

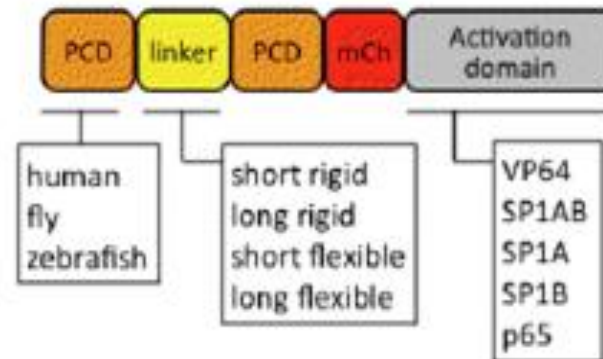
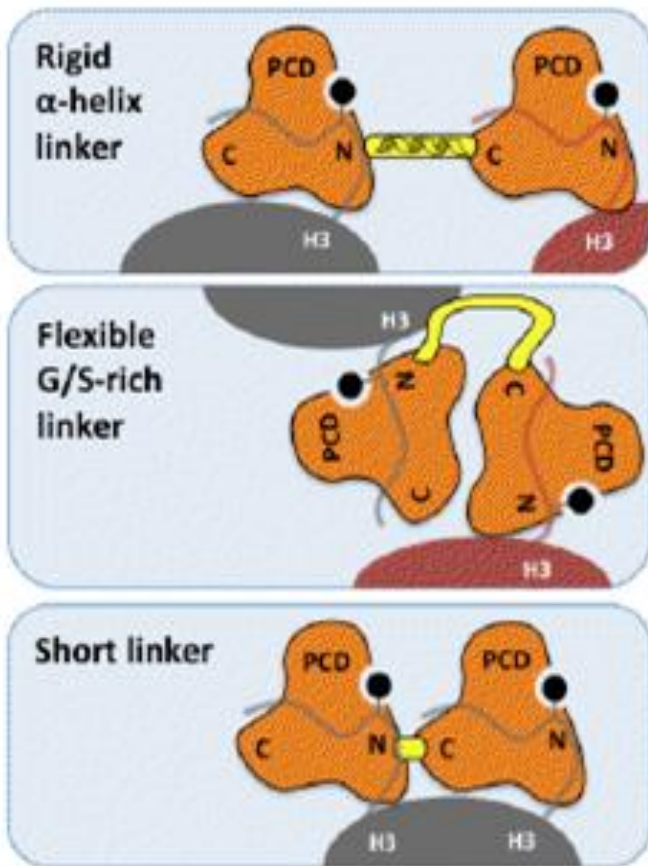
Engineering PC-TFs

