

**The use of molecular methods
to screen *N. gonorrhoeae* isolates
for *penA* (PBP2) alterations
— San Francisco**

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Is the NG mosaic PBP2 (mPBP2) in San Francisco?

- **Using published Ochiai real-time PCR assay for mosaic**
- **Isolates collected for Gonococcal Isolate Surveillance Program (GISP) in 2008**
 - **Men with symptomatic urethritis**
- **187 of 213 collected isolates available**
- **17 of 187 (9.1%) isolates with mPBP2**

PBP2 Sequence Comparisons

Japan Mosaic	301	AVTDMIIEPGSAMKPFTHAKALDSGKVDATDTFNTLPYKIGSATVQDTHVYPTLDVRGIMQ
SF-A (n=6)	301
SF-B (n=11)	301P.....
Wild Type	301I...V.....A..T.LNERL..Q.....PSP.R.....S.....

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Japan Mosaic	361	KSSNVGTSKLSAMFTPKEMYDFYHDLGVGVRMHSGFPGETAGLLRSWRRWQKIEQATMSF
SF-A (n=6)	361
SF-B (n=11)	361N...RP.....
Wild Type	361R.GAE.....E..I.....N...RP.....

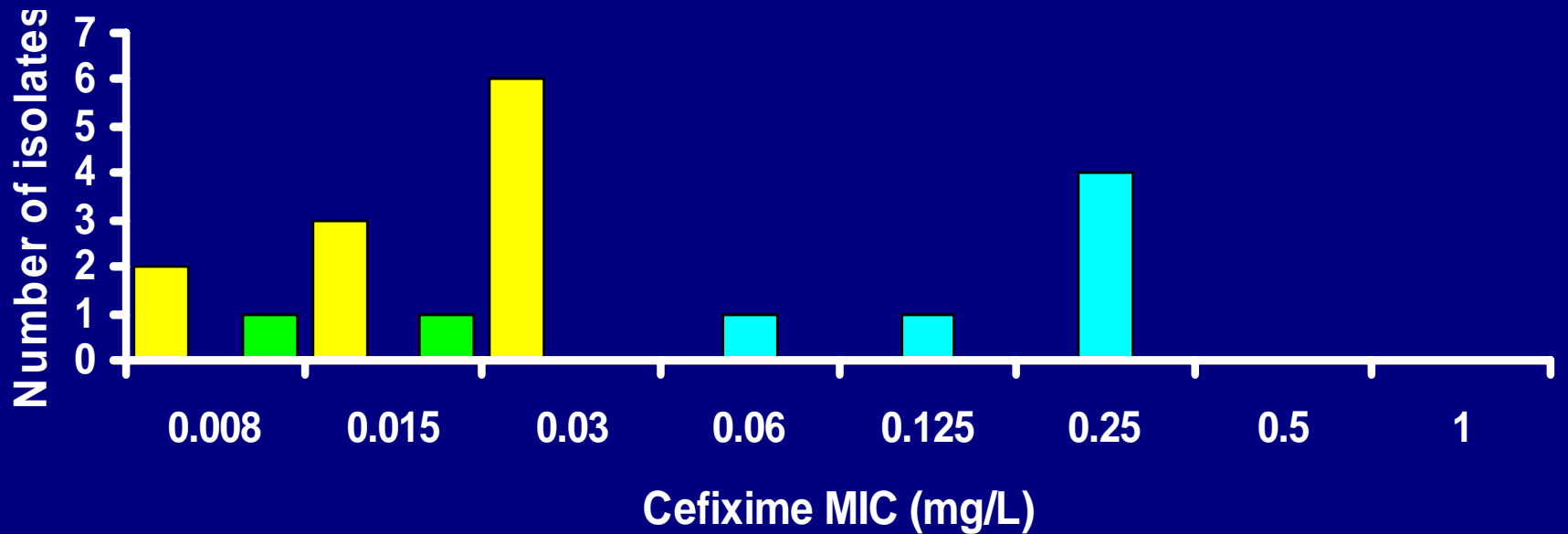
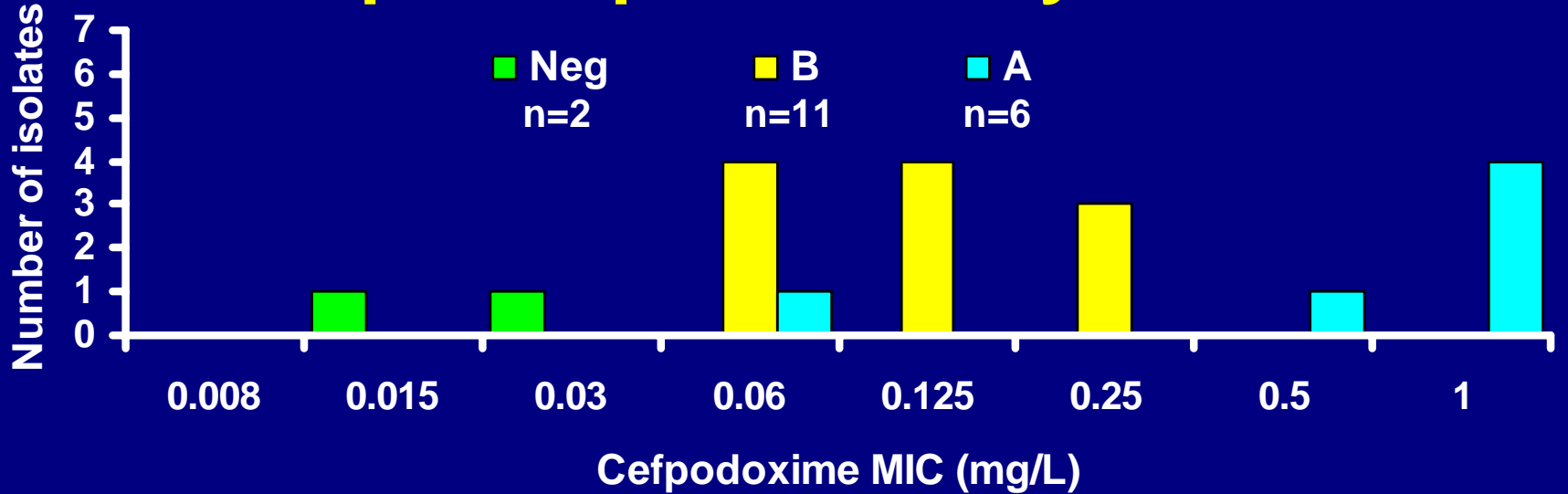
Japan Mosaic	421	GYGLQLSLLQLARAYTVLTHDGELLPVSFQAVAPKGKRVIKASTAKKVRELMVSVTEA
SF-A (n=6)	421
SF-B (n=11)	421A...V.....Q...IF.E...RE..N.....P
Wild Type	421A...V...L.....Q...IF.E...RE..N.....P

