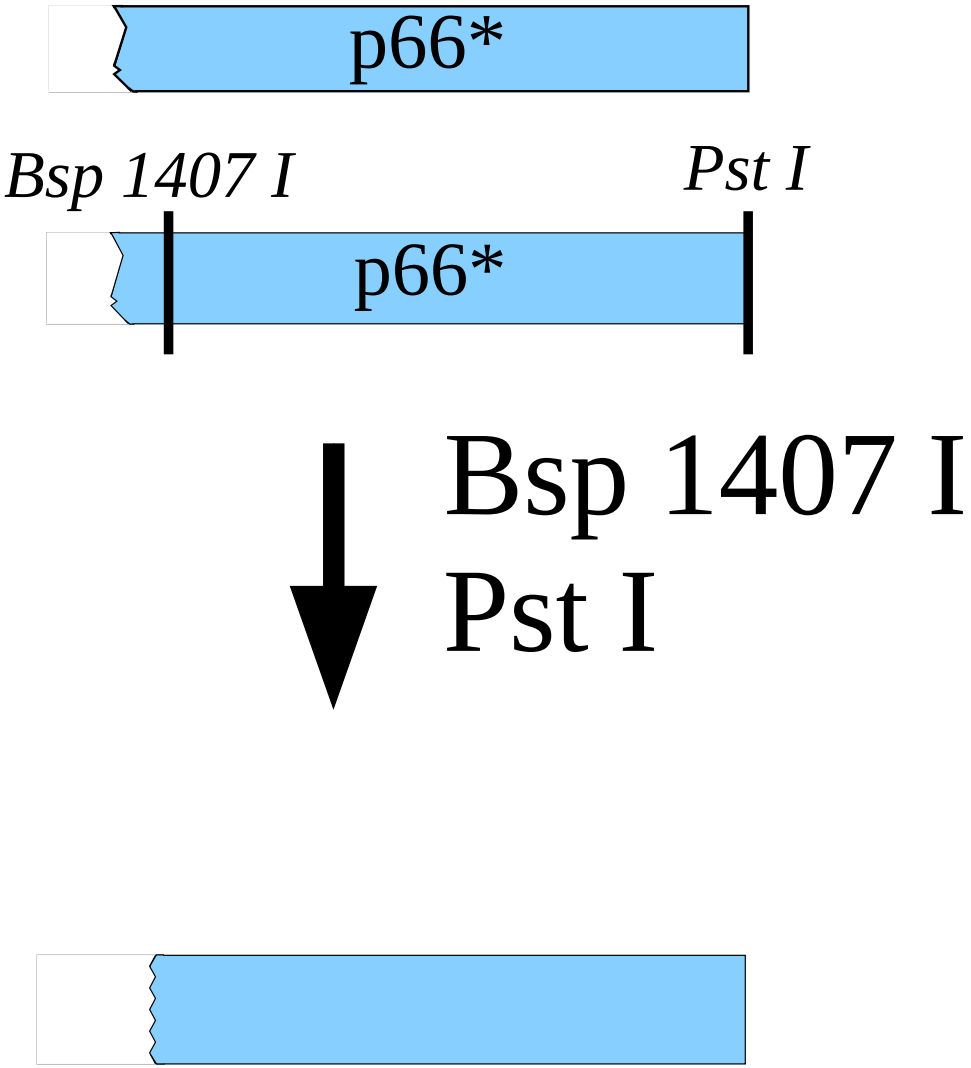
## aims

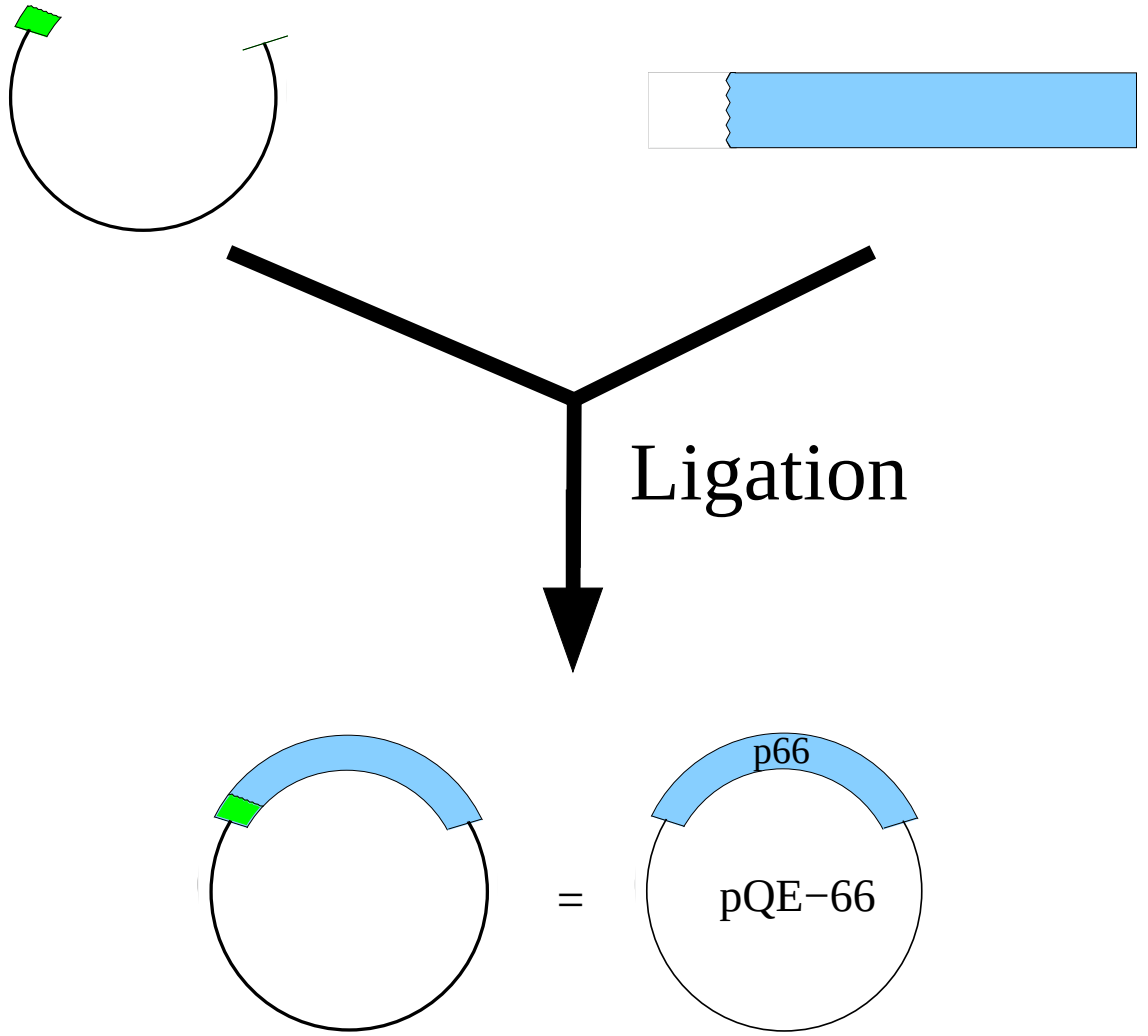
- construction of *p66*
- construction of MB
- provement polymerase-activity of *p66*