Haynes Lab

Brady Laughlin

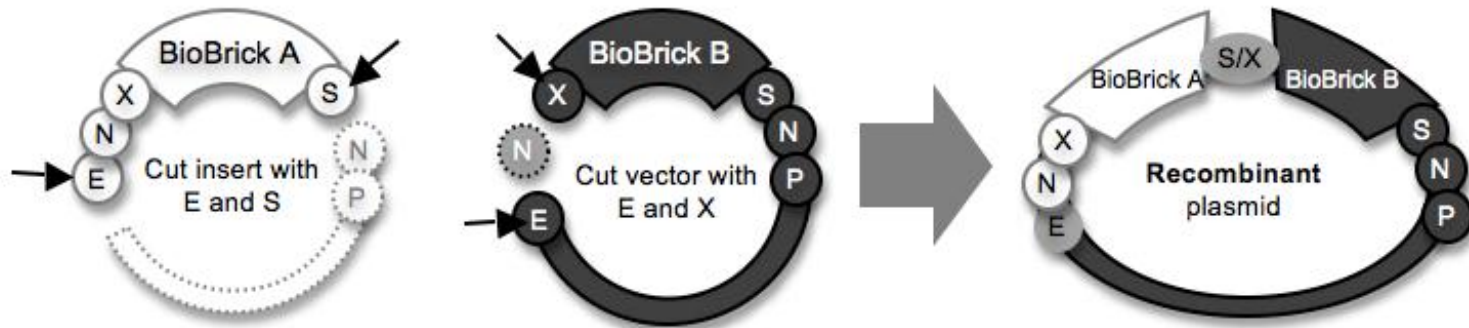
Project Overview

Engineering Pc-TF's with new characteristics

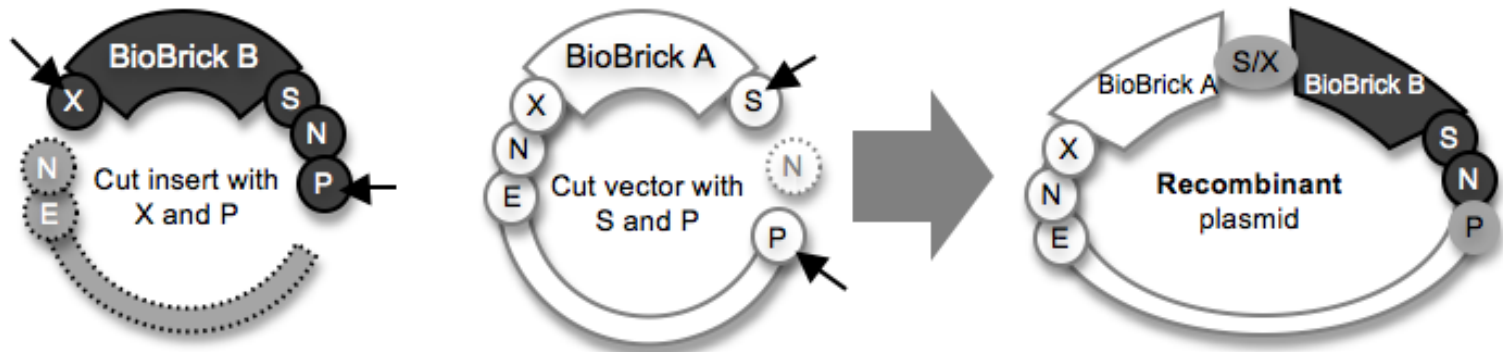


Biobrick Assembly

Ligating a “front insert” in a “front vector” to make A+B



Ligating a “back insert” in a “back vector” to make the same A+B construct



Restriction Digest

DNA ligation involves the creation of a phosphodiester bond between the 3' hydroxyl of one nucleotide and the 5' phosphate of another.

SpeI Restriction
Enzyme

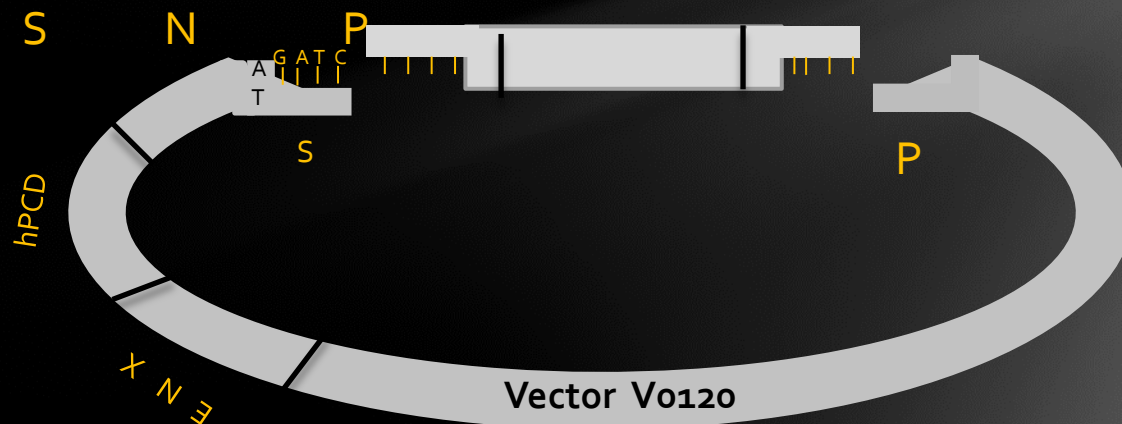
Cut Site					
A	C	T	A	G	T
T	G	A	T	C	A

PstI Restriction
Enzyme

Cut Site					
C	T	G	C	A	G
G	A	C	G	T	C

1. KAH01 SpeI/PstI
2. KAH03 SpeI/PstI
3. KAH04 SpeI/PstI
4. KAH204 XbaI/PstI
5. KAH205 XbaI/PstI
6. KAH206 XbaI/PstI
7. KAH225 XbaI/PstI