Japan Mosaic	481	GGTGTAGAVDGFVGAKTGTARKLVNGRYVDYKHVATFIGFAPAKNPRVIVAVTIDEPTA
SF-A (n=6)	481
SF-B (n=11)	481F...A.N..I.....
Wild Type	481F...A.N.....

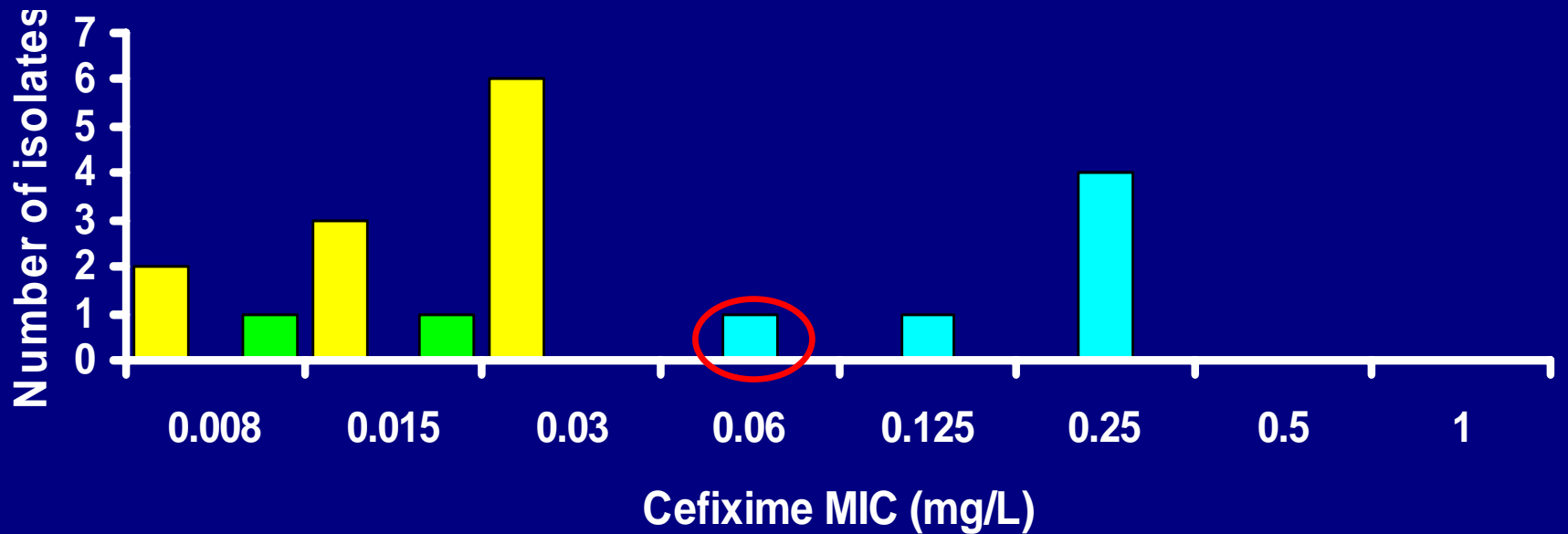
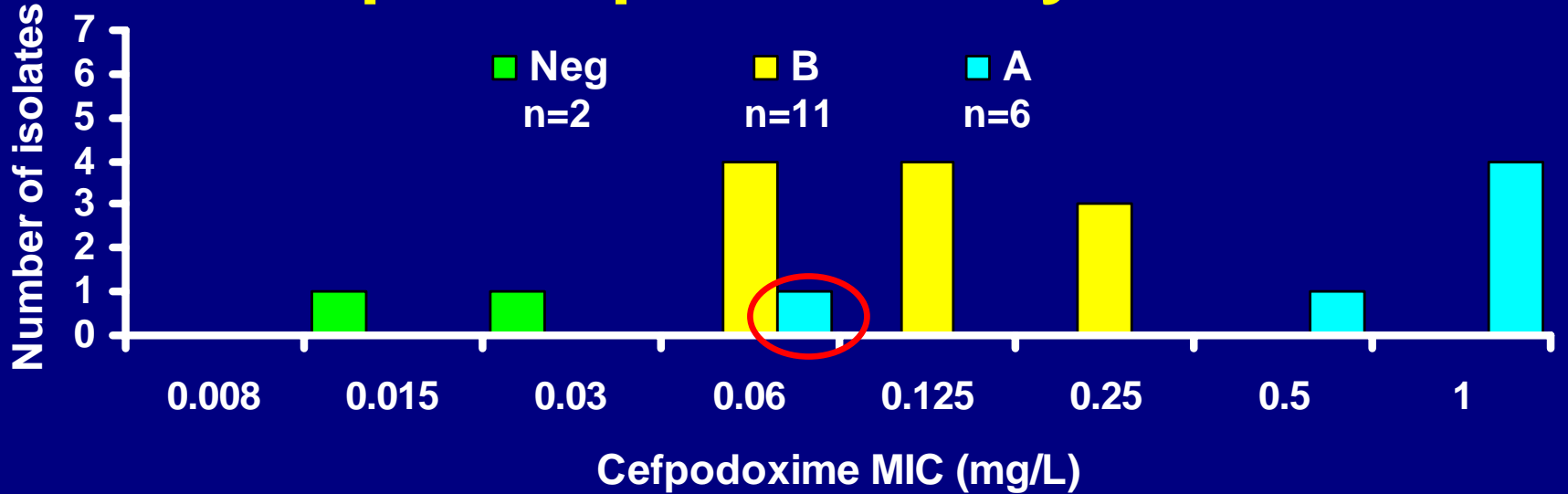
Japan Mosaic	541	NGYYSGVVTGPFVKQVMGGSLNILGVSPKPLTNVAAVKTPS
SF-A (n=6)	541A..P..KI.....I.....-A.....
SF-B (n=11)	541	H...G...A..P..KI.....I.....-A.....
Wild Type	541	H...G...A..P..KI.....I.....-A.....

