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THE ROLE OF T-CELL THERAPY WITHIN COLORECTAL CANCER

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&

PhD. Candidate Michael Magee

Dr. Scott Waldman's Lab

COLORECTAL CANCER

Leading New Cancer Cases and Deaths – 2012 Estimates

Estimated New Cases*		Estimated Deaths	
Male	**Female**	**Male**	**Female**
Prostate 241,740 (29%)	Breast 226,870 (29%)	Lung & bronchus 87,750 (29%)	Lung & bronchus 72,590 (26%)
Lung & bronchus 116,470 (14%)	Lung & bronchus 109,690 (14%)	Prostate 28,170 (9%)	Breast 39,510 (14%)
Colon & rectum 73,420 (9%)	Colon & rectum 70,040 (9%)	Colon & rectum 26,470 (9%)	Colon & rectum 25,220 (9%)
Urinary bladder 55,600 (7%)	Uterine corpus 47,130 (6%)	Pancreas 18,850 (6%)	Pancreas 18,540 (7%)
Melanoma of the skin 44,250 (5%)	Thyroid 43,210 (5%)	Liver & intrahepatic bile duct 13,980 (5%)	Ovary 15,500 (6%)
Kidney & renal pelvis 40,250 (5%)	Melanoma of the skin 32,000 (4%)	Leukemia 13,500 (4%)	Leukemia 10,040 (4%)
Non-Hodgkin lymphoma 38,160 (4%)	Non-Hodgkin lymphoma 31,970 (4%)	Esophagus 12,040 (4%)	Non-Hodgkin lymphoma 8,620 (3%)
Oral cavity & pharynx 28,540 (3%)	Kidney & renal pelvis 24,520 (3%)	Urinary bladder 10,510 (3%)	Uterine corpus 8,010 (3%)
Leukemia 26,830 (3%)	Ovary 22,280 (3%)	Non-Hodgkin lymphoma 10.320 (3%)	Liver & intrahepatic bile duct 6,570 (2%)
Pancreas 22,090 (3%)	Pancreas 21,830 (3%)	Kidney & renal pelvis 8,650 (3%)	Brain & other nervous system 5,980 (2%)
All sites 848,170 (100%)	All sites 790,740 (100%)	All sites 301,820 (100%)	All sites 275,370 (100%)

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

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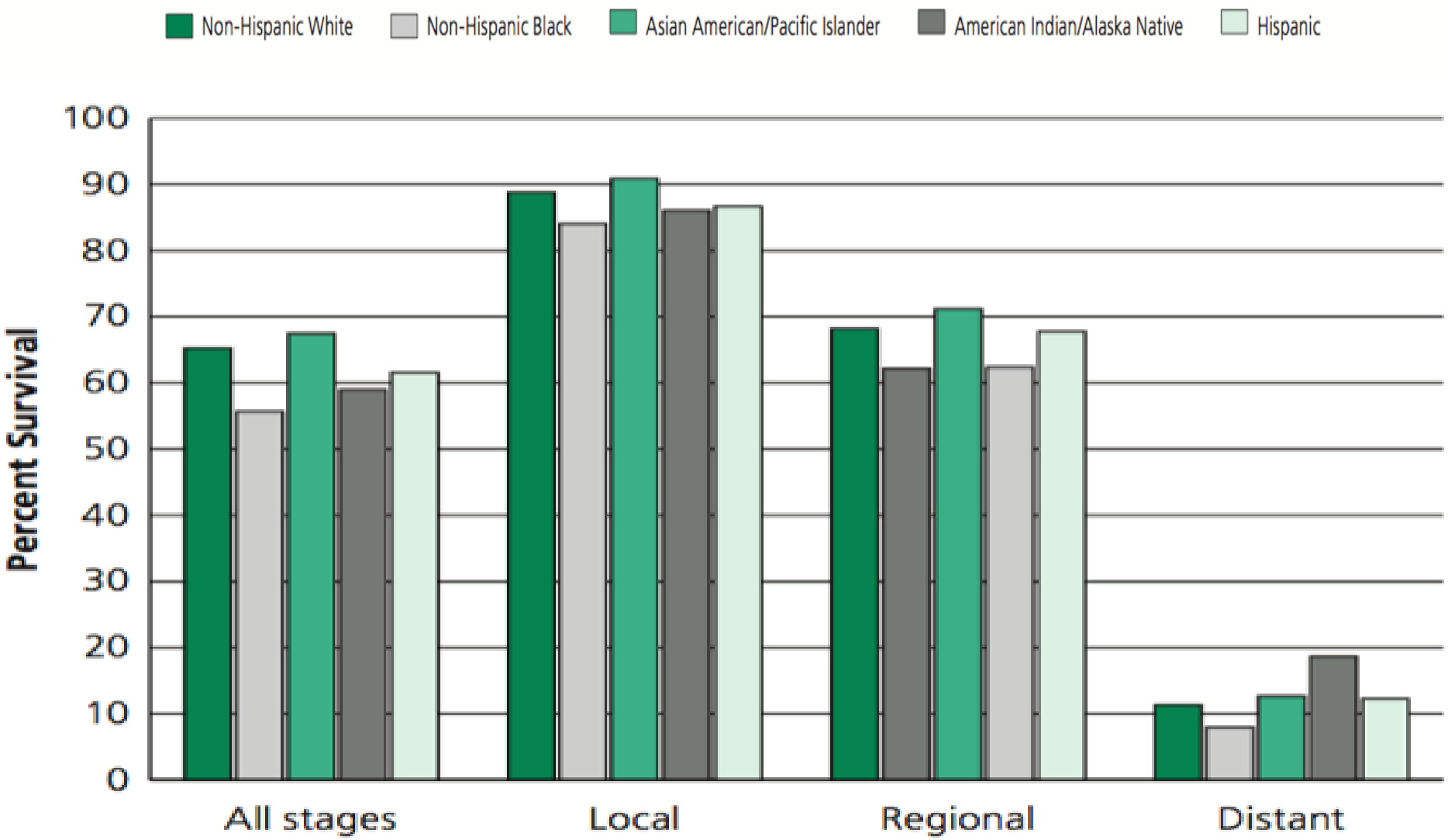
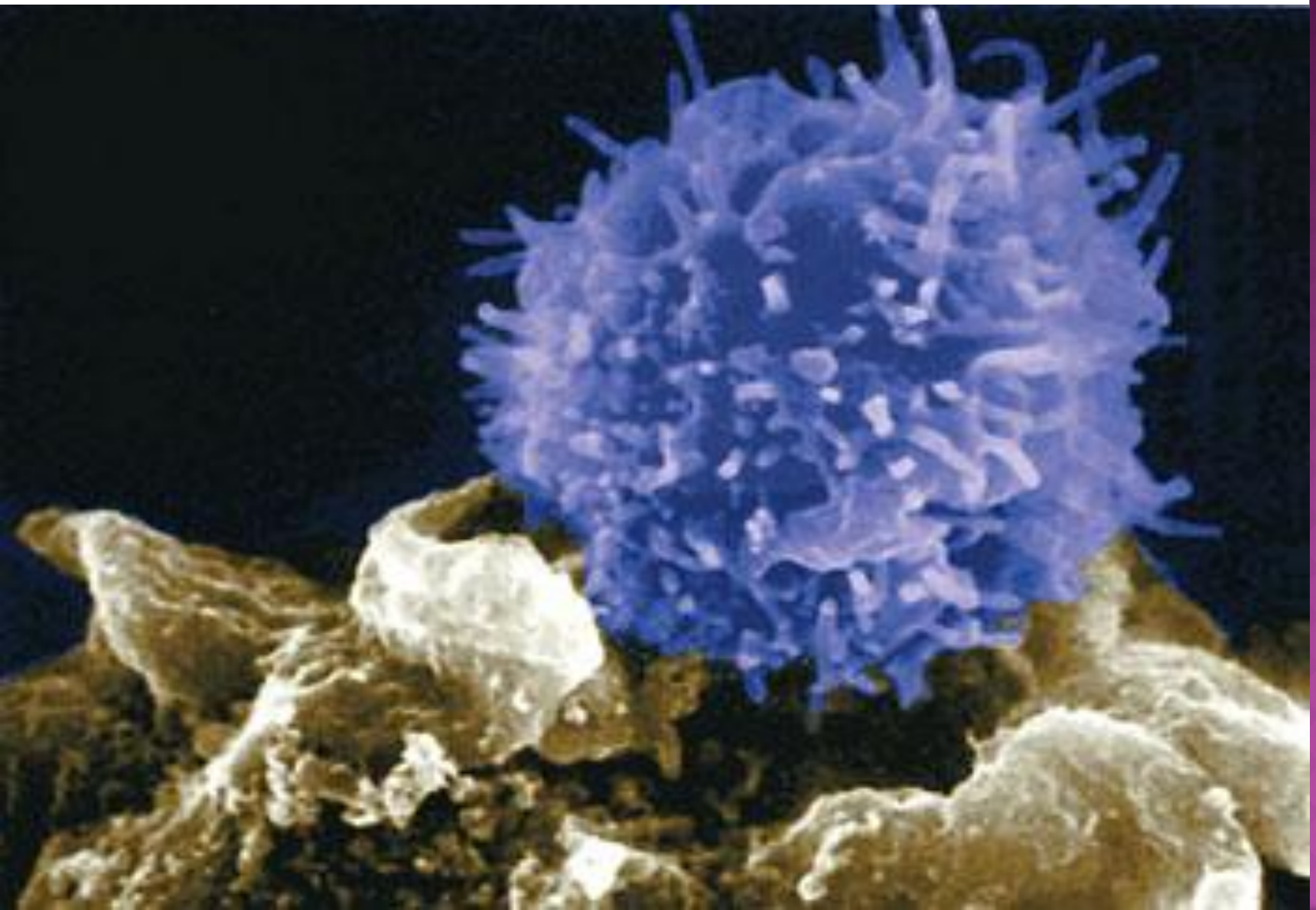
COLORECTAL CANCER STATISTICS