# construction *p66*

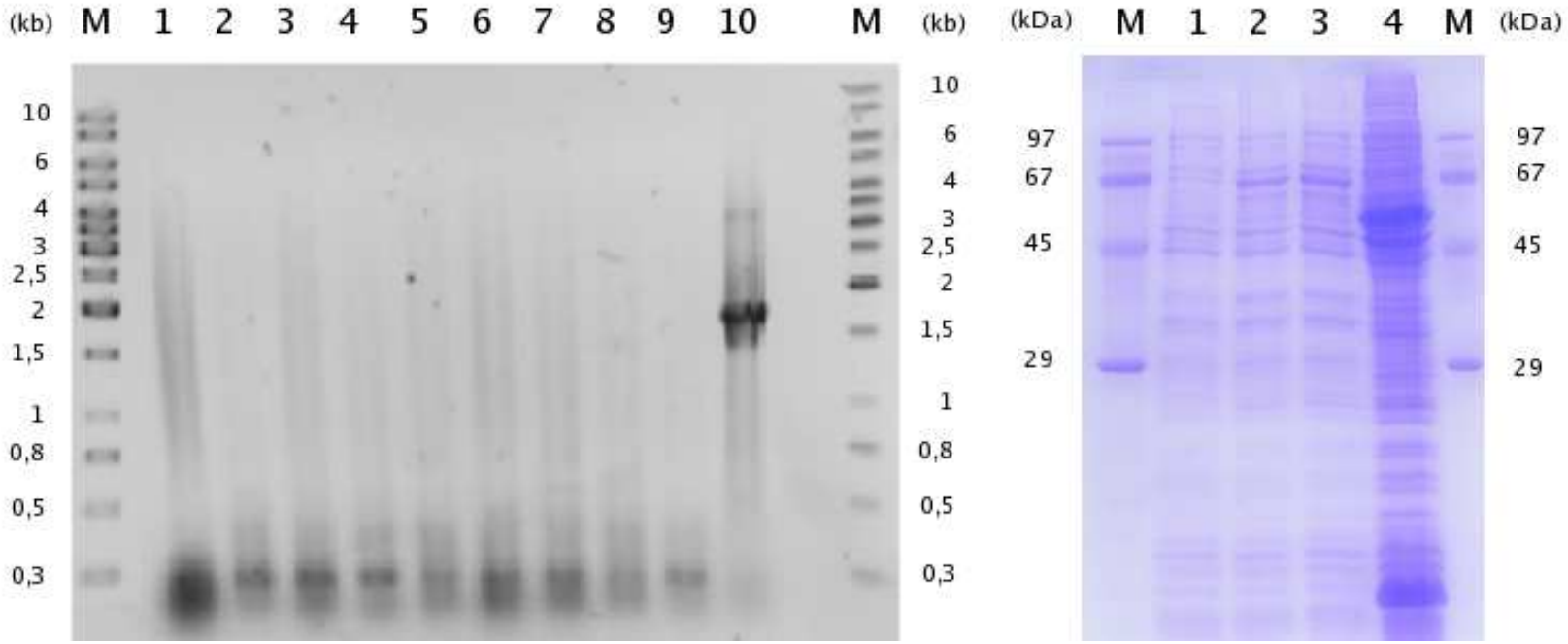



Bsp 1407 I
p51
Pst I
pQE−51
Bsp 1407 I
Pst I

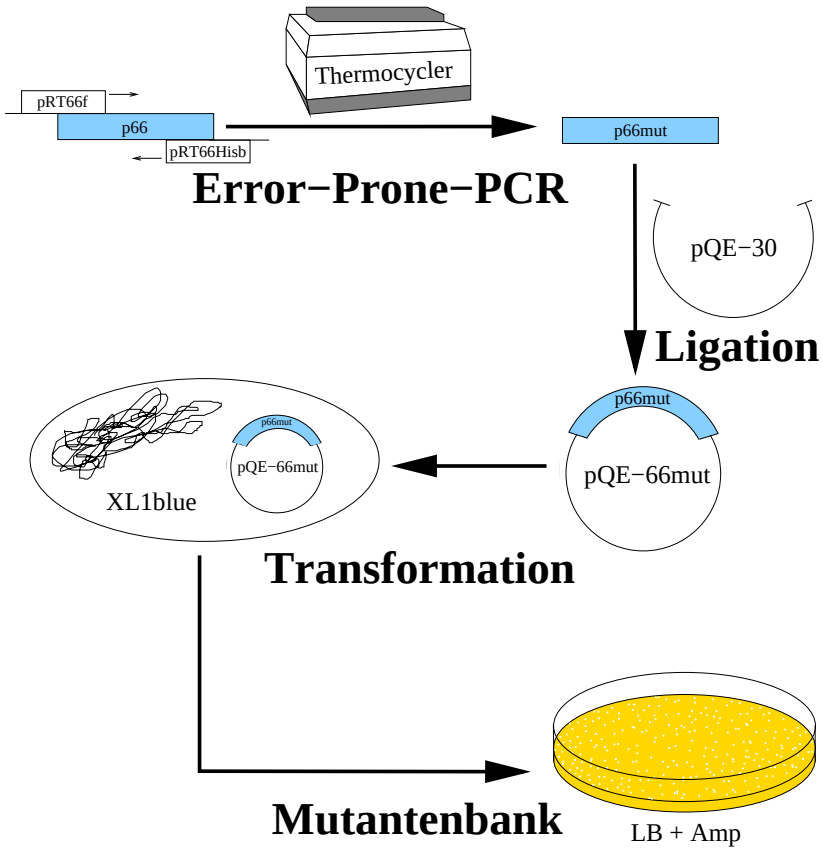

p66*
Bsp 1407 I
Pst I
p66*
Bsp 1407 I
Pst I


Ligation
p66
=
pQE−66

# results


(kb) M 1 2 3 4 5 6 7 8 9 10 M (kb)
10
6
4
3
2,5
2
1,5
1
0,8
0,5
0,3
(kDa) M 1 2 3 4 M (kDa)
97
67
45
29