Back Vector



Restriction Digest

XbaI Restriction Enzyme

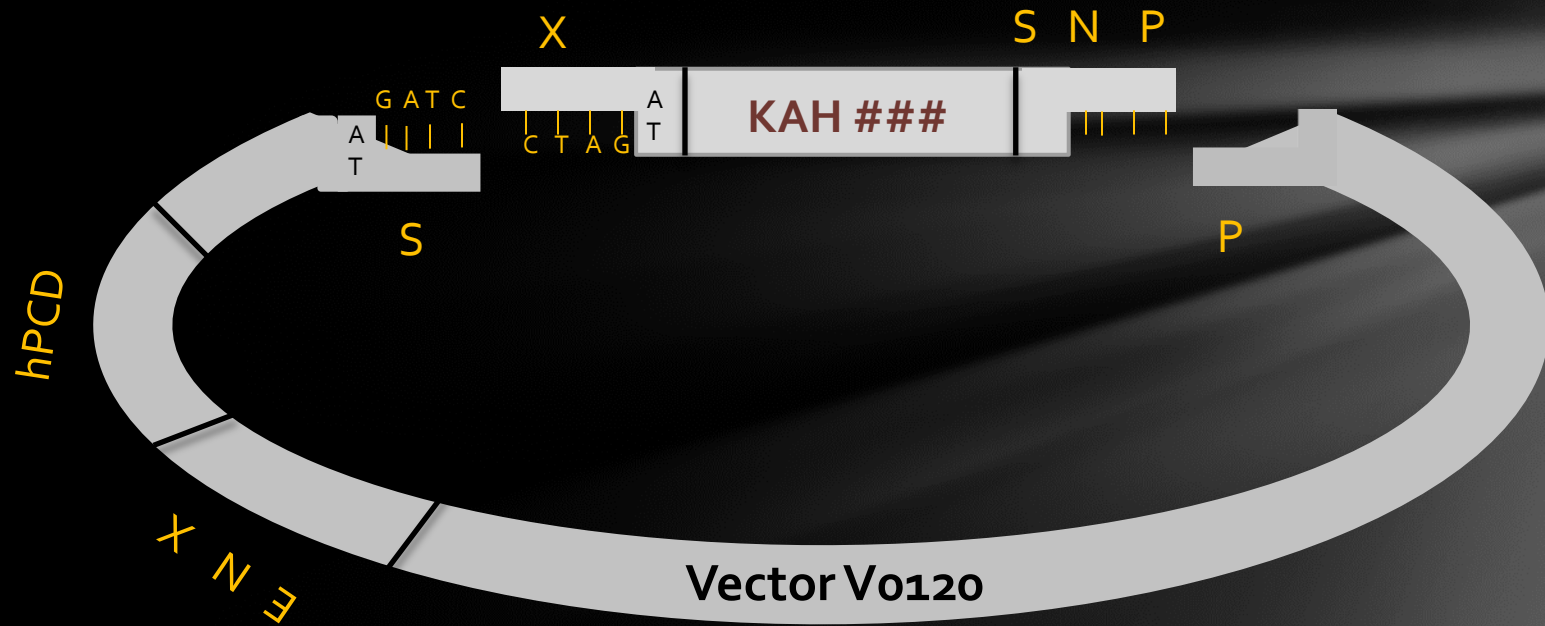
Cut Site						
T/C	T	A	G	A		
A	G	A	T	C/T		

PstI Restriction Enzyme

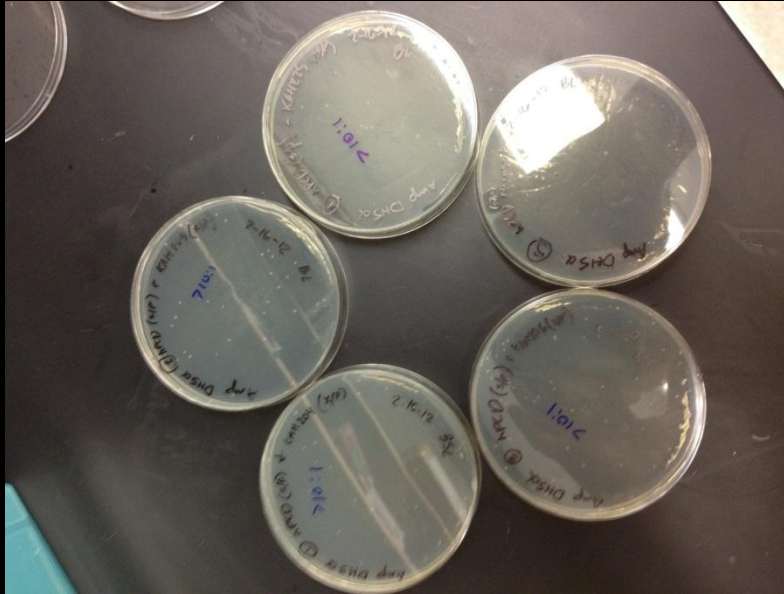
Cut Site						
C	T	G	C	A/G		
G/A	C	G	T	C		