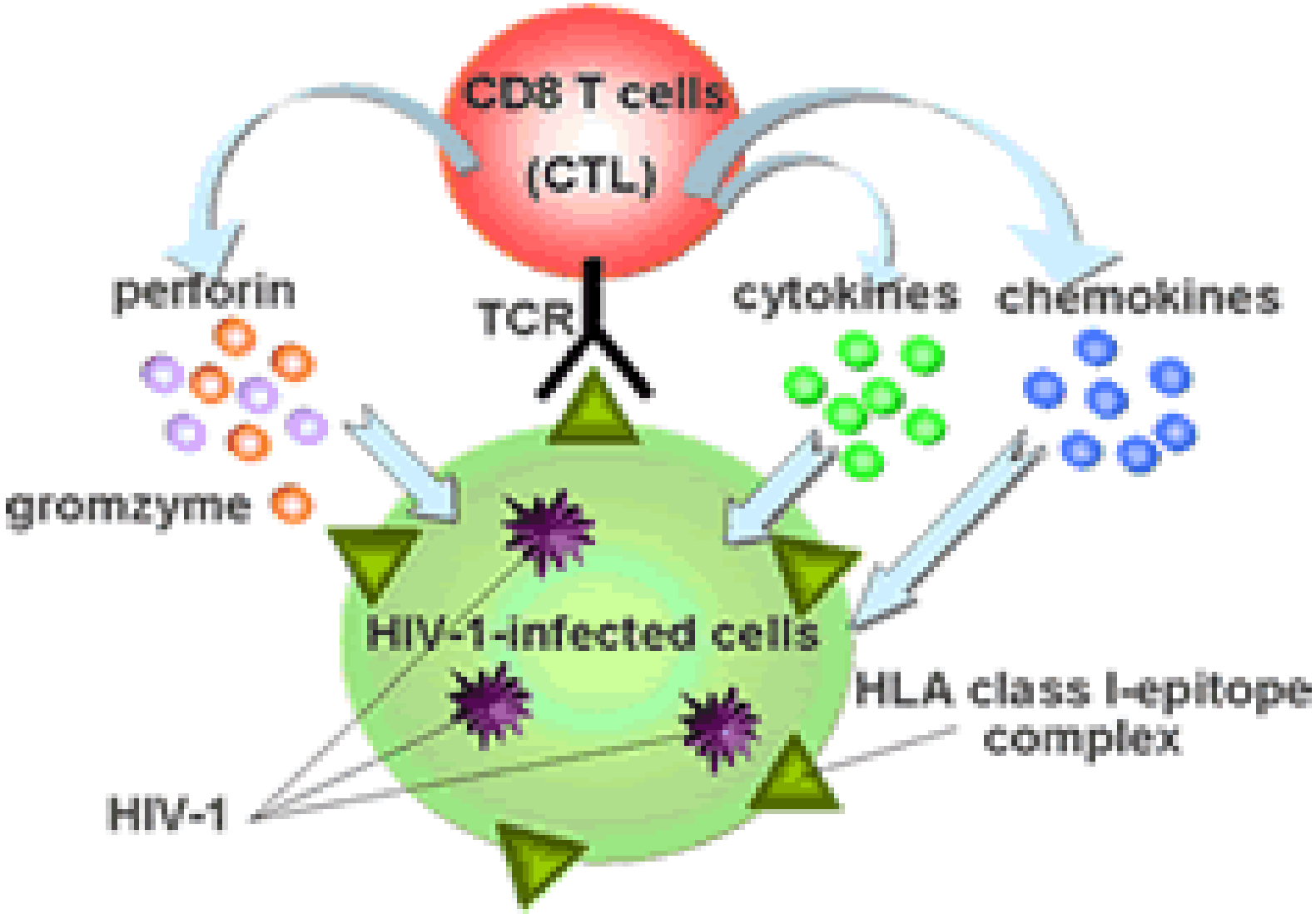
Figure 6. Five-year Colorectal Cancer-specific Survival* by Stage and Race/Ethnicity, 1999-2006



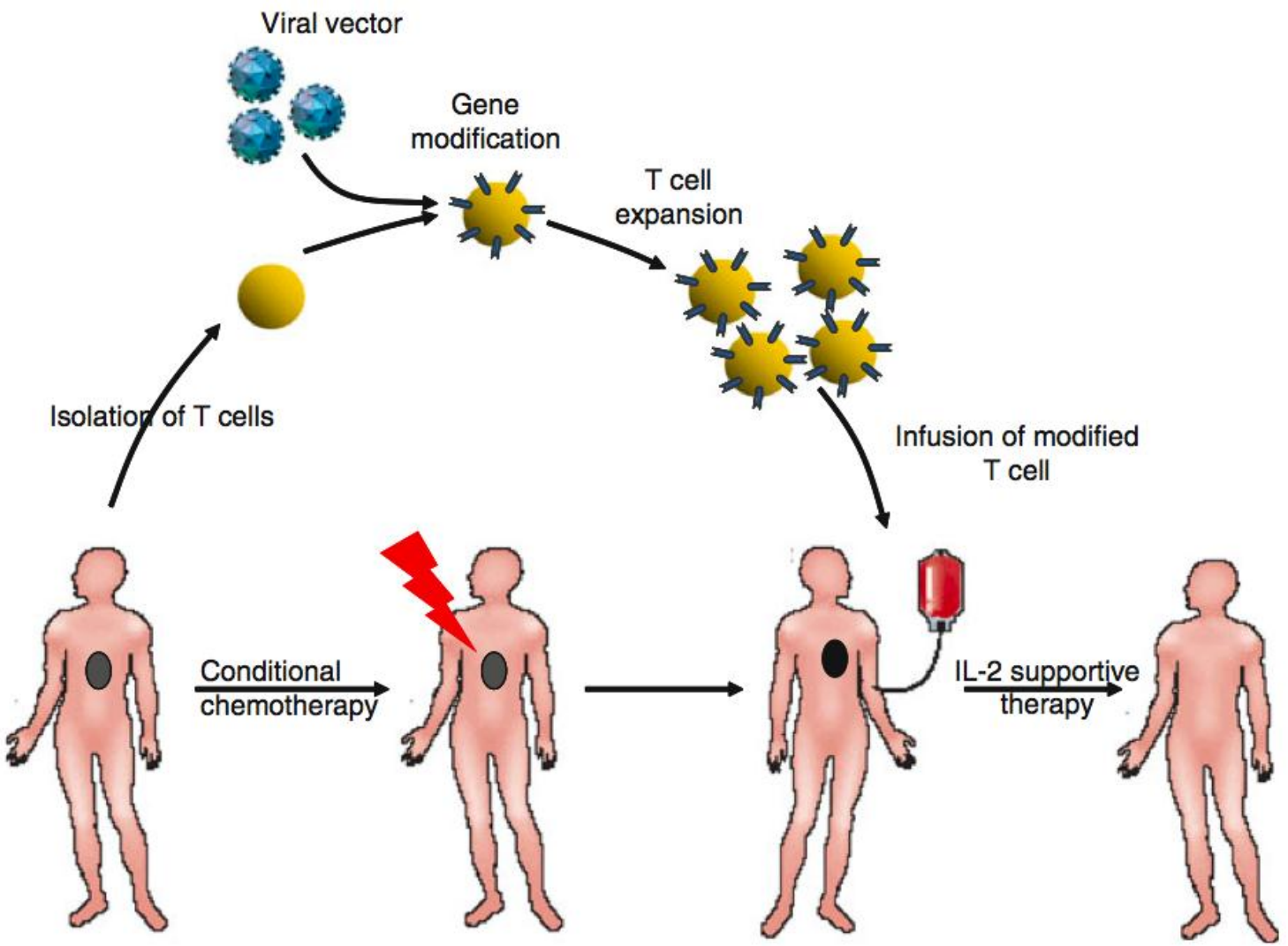
WHAT ARE T-CELLS?



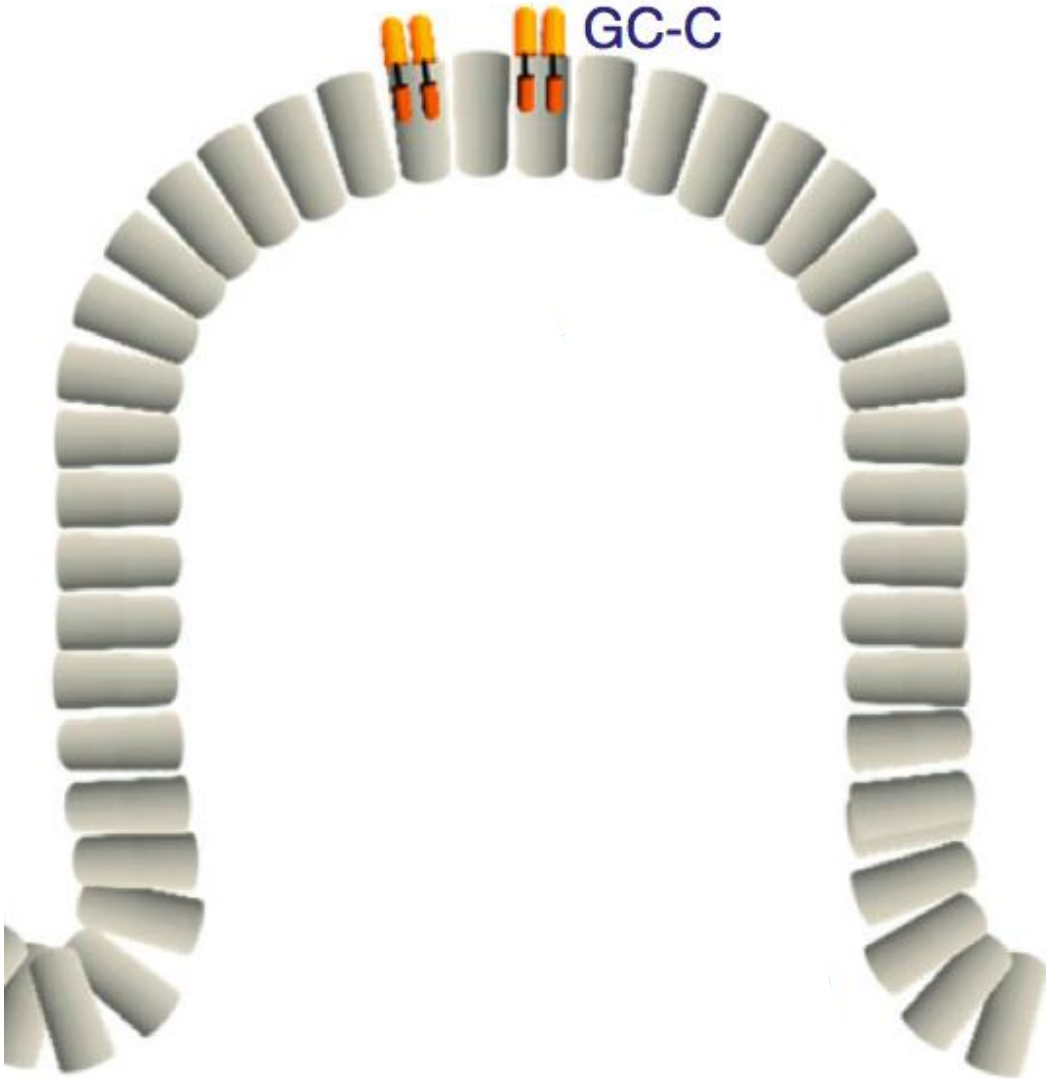
CD8 T-CELLS


CD8 T cells
(CTL)
perforin
TCR
cytokines
chemokines
gromzyme
HIV-1-infected cells
HLA class I-epitope
complex
HIV-1