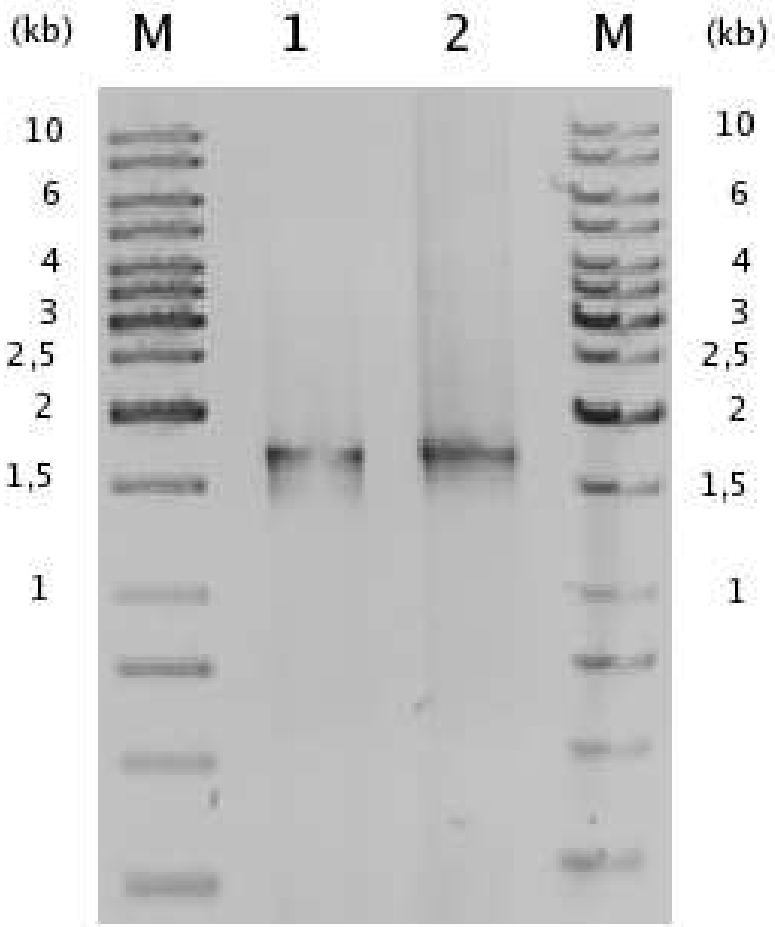
# construction of MB



# Error-Prone-PCR

| PCR 1 | PCR 2 | Error-Prone-PCR | description |
|---|---|---|---|
| | | 676.5ng | 0.2pmol DNA ($5125bp \cdot 660\frac{g}{mol}$) |
| 100ng | 50ng | | DNA |
| 2.5$\mu$l | 1.25$\mu$l | 2.5$\mu$l | Primer p66f (20$\mu$M ) |
| 2.5$\mu$l | 1.25$\mu$l | 2.5$\mu$l | Primer p66Hisb (20$\mu$M ) |
| 10$\mu$l | 5$\mu$l | 10$\mu$l | 10X PCR-Puffer |
| 0.25$\mu$l | 0.125$\mu$l | 0.2$\mu$l | dATP (100mM ) |