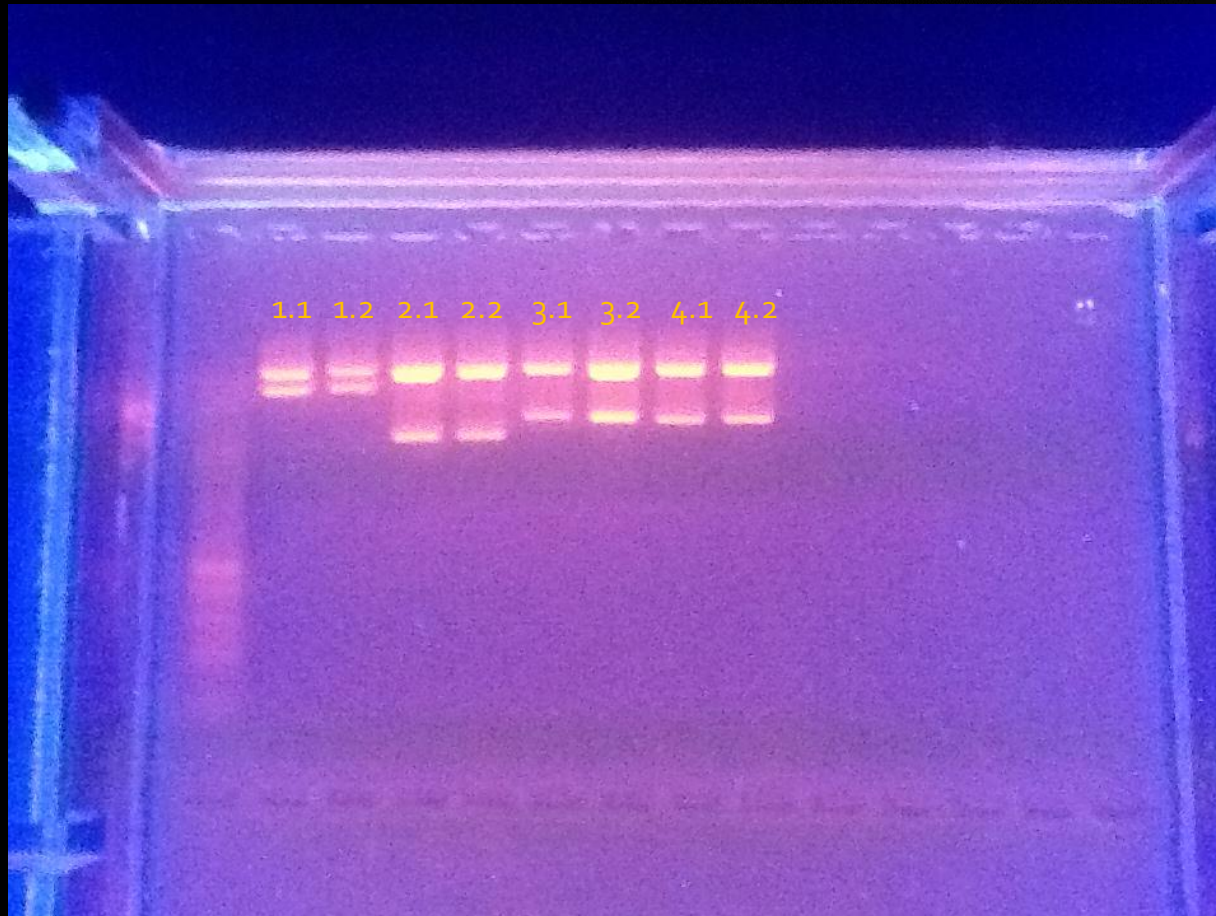
How Ligation Works



Ligation Slides



RestrictionDigest: Assembly Check



Progress

[PCD] : mCh : [AD]				
PCTF046	1053	hPCD : mCh : VP64	BBa_S04802	✓
PCTF047	2520	hPCD : mCh : SP1AB	BBa_S04803	✓
PCTF048	1443	hPCD : mCh : SP1A	BBa_S04804	✓
PCTF049	1224	hPCD : mCh : SP1B	BBa_S04805	✓
PCTF050	1686	hPCD : mCh : p65	BBa_S04806	✓
PCTF051	1053	flyPCD : mCh : VP64	BBa_S04807	✓
PCTF052	2520	flyPCD : mCh : SP1AB	BBa_S04808	
PCTF053	1443	flyPCD : mCh : SP1A	BBa_S04809	
PCTF054	1743	flyPCD : mCh : SP1B	BBa_S04810	
PCTF055	1686	flyPCD : mCh : p65	BBa_S04811	
PCTF056	1053	fshPCD : mCh : VP64	BBa_S04812	✓
PCTF057	2520	fshPCD : mCh : SP1AB	BBa_S04813	✓
PCTF058	1443	fshPCD : mCh : SP1A	BBa_S04814	✓
PCTF059	1743	fshPCD : mCh : SP1B	BBa_S04815	✓
PCTF060	1686	fshPCD : mCh : p65	BBa_S04816	✓