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Oral Cephalosporin MIC by PBP2 allele



Oral Cephalosporin MIC by PBP2 allele



Major Question: Treatment failure in San Francisco?

- **Active treatment follow-up for persons at risk for failure**
- **Identify persons at risk for treatment failure**
- **Not limited to culture isolates**

San Francisco STD Clinic

NG Testing

- All screening is with Aptima Combo 2 (AC2)
- Routine screening of MSM at exposed sites: rectal, pharyngeal, urethral/urine
- Cultures only collected for GISP (symptomatic urethritis)
- 2008:
 - 20,571 AC2 tests; 992 positive
 - 471 Cultures; 213 positive

New Assay Needed

- No local resources for additional cultures or susceptibility testing
- GISP results take months to return
- Ochiai assay cross-reacts with *N. meningitidis* and cannot distinguish 2 mosaic alleles found (currently) in San Francisco

New Assay

- **Melting curve PCR assay to:**
 - Distinguish SF-A from SF-B and *N. meningitidis*
 - Use on specimens collected for AC2 (urine, swabs)
 - Roche MagnaPure DNA purification

Real-time PCR Assays for Mosaic PBP2

Japan Mosaic 241 QRIQTLAYEELNKAVEYHQAKAGTVVVLDAFTGEILALVNTPAYEPNKPQADSEQRRNR
 SF-A (n=6) 241
 SF-B (n=11) 241 A D . . R . . R
 Wild Type 241 A D . . R . . R

Japan Mosaic 301 AVTDMIEPGSAMKPFETIAKALDSGKVDATDTFNITPYKIGSATVQDTHVYPTLDVRGIMQ
 SF-A (n=6) 301
 SF-B (n=11) 301 P
 Wild Type 301 I . . V A . . T . LNERL . . Q PSP . R S

312 316

Japan Mosaic 361 KSSNVGTSKLSAMFTPKEYDFYHDLGVGVVMHSGFPGETAGLLRSWRRWQKIEQATMSF
 SF-A (n=6) 361
 SF-B (n=11) 361 N RP
 Wild Type 361 R . GA E E . . I N RP

Japan Mosaic 421 GYGLQLSLLQLARAYTVLTHDGELLPVSFQAVAPKGRVIKASTAKKVRELMVSVTEA
 SF-A (n=6) 421
 SF-B (n=11) 421 A V Q . . IF . E . . RE . . N P
 Wild Type 421 A V . . L Q . . IF . E . . RE . . N P

Japan Mosaic 481 GGTGTAGAVDGFVDGAKTGTARKLVNGRYVDYKXVATFIGFAPAKNPRVIVAVTIDEPTA
 SF-A (n=6) 481
 SF-B (n=11) 481 F A . N . . I
 Wild Type 481 F A . N