| 0.25$\mu$l | 0.125$\mu$l | 0.5$\mu$l | dCTP (100mM ) |
| 0.25$\mu$l | 0.125$\mu$l | 0.2$\mu$l | dGTP (100mM ) |
| 0.25$\mu$l | 0.125$\mu$l | 0.5$\mu$l | dTTP (100mM ) |
| - | - | 1$\mu$l | $MnCl_2$ 100X |
| ad 98$\mu$l | ad 49$\mu$l | ad 98$\mu$l | d$H_2O$ |

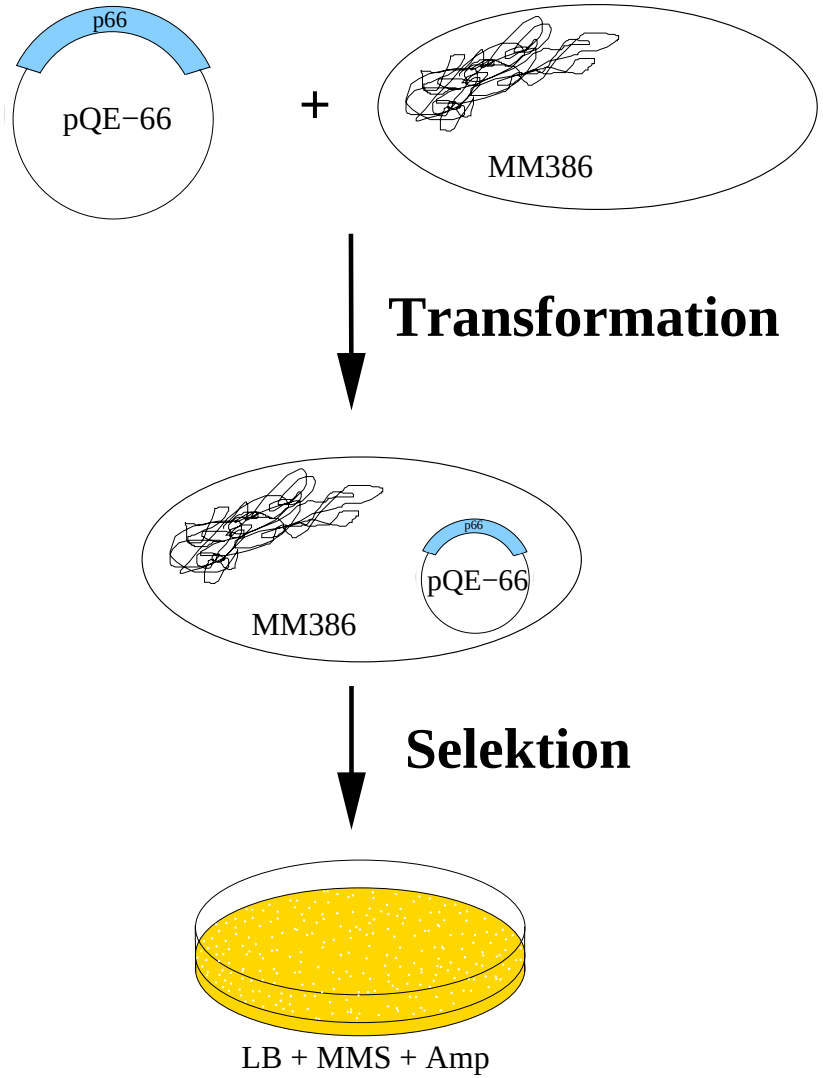
# results



# determine cfu

| approach | $\mu$l | colonies | cfu |
|---|---|---|---|
| 1 | 200 | 304 | $1,31 \cdot 10^3$ |
| 2 | 100 | 148 | $2,47 \cdot 10^3$ |
| 3 | 200 | 1200 | $3,00 \cdot 10^3$ |