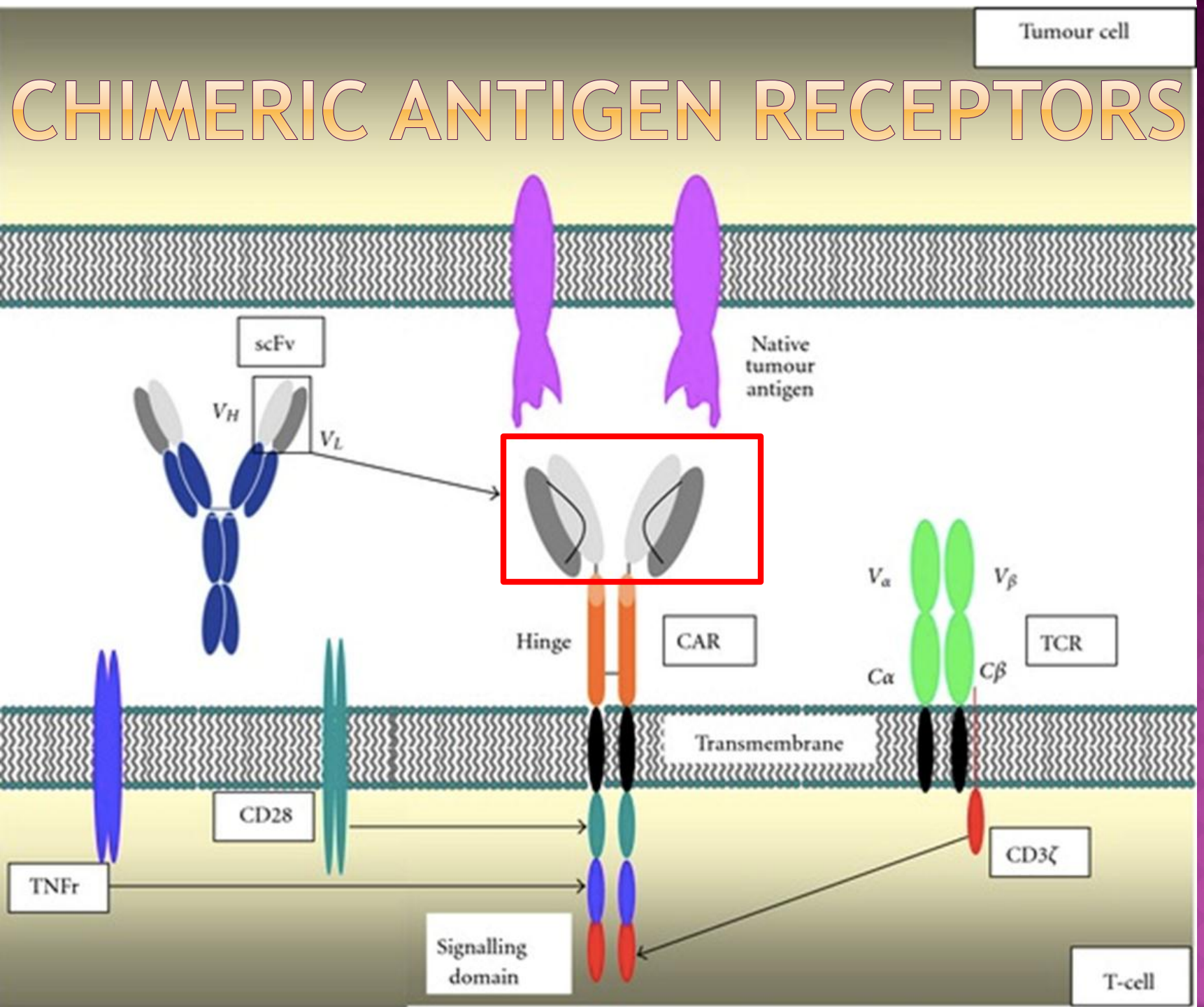
ADOPTIVE T-CELL THERAPY



GUANYLYL CYCLASE C (GCC)

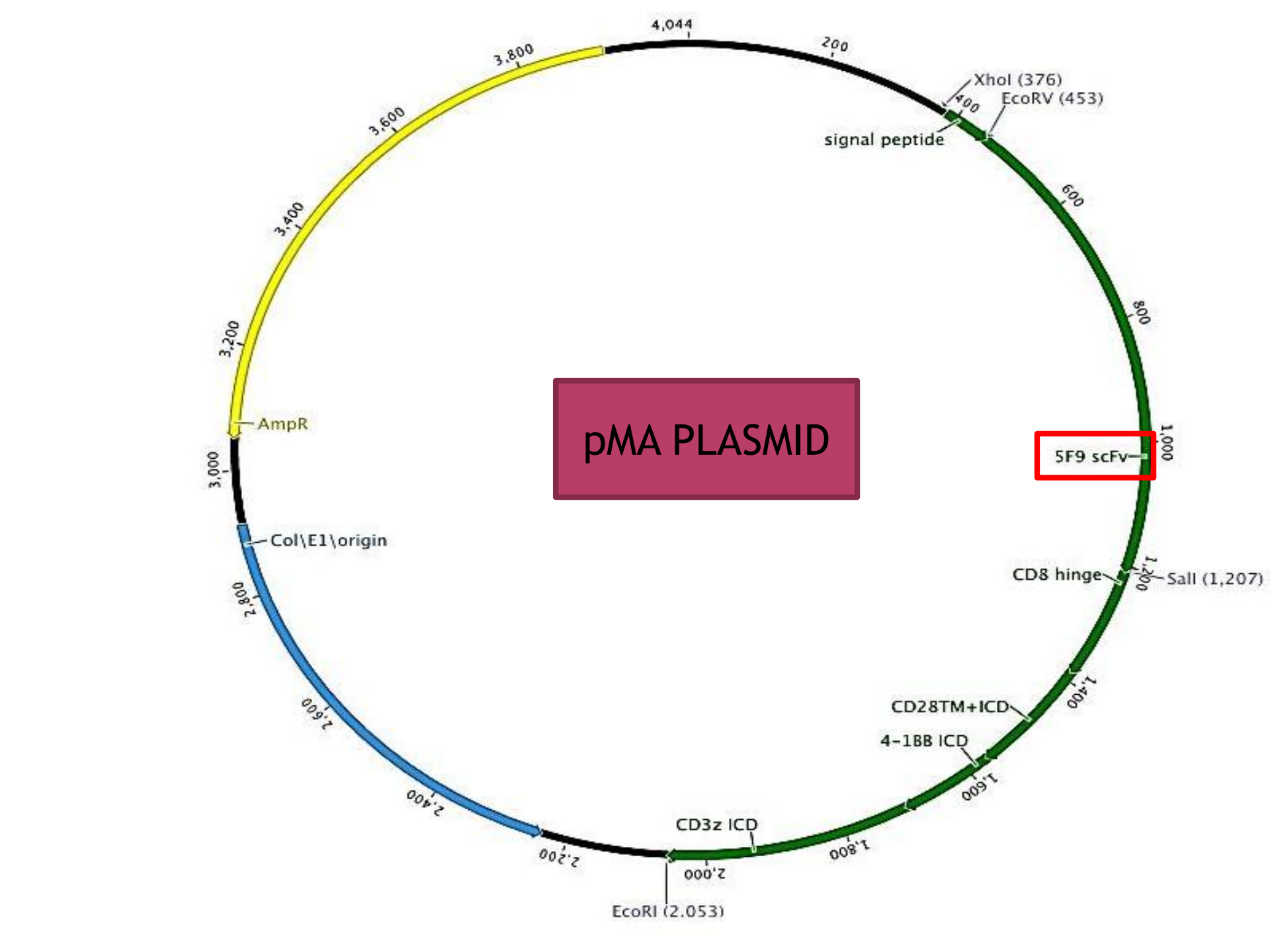


CHIMERIC ANTIGEN RECEPTORS

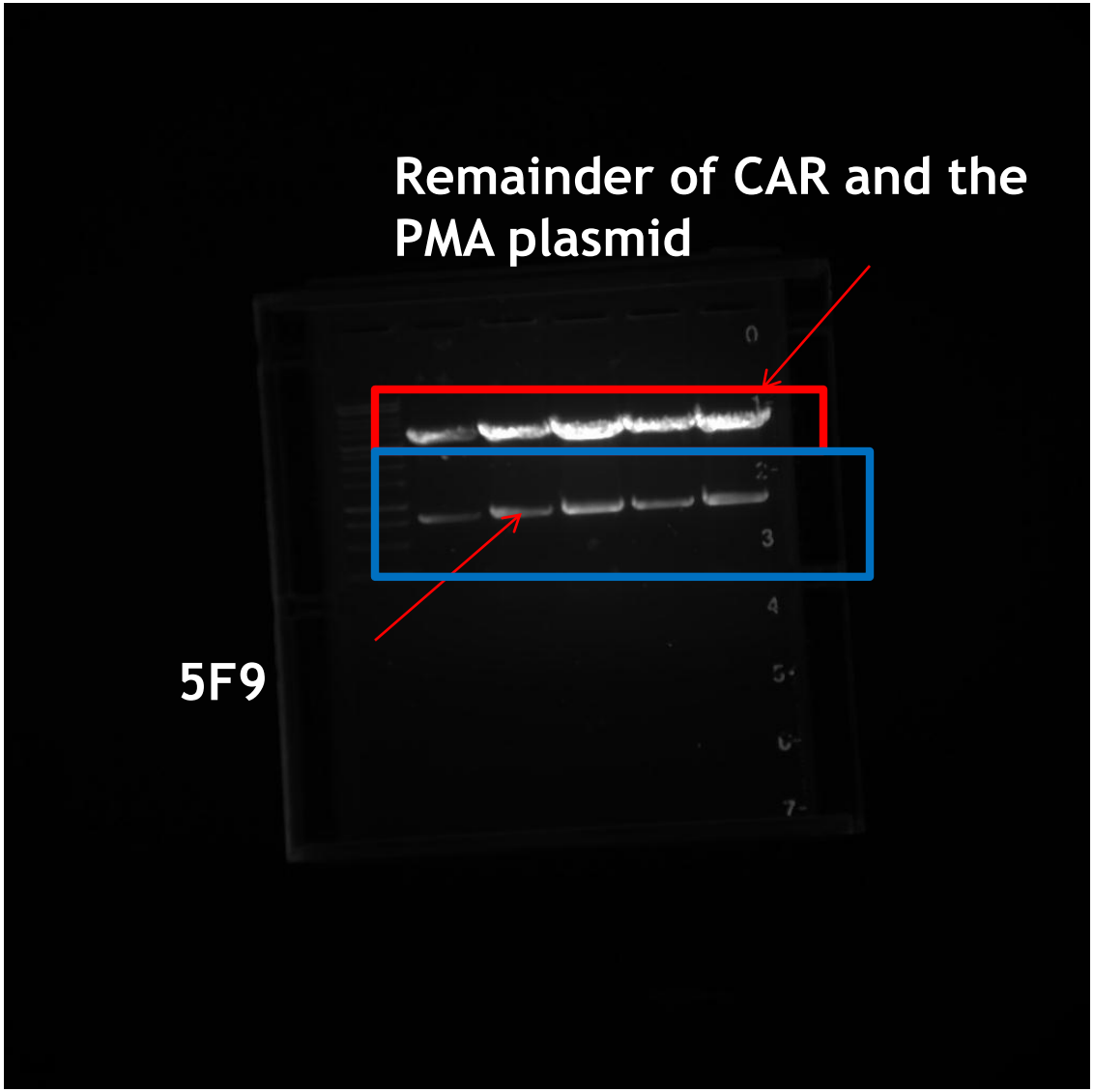


OVERVIEW

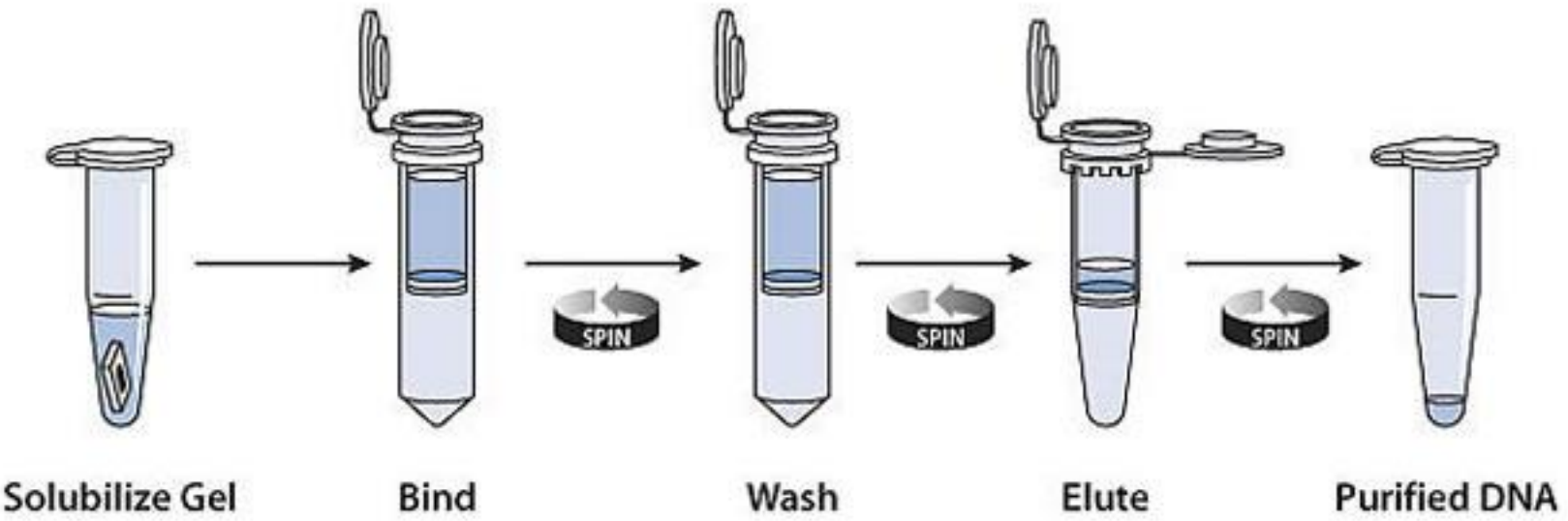
- Create 12 CAR PMA PLASMIDS
  - **Abx012**
  - **Abx020**
  - **Abx106**
  - **Abx198**
  - **Abx221**
  - **Abx229**
  - **Abx338**
  - **Abx393**
  - **6H8**
  - **8C2**
  - **10C10**
  - **10D3**
- Transfer CAR's to retroviral (MIG) plasmid
- Transduce T-cells with retrovirus to obtain T-cells expressing CAR DNA
- T-cell activation