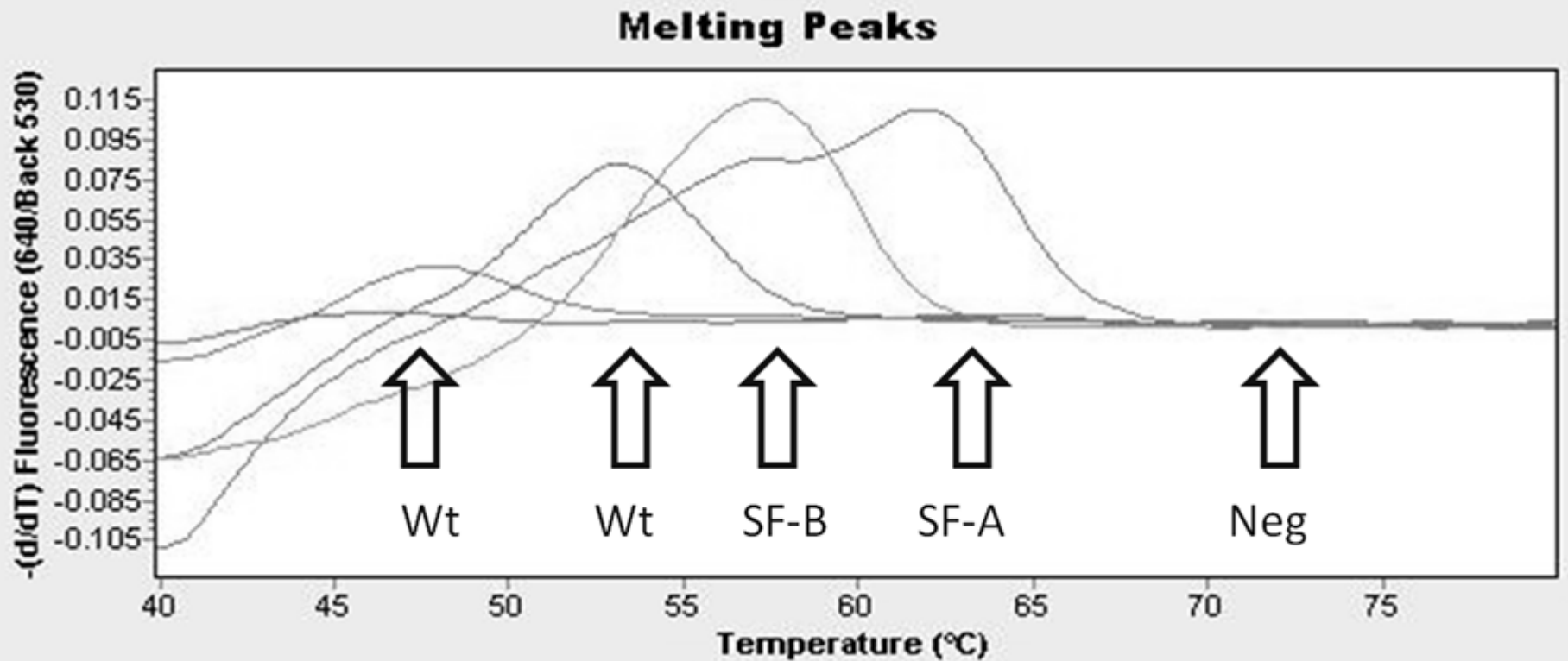
Japan Mosaic 541 NGYYSEVVTGPFVFKQVMGGSLNLLGVSPKPLTNVAAVKTPS
 SF-A (n=6) 541 A . . P . . KI I -A
 SF-B (n=11) 541 H . . G . . A . . P . . KI I -A
 Wild Type 541 H . . G . . A . . P . . KI I -A