provement polymerase activity of *p66*


p66
pQE−66
+
MM386
Transformation
MM386
p66
pQE−66
Selektion
LB + MMS + Amp

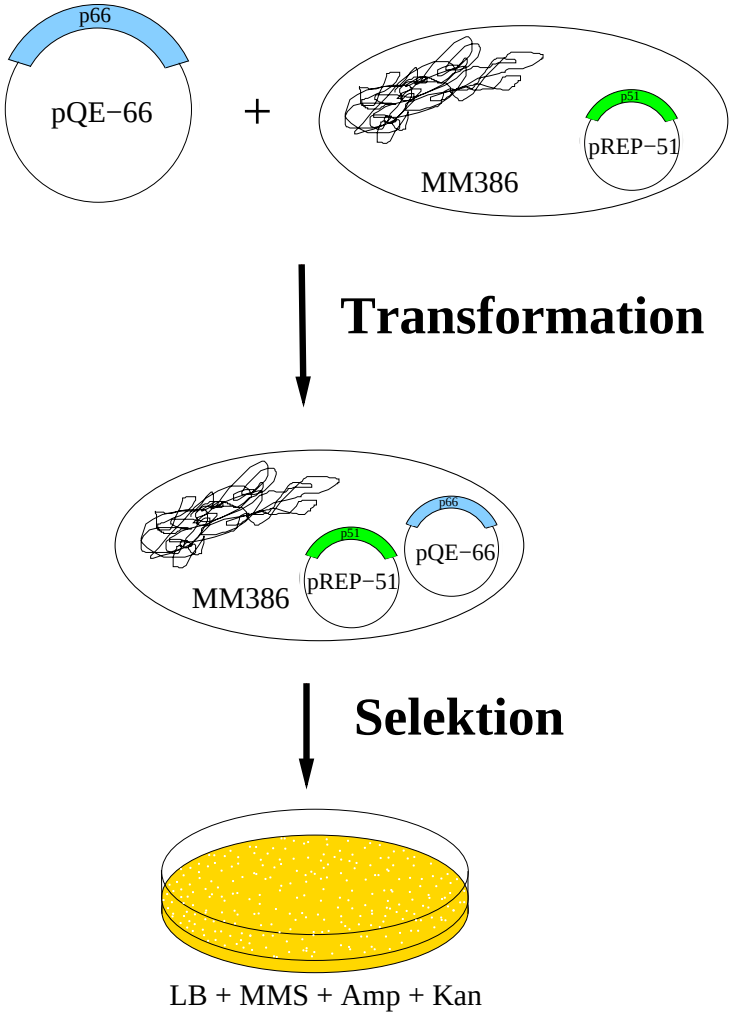
# results

| | no plasmid | no plasmid | | *p66* | *p66* | *p66* | |
|---|---|---|---|---|---|---|---|
| | LB | LB+MMS | | LB+Amp | Amp(FK) | LB+MMS+Amp | |
| | 42°C | 37°C | 42°C | 42°C | 37°C | 37°C | 42°C |
| Versuch | 50$\mu$l | 50$\mu$l | 100$\mu$l | 50$\mu$l | 100$\mu$l | 50$\mu$l | 100$\mu$l |
| 1 | Rasen | >1000 | >1000 | Rasen | 0 | 0 | 0 |
| 2 | Rasen | Rasen | Rasen | 2200 | 17 | 0 | 0 |
| 3 | Rasen | Rasen | Rasen | Rasen | 0 | 0 | 0 |
| expected | Rasen | Rasen | 0 | Rasen | | Rasen | some clones |

# MM386-Test

| *E. coli* | $\mu$l | °C | MMS | colonies | cfu |
|---|---|---|---|---|---|
| MM386 | 100 | 42 | + | 0 | |
| MM386 | 25 | 42 | + | 0 | |
| MM386 | 25 | 37 | − | Rasen | |
| MM386 $\cdot 10^{-5}$ | 50 | 37 | − | $\sim 500$ | $\sim 1,0 \cdot 10^9$ |
| MM386 $\cdot 10^{-6}$ | 50 | 37 | − | $\sim 320$ | $\sim 6,4 \cdot 10^9$ |