pMA PLASMID
4,044
200
XhoI (376)
400
EcoRV (453)
signal peptide
600
800
1,000
5F9 scFv
1,200
SalI (1,207)
CD8 hinge
1,400
CD28TM+ICD
4-1BB ICD
1,600
1,800
CD3z ICD
2,000
EcoRI (2,053)
2,200
2,400
2,600
2,800
Col\E1\origin
3,000
AmpR
3,200
3,400
3,600
3,800

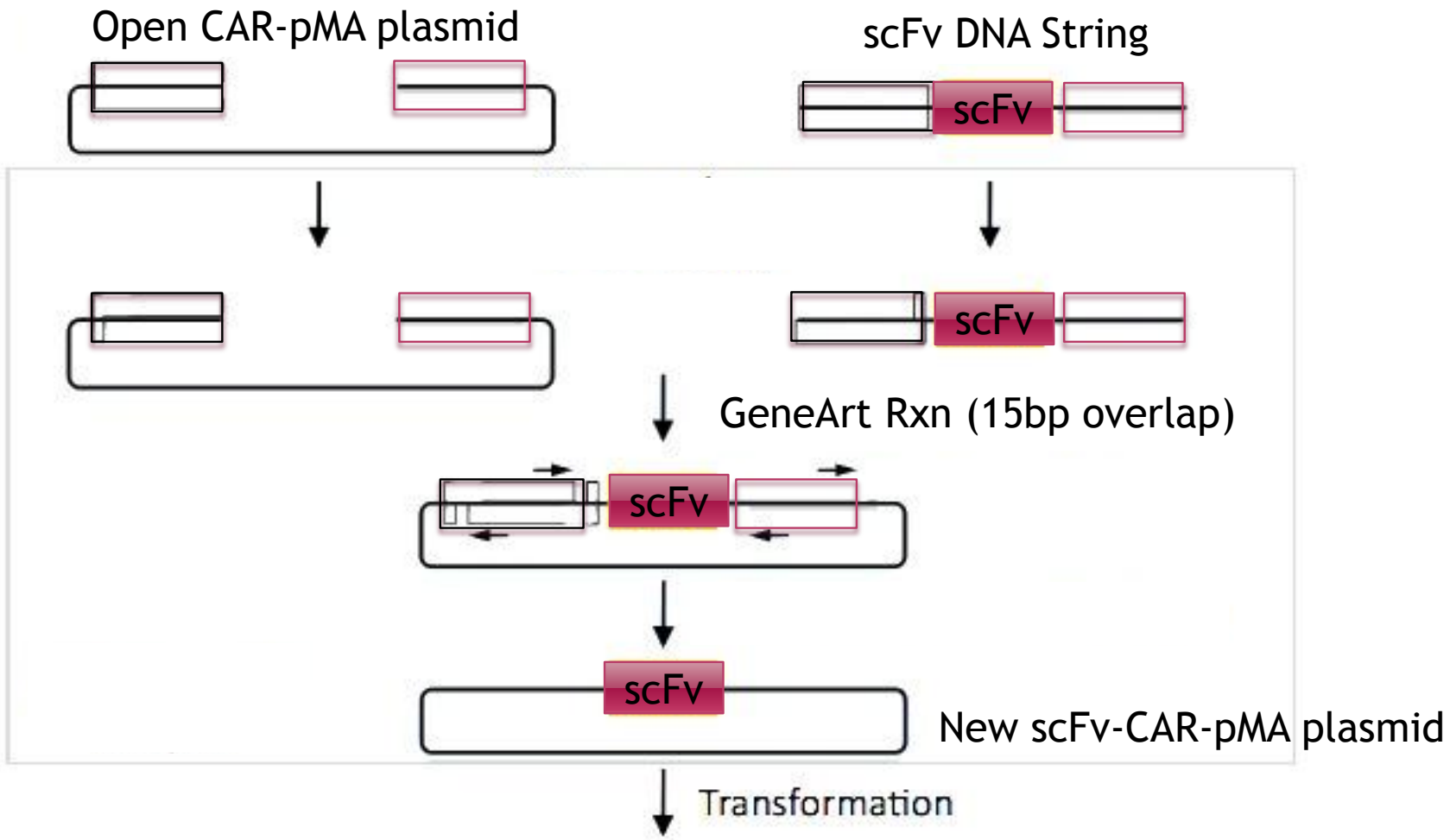
DIGESTION REACTION



