A. Time (hour)

| Time (hour) | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
|------------|---|---|---|---|---|----|----|----|----|
| 
| OD<sub>600</sub> | 0.001 | 0.01 | 0.1 | 1 |

B. Dilution factor

| Dilution factor | -1 | -2 | -3 | -4 | -5 |
|-----------------|----|----|----|----|----|
| 
| WT | P<sub>spxB</sub>-19AJ | WT | P<sub>spxB</sub>-19AJ | WT | P<sub>spxB</sub>-19AJ | WT | P<sub>spxB</sub>-19AJ | WT | P<sub>spxB</sub>-19AJ | WT | P<sub>spxB</sub>-19AJ | WT | P<sub>spxB</sub>-19AJ | WT | P<sub>spxB</sub>-19AJ |
|-----------------|----|----|----|----|----|
| 
| CPS19A | CPS19B | CPS19C | CPS19C | CPS19A | CPS19B | CPS19C | CPS19C | CPS19A | CPS19B | CPS19C | CPS19C | CPS19A | CPS19B | CPS19C | CPS19C | CPS19A | CPS19B | CPS19C | CPS19C |

C. Blot: Anti-serotype 19 CPS antibodies

D. Relative amount of type 19 CPS (%)

| Relative amount of type 19 CPS (%) | 0 | 20 | 40 | 60 | 80 | 100 |
|-----------------------------------|---|----|----|----|----|-----|
| WT | P<sub>spxB</sub>-19AJ |
| CPS19B | |
| CPS19C | |

E. Blot: Anti-FLAG antibodies

F. Relative amount of protein expression (%)

| Relative amount of protein expression (%) | 0 | 20 | 40 | 60 | 80 | 100 |
|-------------------------------------------|---|----|----|----|----|-----|
| WT | D64A | G71R |
| CPS19C | P<sub>spxB</sub>-19AJ |

G. Serotype 19C CPS synthesis pathway

- Cps19AJ
- Cps19C
- WchA
- WchO
- WchQ
- WchS
- WchR
- WchU
- Cps19CH
- Polymerization
- Ligation

**Pathway Components:**

- Glucopyranose
- Rhamnose
- N-acetylmannosamine
- Ribofuranose

**Cytoplasm**