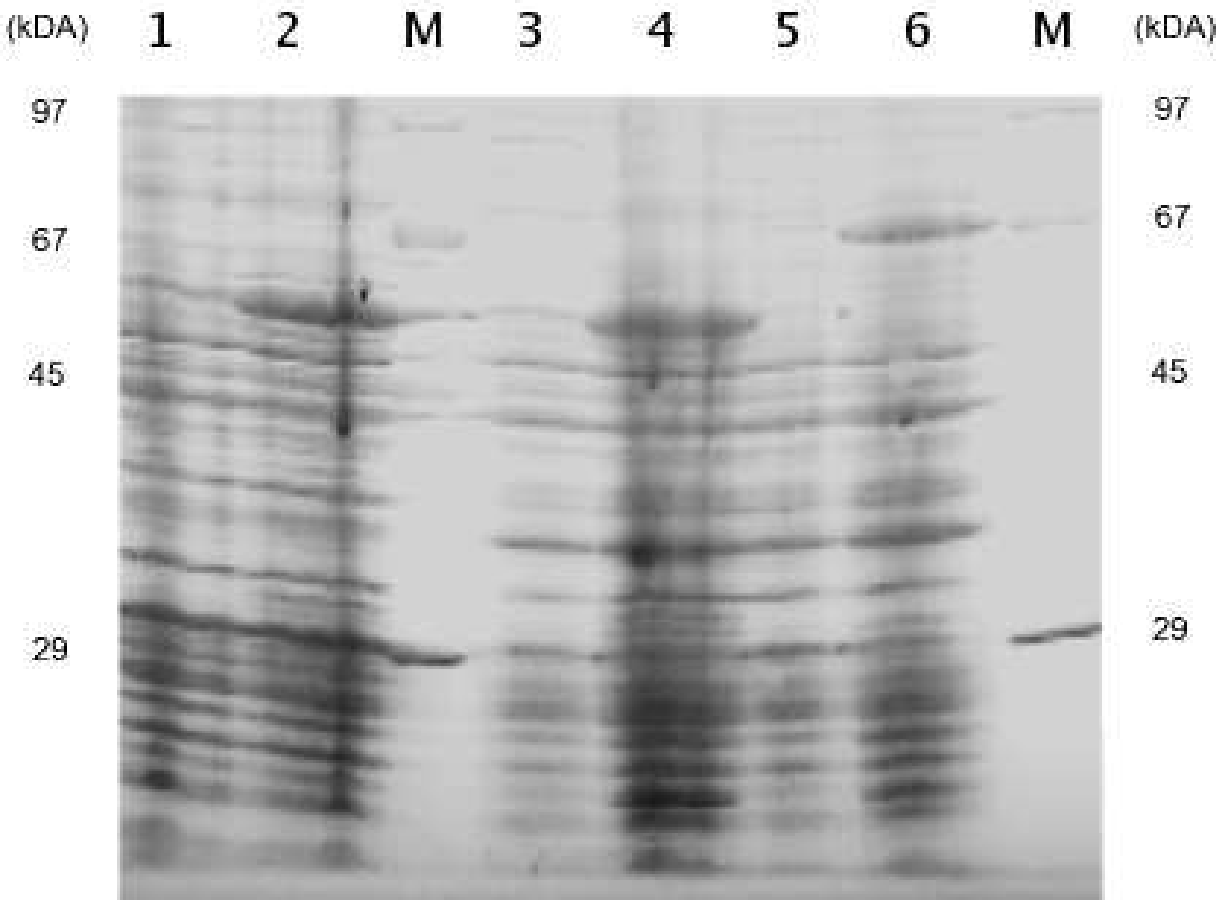
# polymerase activity of *p66*- 2. approach