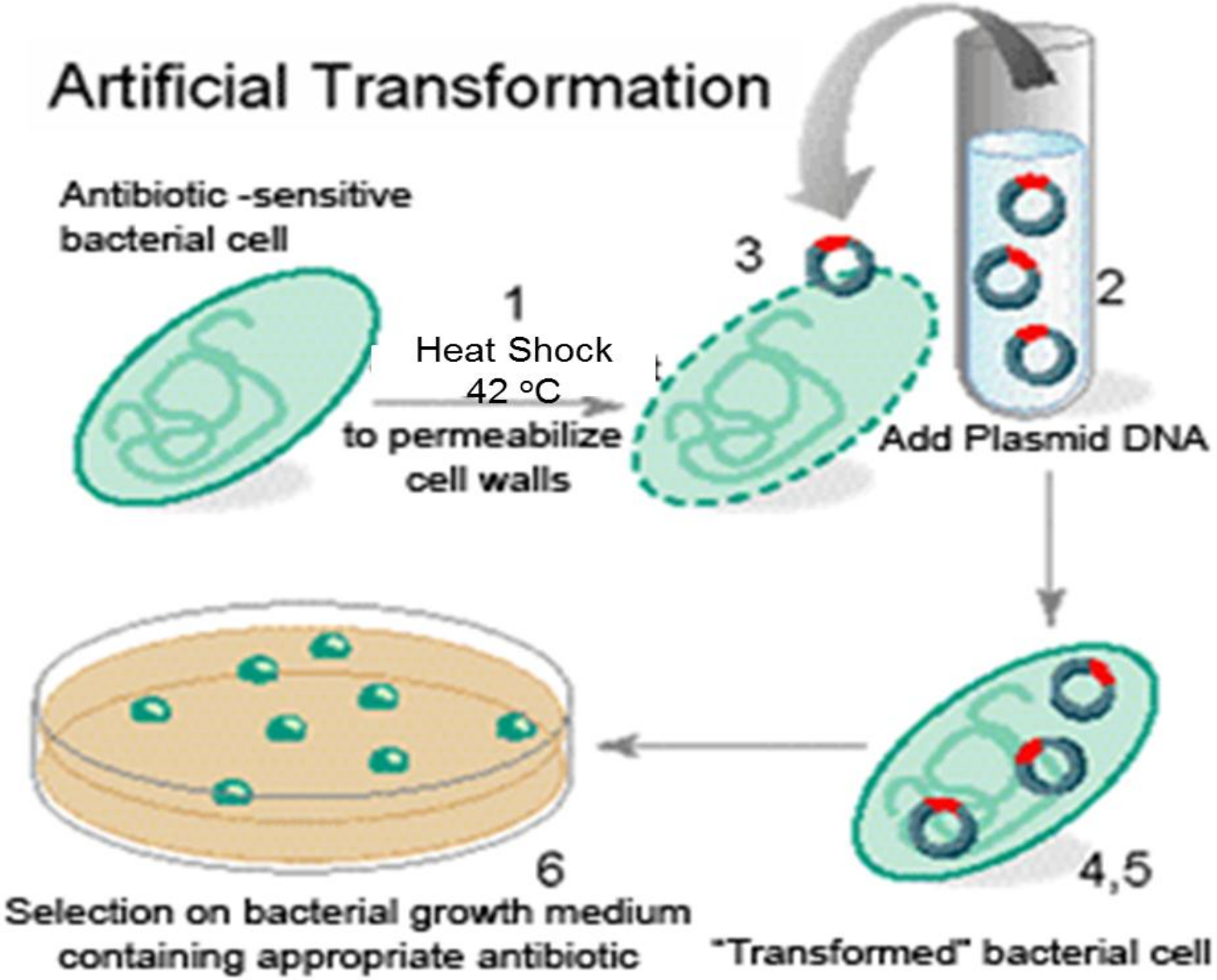
GEL EXTRACTION



GENEART ASSEMBLY



TRANSFORMATION REACTION



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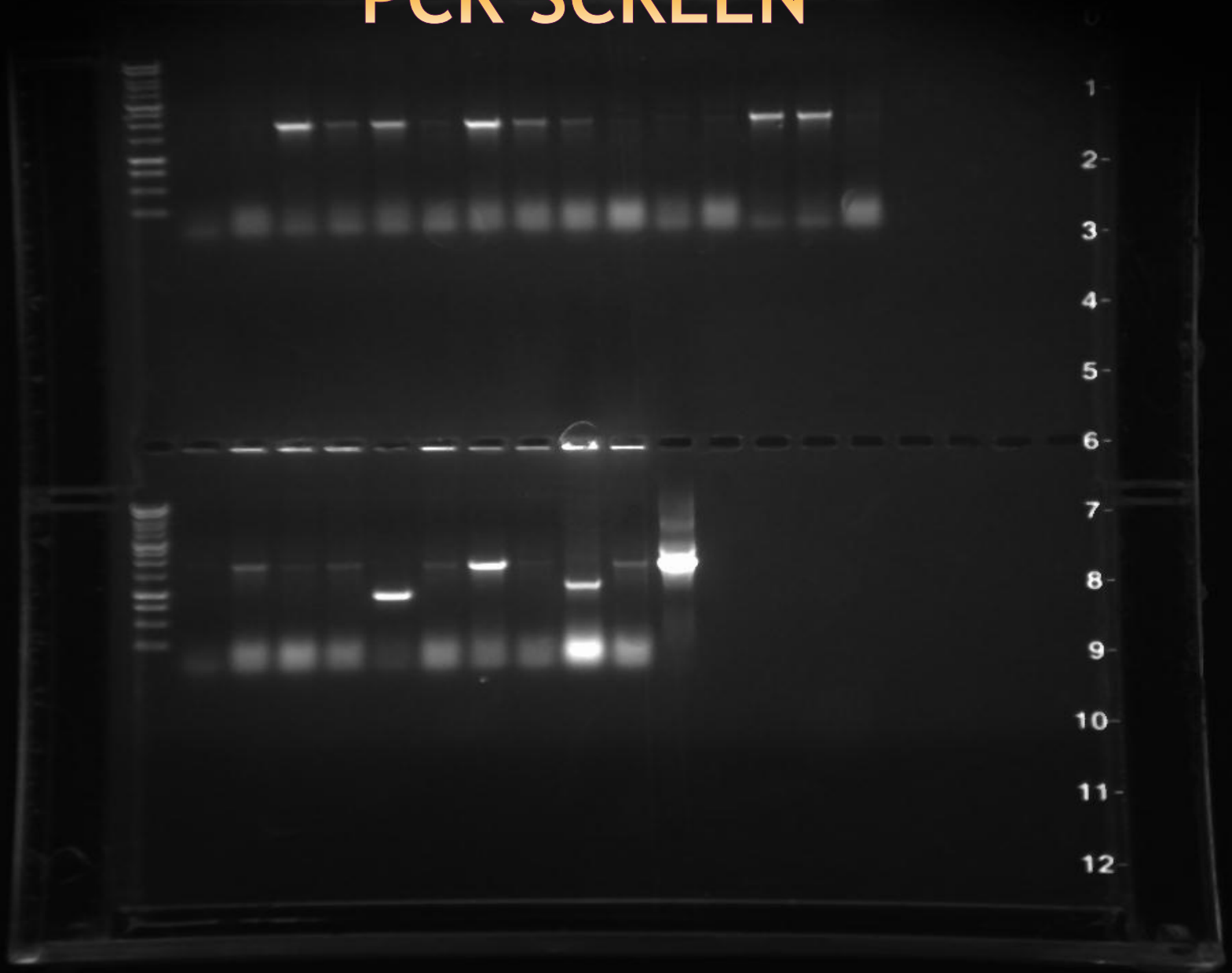
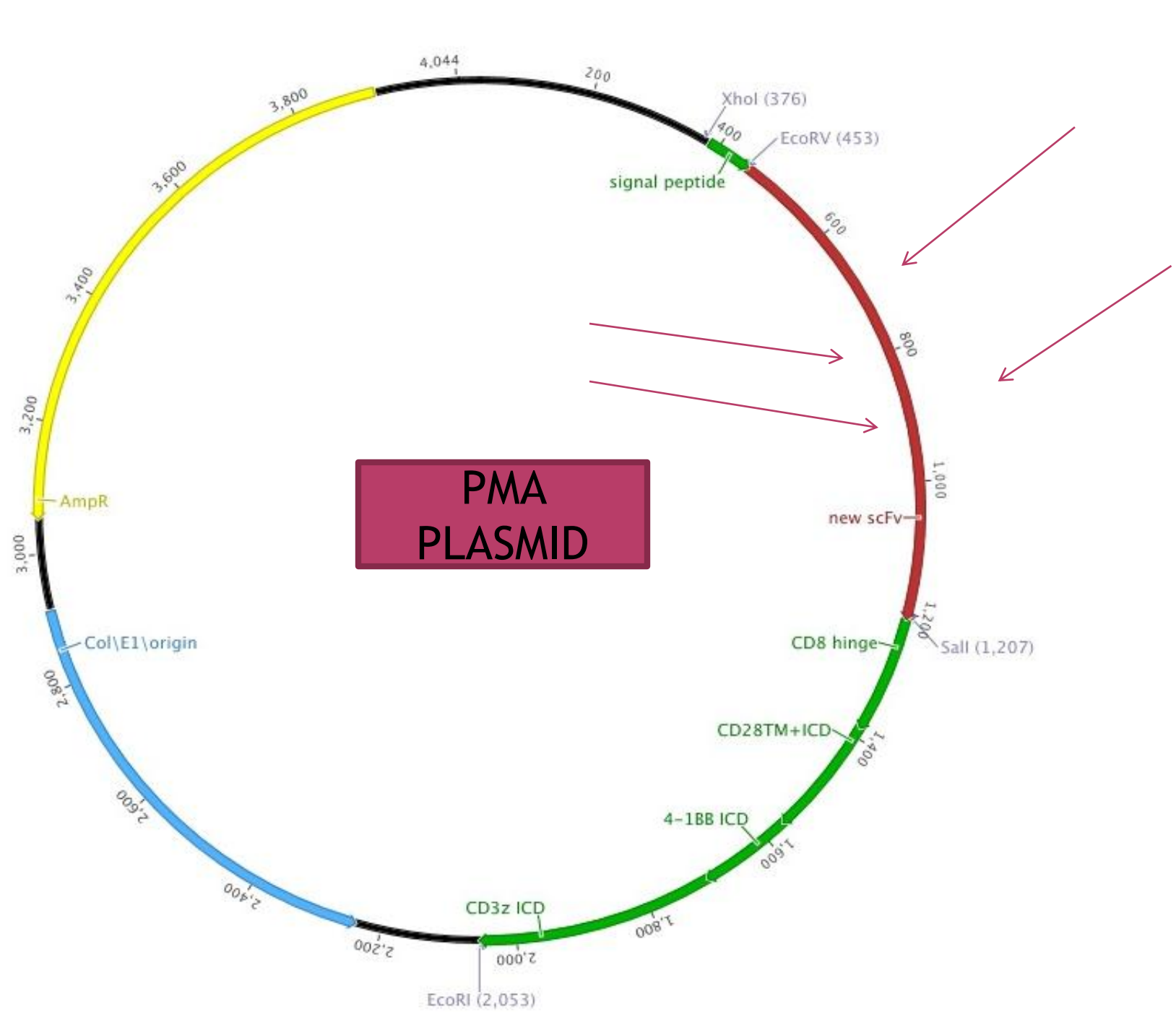
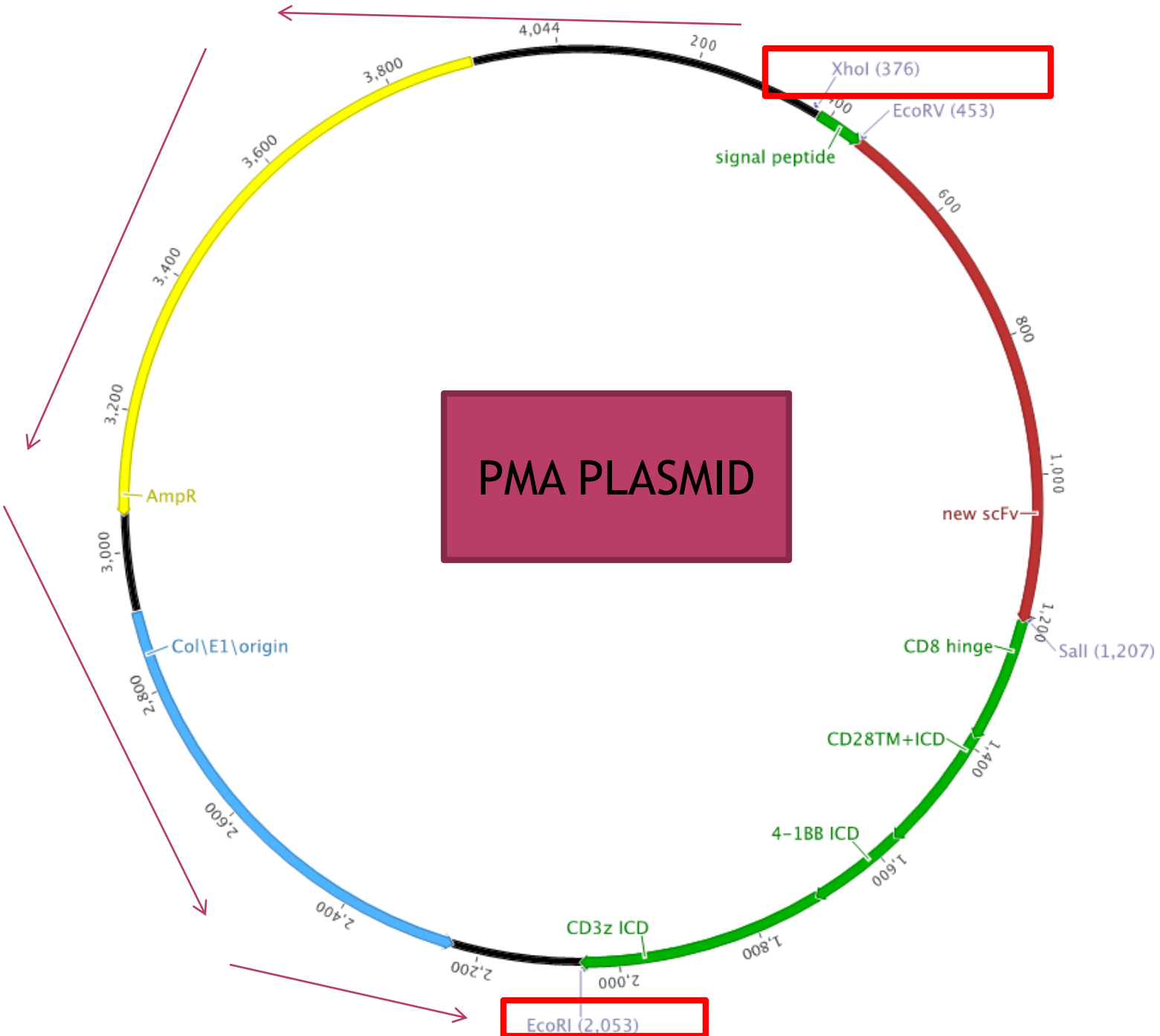
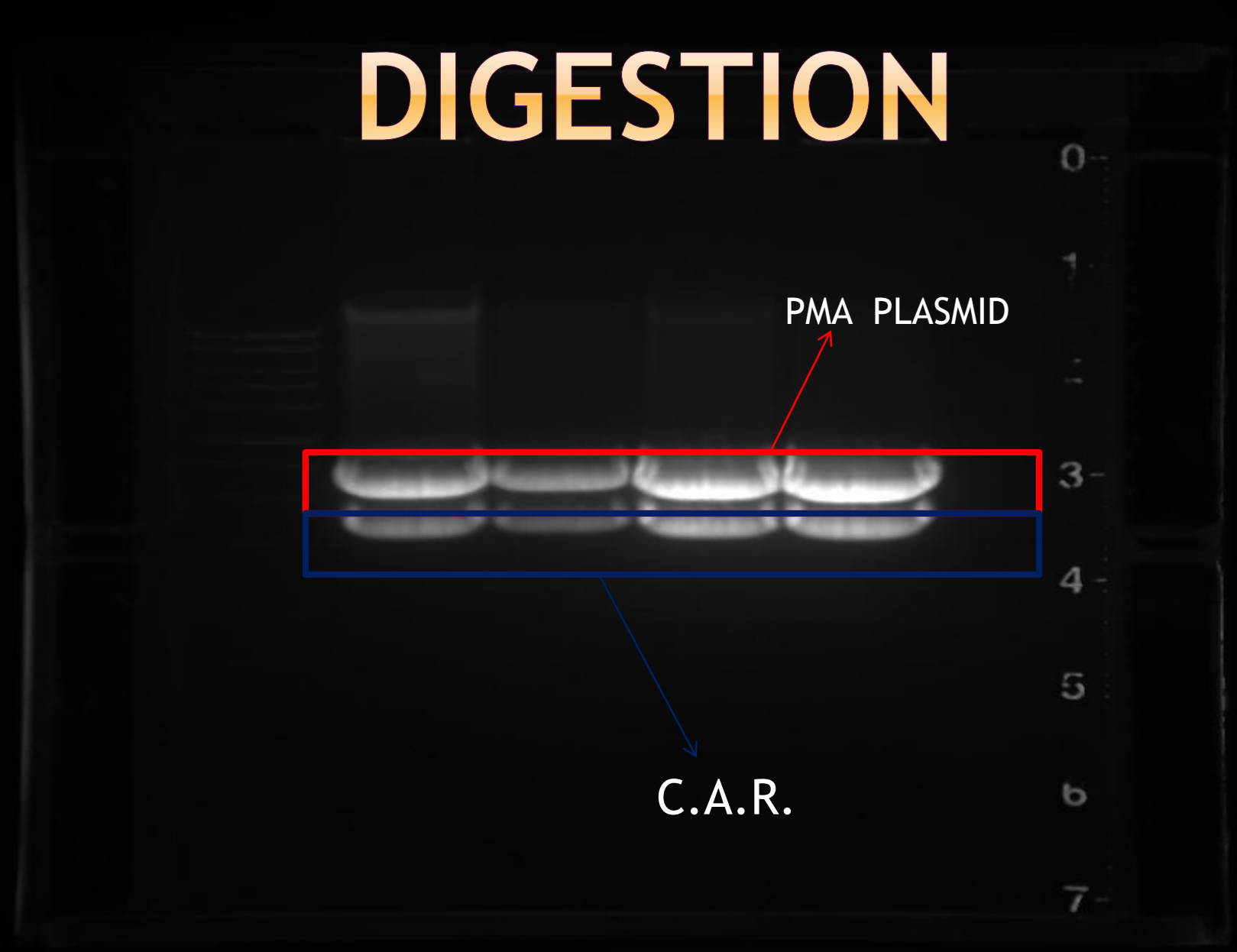
PCR SCREEN