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Ochiai

Wu

Melting Peaks



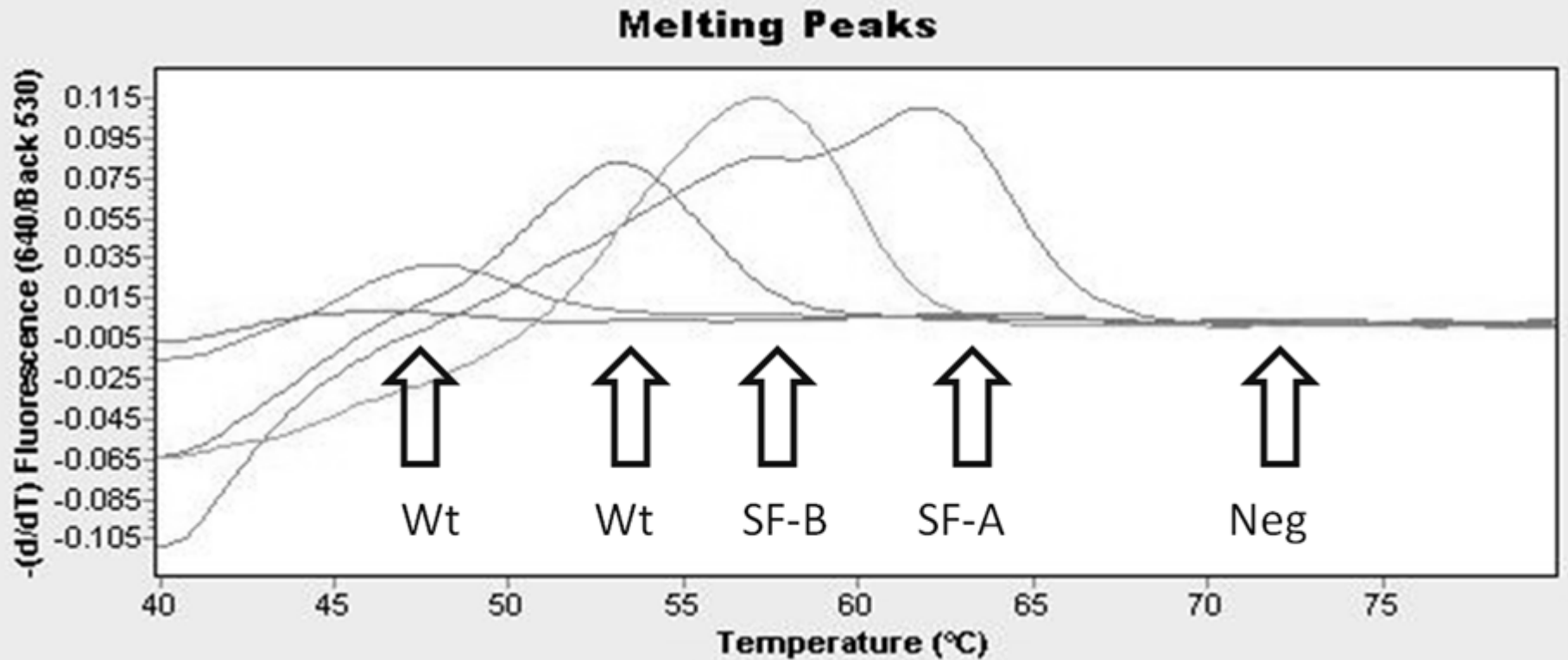
New PCR Validation

- **Mosaic vs. NonMosaic**
 - 100% agreement with Ochiai assay (n=134)
- **Mosaic Allele**
 - 100% agreement with sequencing (n=29)
- **Specimens negative for NG on AC2:**
 - Urine, vaginal, rectal specimens (n= 25 each)
 - all negative
 - Pharyngeal specimens:
 - 18 of 25 (72%) NonMosaic
 - 2 of 25 (8%) SF-B/*N. meningitidis*

PCR Results for Commensals

<u>Species</u>	<u>PCR result</u>
<i>N. cinerea</i>	SF-B
<i>N. meningitidis</i>	SF-B
<i>N. mucosa</i>	NonMosaic
<i>N. sicca</i>	NonMosaic
<i>N. lactamica</i>	NonMosaic

Melting Peaks



Current (Evolving) Protocol

- All symptomatic patients with positive AC2 for NG infection at any site
- Tested using new (Wu) real time PCR assay
- SF-A are confirmed by Ochiai real time PCR
- Patients with SF-A are contacted for follow-up of treatment results and interview

Real time Screening Results

- **Started mid April 2009**
- **52 specimens from symptomatic patients**
 - 45 urine; 4 rectal; 2 pharyngeal; 1 vaginal
 - 14 (27%) did not amplify
 - 34 (65%) non-mosaic
 - 2 (4%) SF-B (partial) mosaic
 - 2 (4%) SF-A (complete) mosaic

Conclusions

- **Mosaic PBP2 is present in San Francisco**
- **Appears to be associated with increased oral cephalosporin MIC**
 - (with chromosomal pen/tet resistance)
- **Molecular assay can be used to screen clinical specimens for resistance markers**
- **Still no treatment failures identified**

Unanswered Questions and Future Directions

- Is there treatment failure associated with mPBP2 in SF?
- How best to use AC2 as a test of cure?
- What other genes are involved?
- What is the role played by pharyngeal infection and commensal *Neisseria* species?
- Strain typing by NG-MAST

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