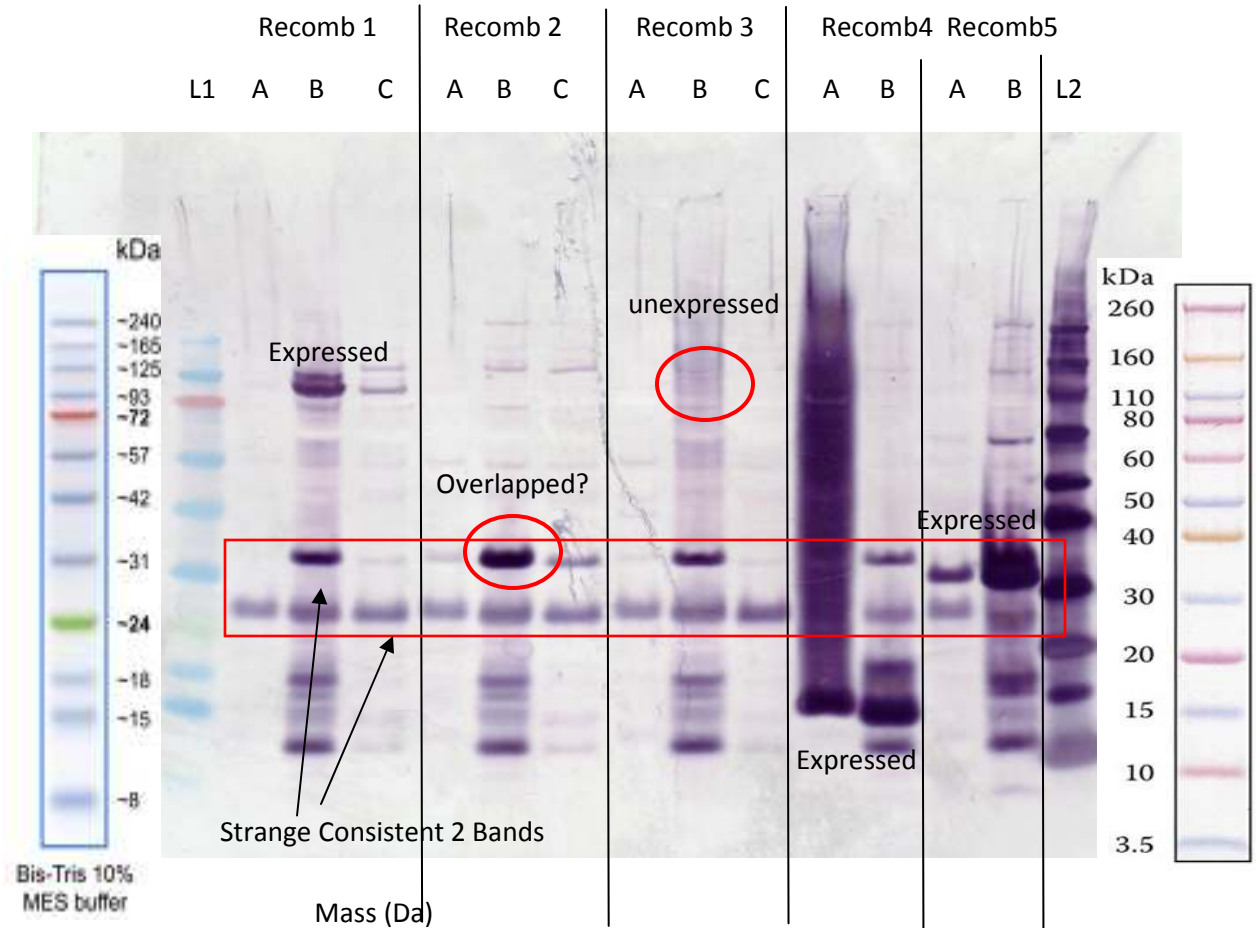
Western Blot 21-03-2011 ADRIAN K



	Mass (Da)		
Recomb 1	83KDa	A	SMART protein extracted Supernatant
Recomb 2	32KDa	B	UREA + lysate
Recomb 3	89KDa	C	UREA supernatat
Recomb 4	12KDa		
Recomb 5	30KDa		

Expression Condition: 37C, 4hr 30 Minutes, LB with 0.5% Glucose, 125rpm, 1mM IPTG

Protein Extraction: SMART^{tm} Bacteria Protein Extraction Solution Cat. No. 17511,

http://eshop.intronbio.com/pds/SMART_bacterial_Kit.pdf, using 2ml spin down pellet

Supernatant extracted: A; Insoluble fraction: proceed with inclusion body protocol.

Inclusion Body protocol: +200µl UREA Buffer, Suspend thoroughly, and vortex, spin down, take 100µl as "C: UREA supernatant", the remaining 100µl with lysate resuspend together "B: UREA + lysate"

UREA Buffer: 8 M Urea , 50 mM Tris-HCl (pH 8.0), 1 mM EDTA

Western Blot:

HisDetector Western Blot Kit, AP Colorimetric, Cat No 25-00-01

https://www.kpl.com/docs/datasheet/250001.pdf

Problem Arise

*limited lanes to run on pre-cast gel, Invitrogen MES Bis-Tris.

Consistent background protein detected on ~25kDa

Unconsistent background protein detected at ~32kDa, only in UREA treated lysate, B.

Possible overlapped Recombinant 2 with background on lane B, Recombinant 2?