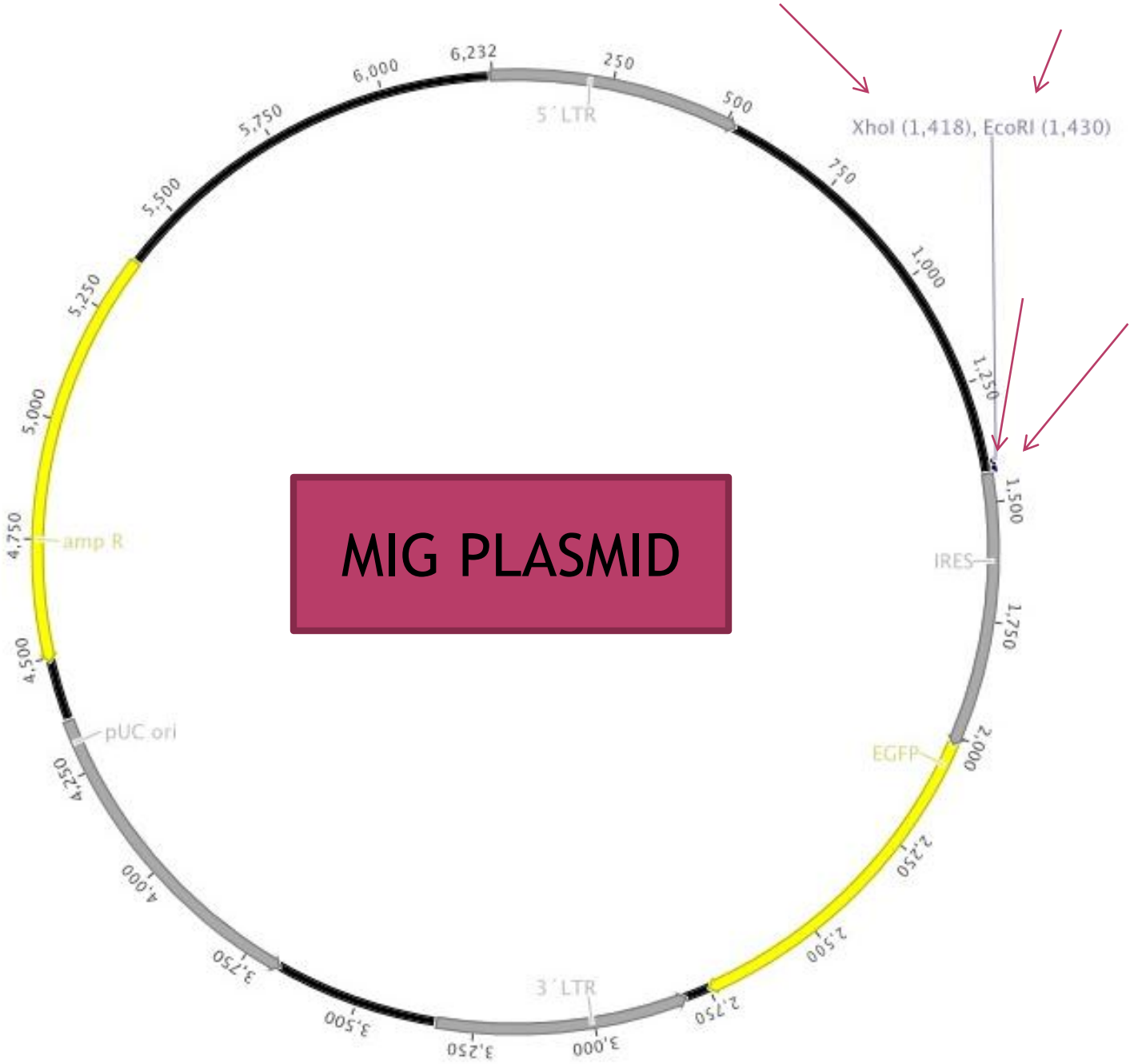



PMA
PLASMID
4,044
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
2,200
2,400
2,600
2,800
3,000
3,200
3,400
3,600
3,800
XhoI (376)
EcoRV (453)
signal peptide
new scFv
SalI (1,207)
CD8 hinge
CD28TM+ICD
4-1BB ICD
CD3z ICD
EcoRI (2,053)
Col\E1\origin
AmpR


PMA PLASMID
4,044
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
2,200
2,400
2,600
2,800
3,000
3,200
3,400
3,600
3,800
XhoI (376)
EcoRV (453)
signal peptide
new scFv
SalI (1,207)
CD8 hinge
CD28TM+ICD
4-1BB ICD
CD3z ICD
EcoRI (2,053)
Col\E1\origin
AmpR

DIGESTION




MIG PLASMID
XhoI (1,418), EcoRI (1,430)
5´LTR
IRES
EGFP
3´LTR
amp R
pUC ori
6,232
250
500
750
1,000
1,250
1,500
1,750
2,000
2,250
2,500
2,750
3,000
3,250
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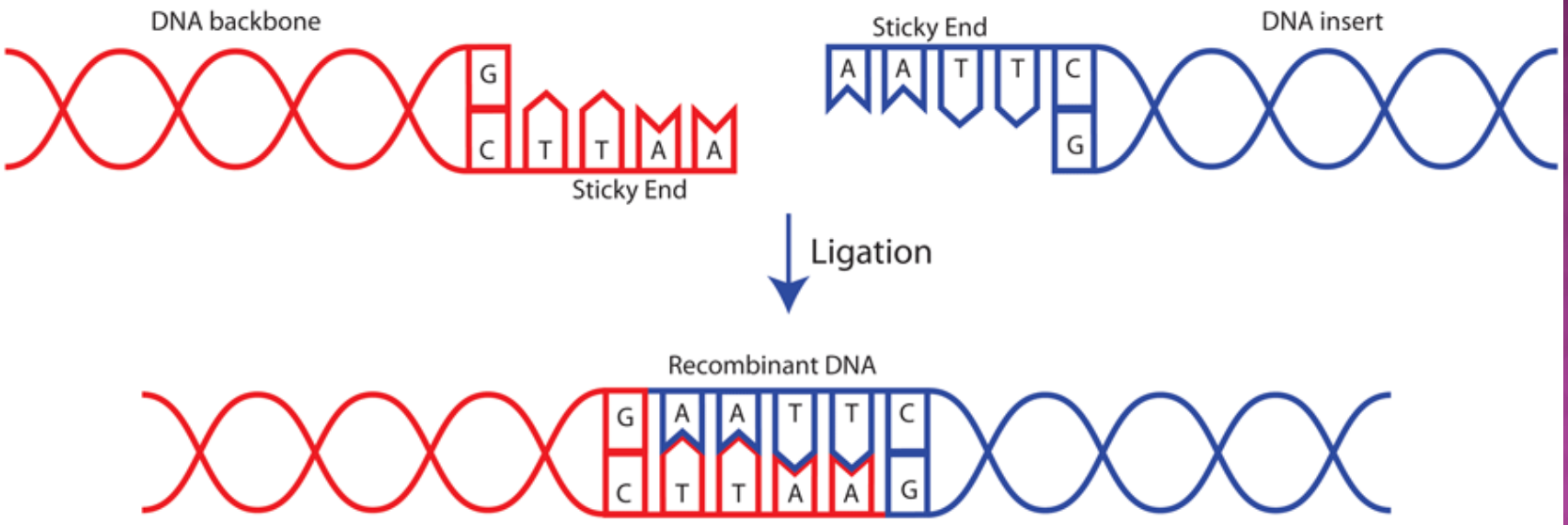
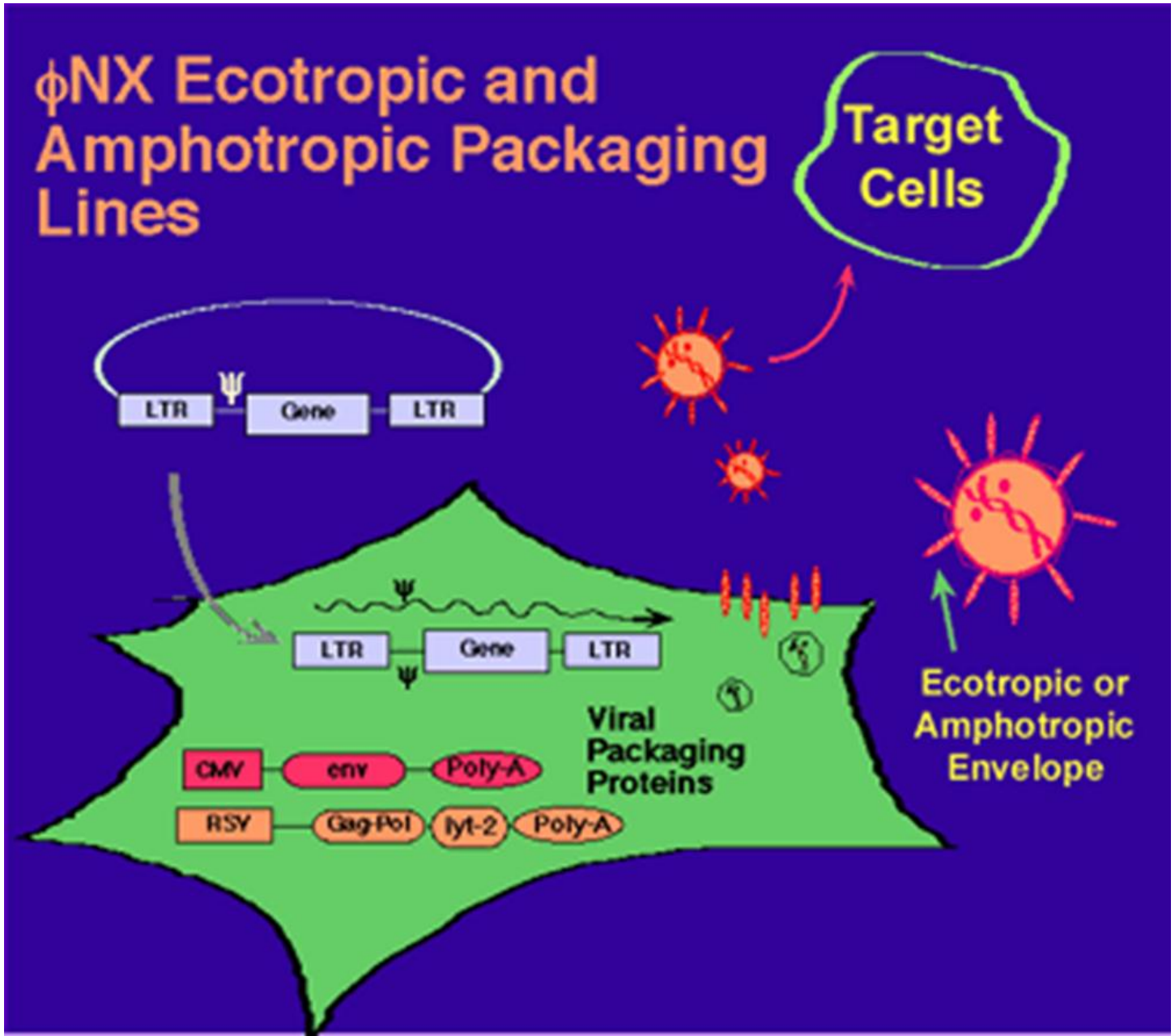
6,000