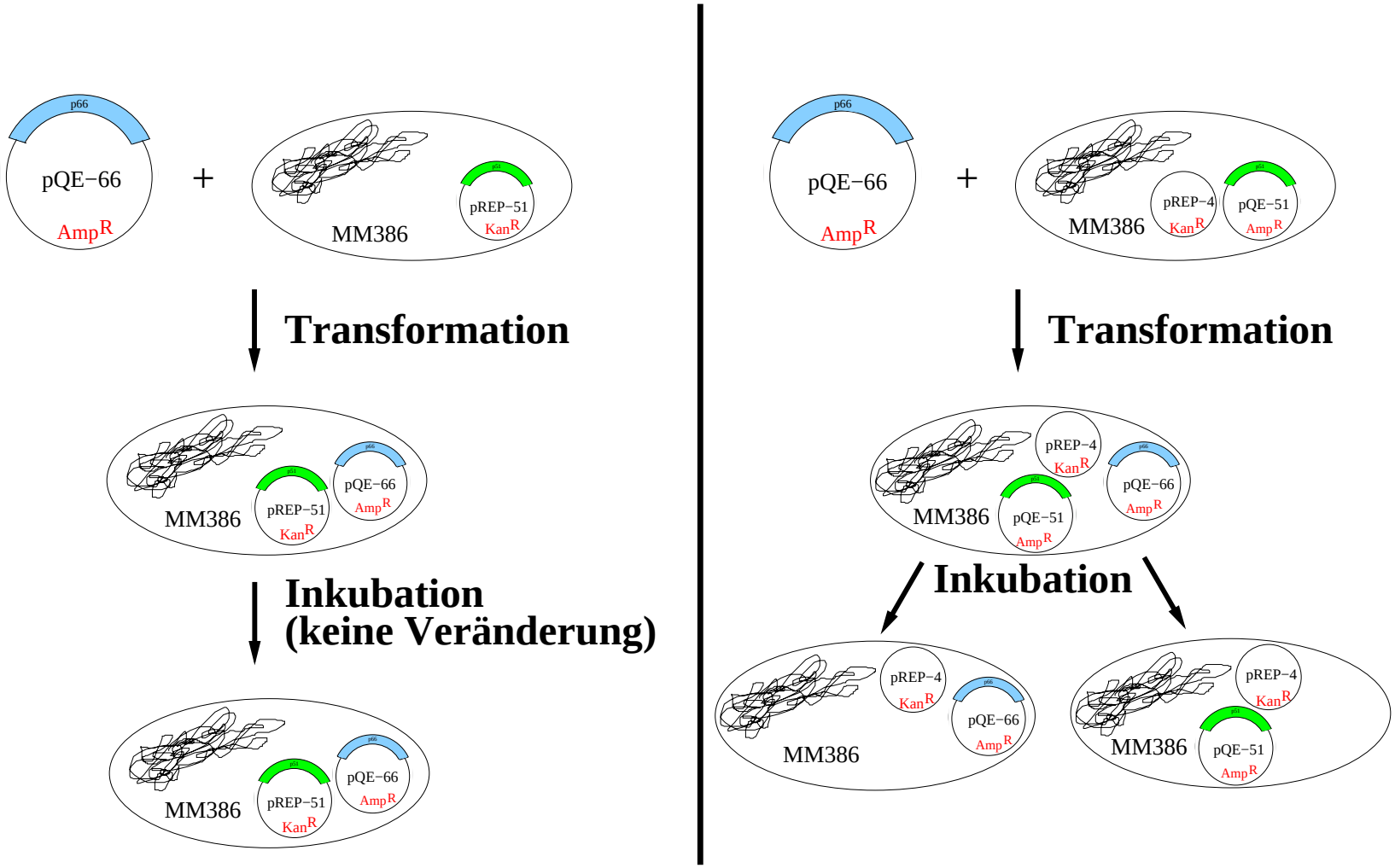
# results - 2. approach

| | no plasmid | no plasmid | | *p51*, *p66* | *p51*, *p66* | |
|---|---|---|---|---|---|---|
| | LB | LB+MMS | | LB+Amp+Kan | LB+MMS+Amp+Kan | |
| | 42°C | 37°C | 42°C | 42°C | 37°C | 42°C |
| Versuch | 50$\mu$l | 50$\mu$l | 100$\mu$l | 50$\mu$l | 50$\mu$l | 100$\mu$l |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | Rasen | Rasen | 0 | Rasen | 0 | 0 |
| expected | Rasen | Rasen | 0 | Rasen | Rasen | some clones |

# sample tests



# possible explanation



# further views