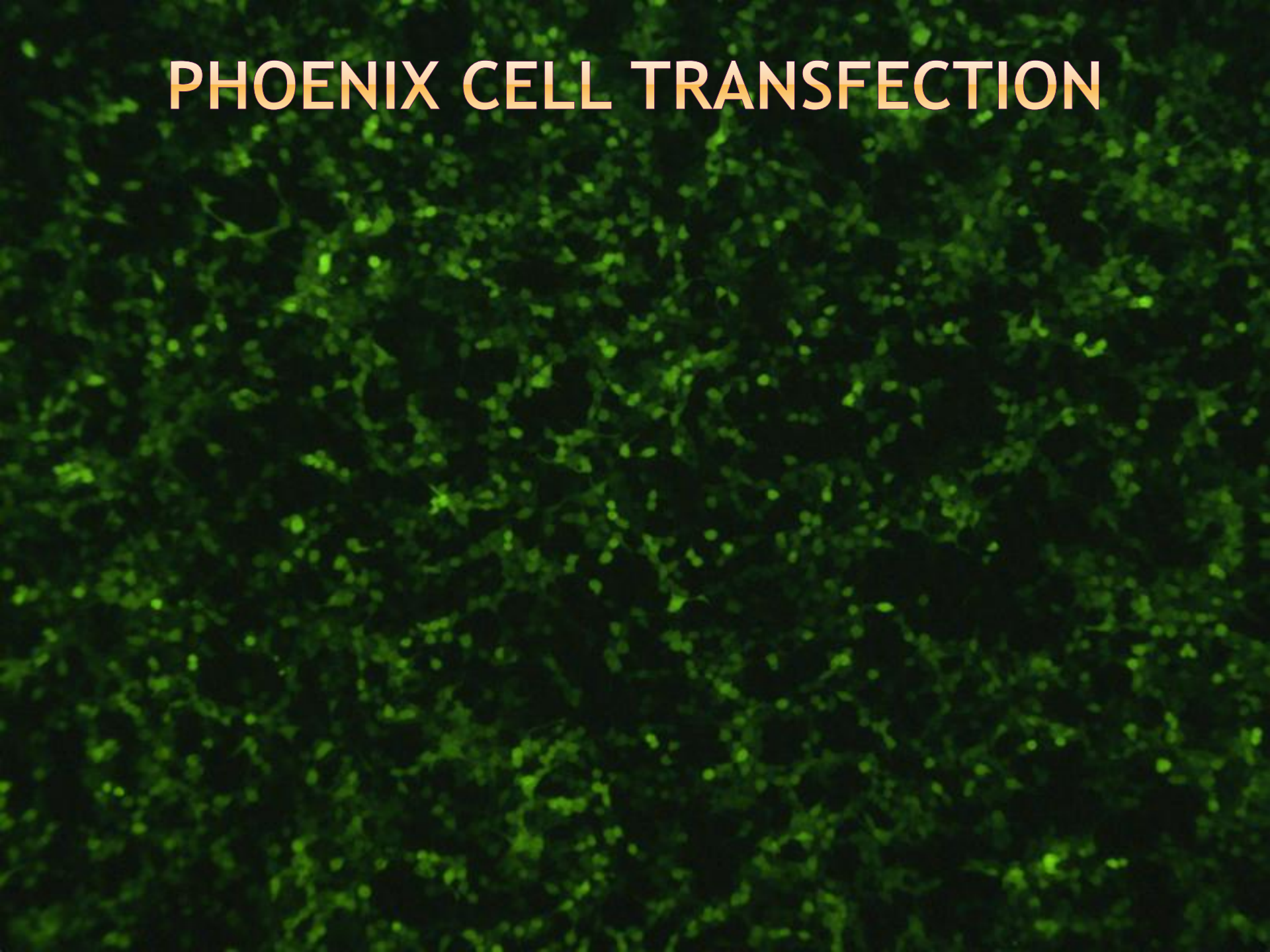
DNA LIGATION



PHOENIX CELLS

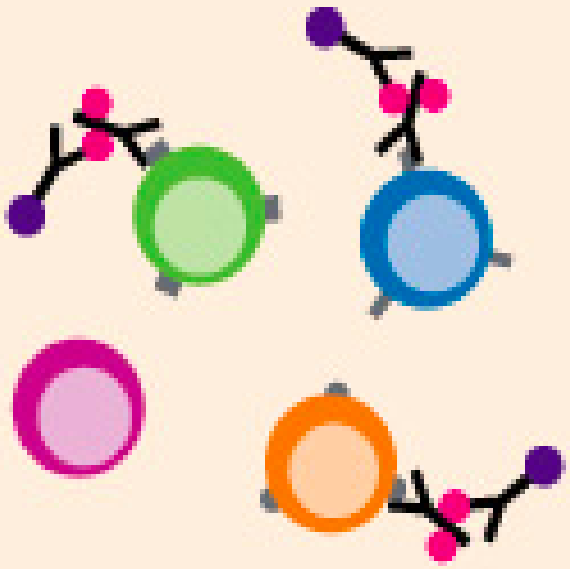


PHOENIX CELL TRANSFECTION

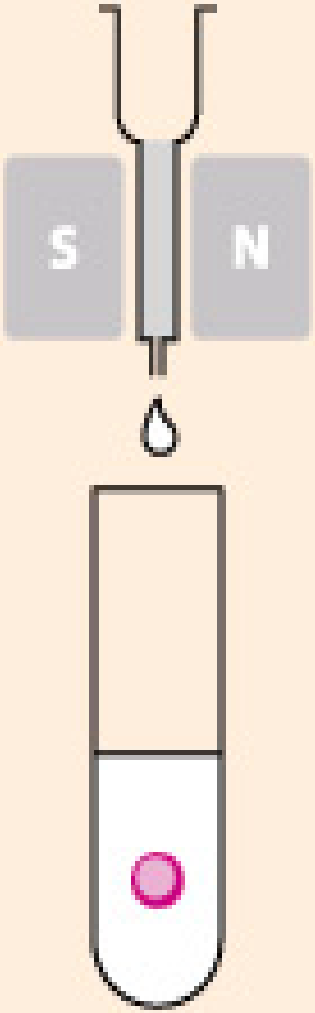


MACS T-CELL PURIFICATION

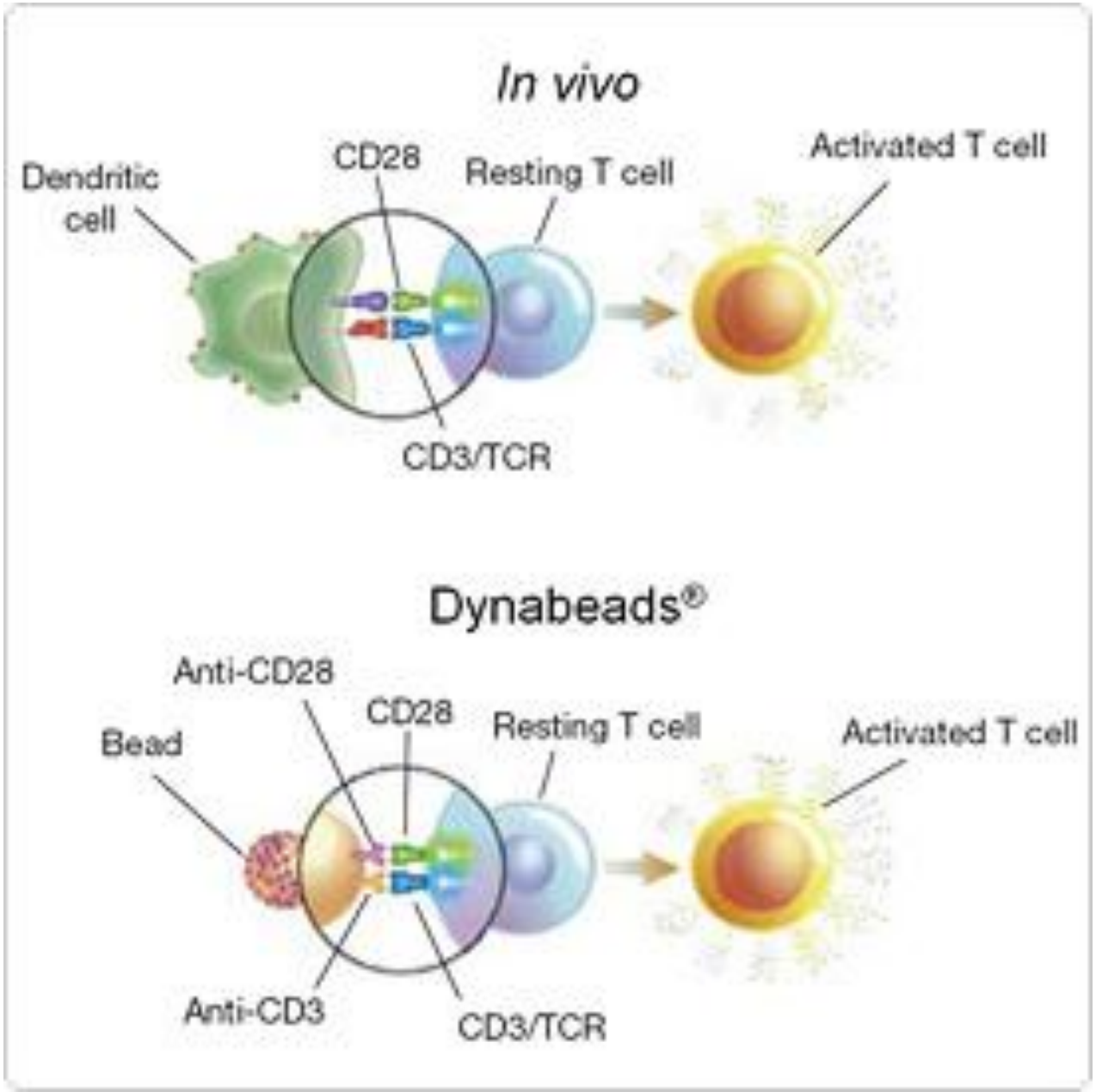
Magnetic labeling



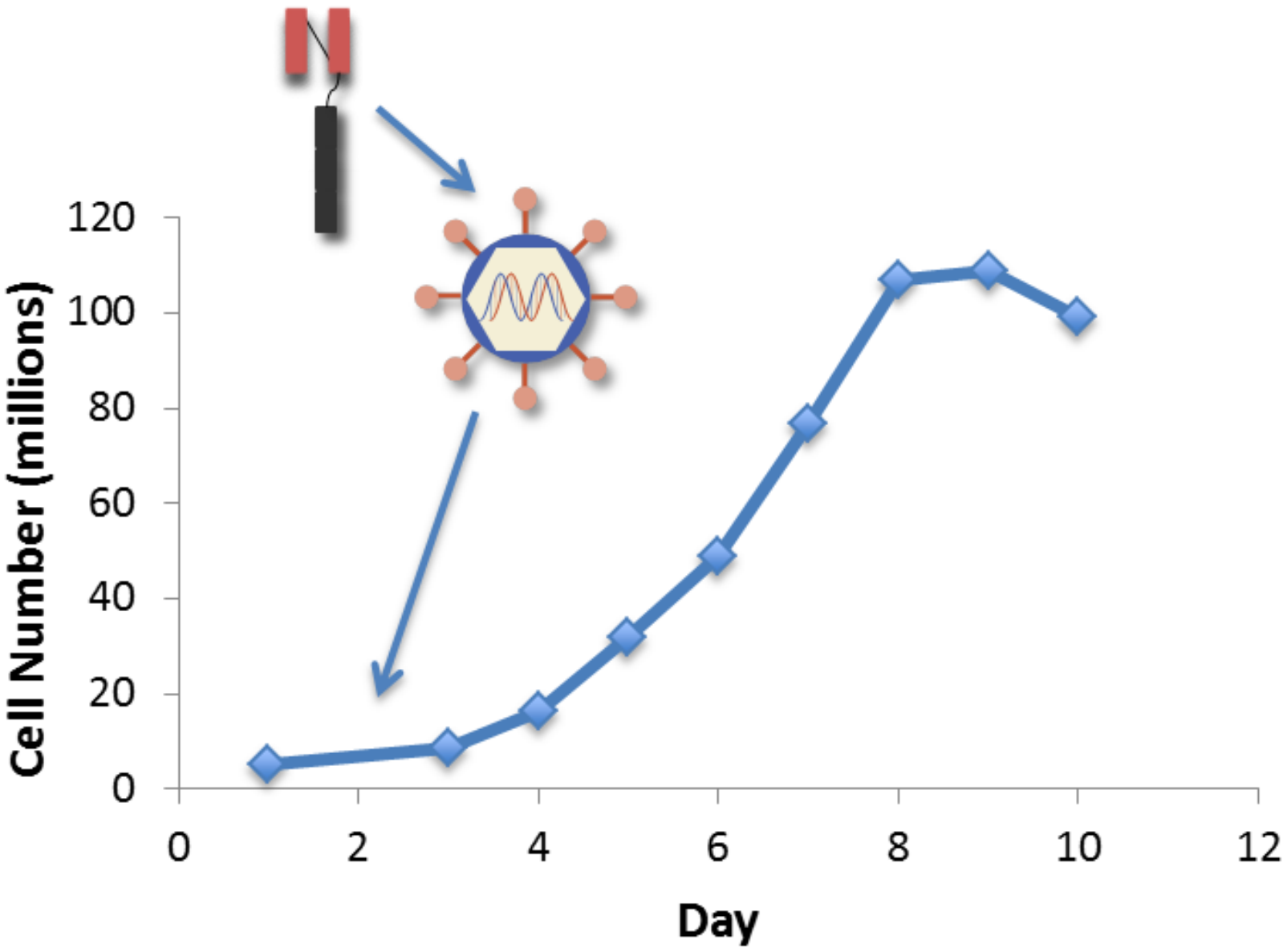
Magnetic separation



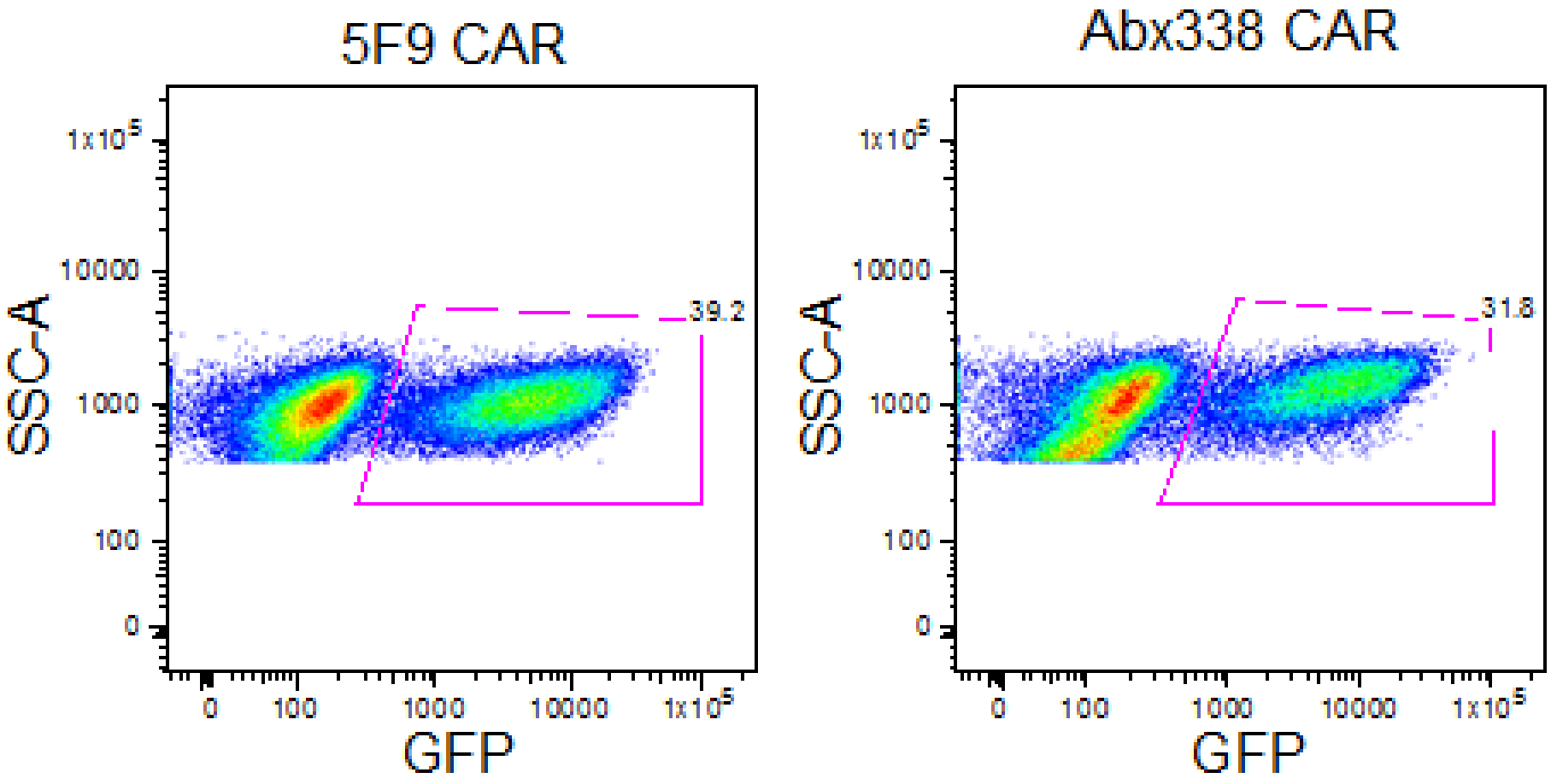
T CELL STIMULATION



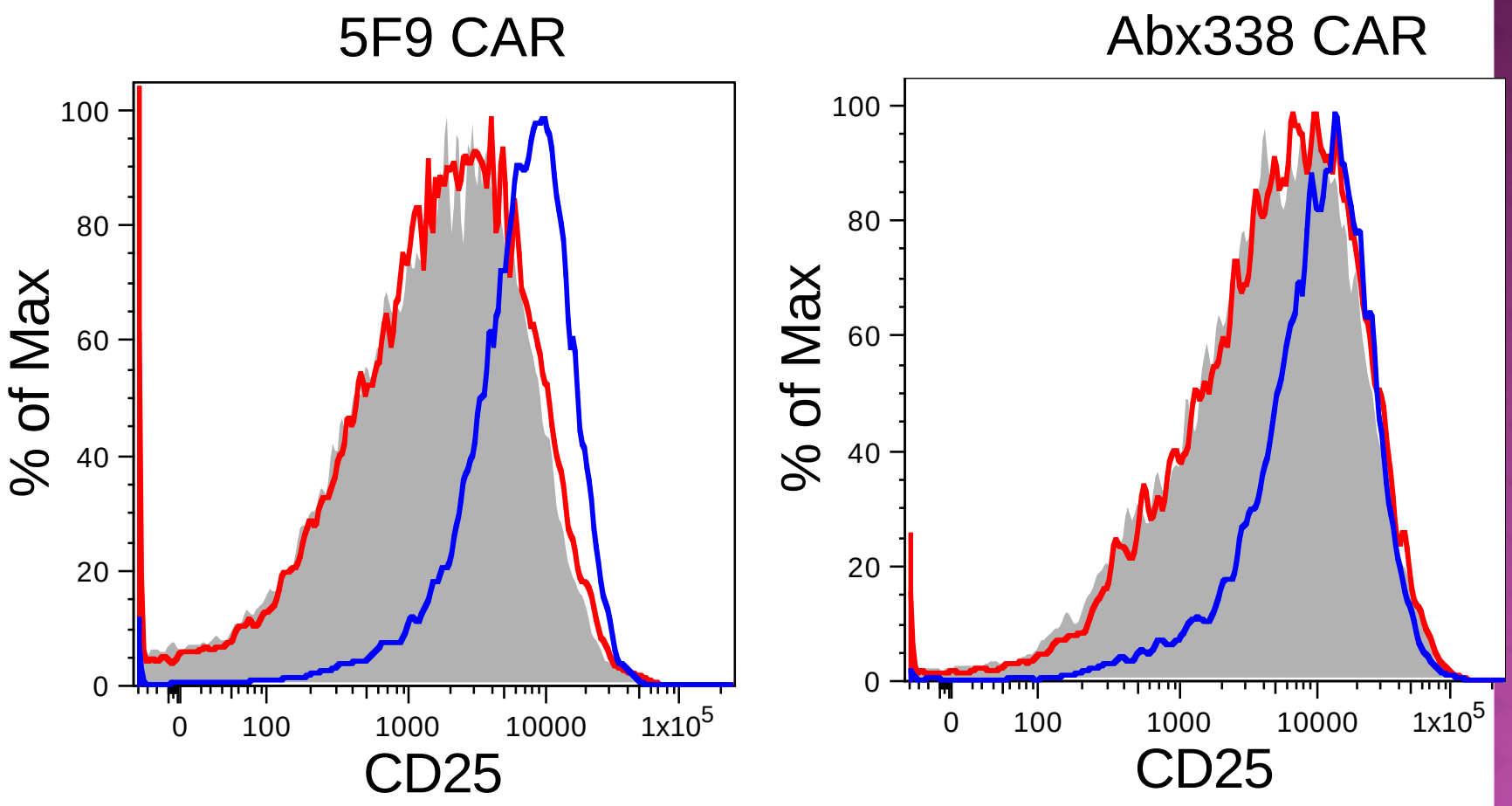
T-CELL EXPANSION



T Cell Transduction Efficiency



CAR INDUCTION OF CD25 EXPRESSION



CONCLUSION

- CREATE 12 CAR PMA PLASMIDS
- TRANSFER CAR'S FROM PMA PLASMID TO RETROVIRAL (MIG) PLASMID
- TRANSDUCE T-CELLS WITH RETROVIRUS TO OBTAIN T-CELLS EXPRESSING CAR DNA
- T-CELL ACTIVATION