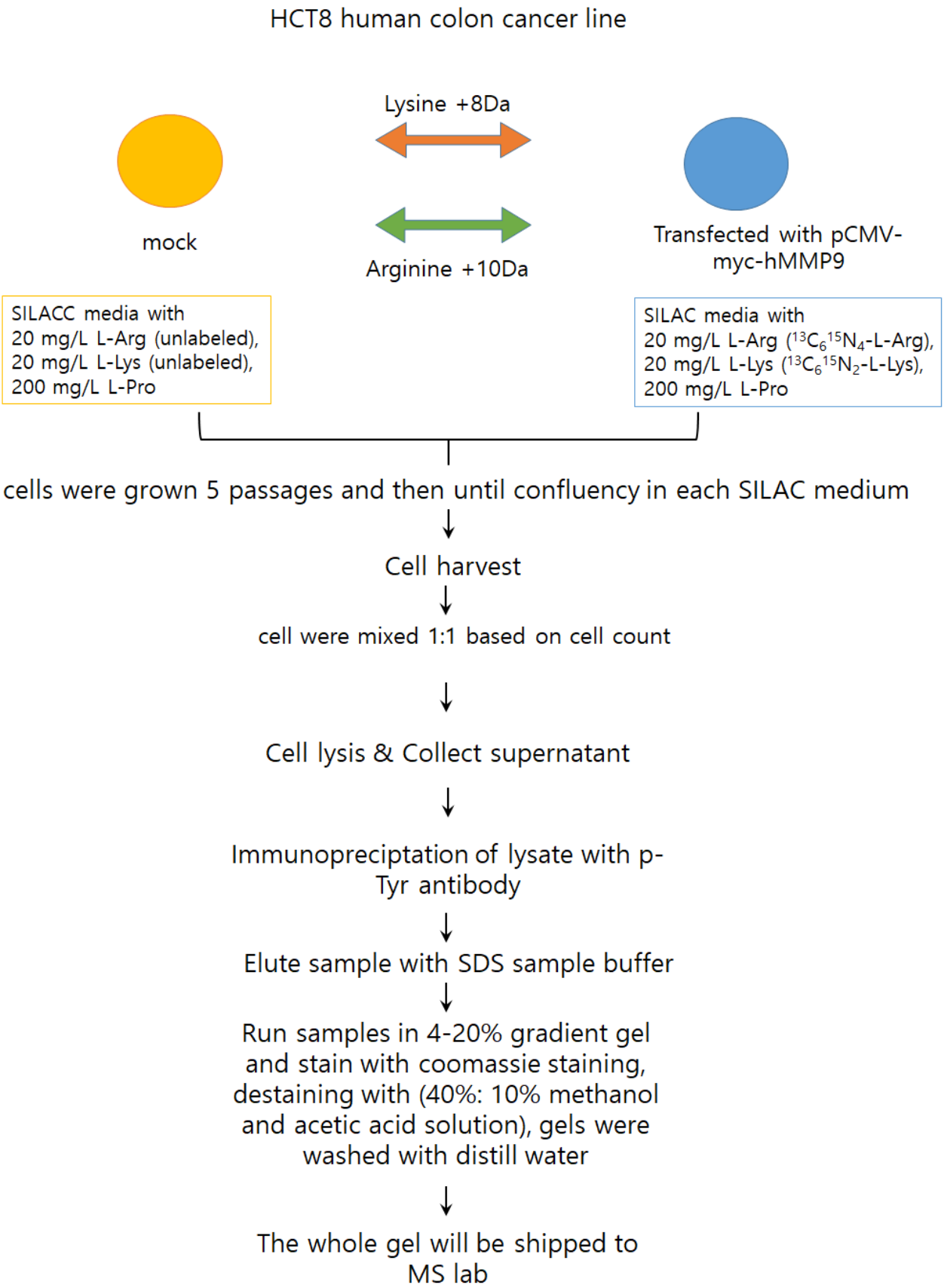
1. Goal: We want to see the global phosphotyrosine change upon overexpression of MMP9

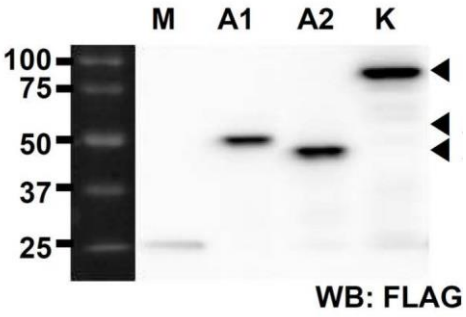


Requests from the MS lab

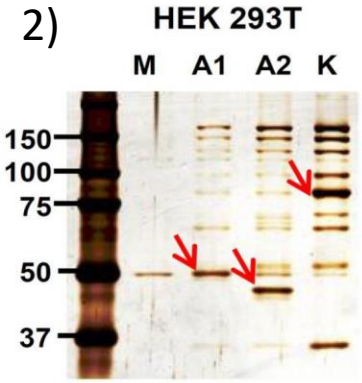
- All procedures are very clear. Please be aware of the followings
- Provide protein sequence information for the construct myc-hMMP9 from the initiator Met to the last residue, the exact sequence of your construct itself. Only the accession number of hMMP9 in the public database is not enough.
- Don’t freeze the gel, put it in a plastic bag with a tiny amount of water to keep the gel wet during delivery (Don’t use too much water, or your precious gel would be broken to pieces), remove all the air bubbles inside, seal the bag completely.
- Take a photo for the SDS gel before shipping, and send it to us through mail.

2. Goal: I am going to find novel binding partner proteins of Protein ‘K’

1)



2)



Silver staining

3)

Coommassie staining

Each of proteins A1, A2, K was cloned into an expression vector with FLAG-SBP (Streptavidin binding peptide) tag → Transfection of HEK 293T → Cell growth → Cells were harvested at a confluence of 90~100% and lysed by NETN buffer containing 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40, Protease Inhibitor Cocktail (Roche Diagnostics) and Phosphatase Inhibitor Cocktail (Roche Diagnostics) → Cell lysis → Collect supernatant → Bradford assay

60 µl of streptavidin agarose beads (Thermo Scientific) in Phosphate buffered-Saline (PBS) was activated and equilibrated by 600 µl of NETN buffer twice. → 2 mg of protein was added to the agarose bead and mixed by a rotator (10 rpm, 2 h, 4 °C). → The beads were washed three times with NETN buffer. → Bound proteins were incubated on the top of a 0.22 µm PVDF filter (Millipore, Billerica, MA) for 2 min on ice with 30 µl of biotin solution (approx. 0.82 mM Biotin in NETN buffer) and were eluted by centrifugation (2,000 rpm, 2 min, 4 °C). The elution step was repeated twice.

1) Cell extract was analyzed by SDS-PAGE and FLAG western blot.
2) One-tenth of elution was visualized by SDS-PAGE and silver staining.
3) The remaining 90% of eluted proteins was shortly run on the SDS-PAGE and the gel was stained with Coommassie Brilliant Blue solution containing ethanol instead of methanol

Response from the MS lab

- It is perfect!!
- If you send us the gel, then we will cut the whole lane of protein ‘K’ and perform in-gel digestion. If the gel piece is too big to accommodate for a single in-gel digestion procedure, we will break it into several, perform in-gel digestion separately, combine all the resultant peptides, and do the LC-MS/MS

3. 시료를 의뢰하는 공식적인 절차가 있나요?

없습니다.

하지만 아래와 같은 정도의 정보는 기본적으로 필요합니다.

그리고 시료의 분석 목적을 정확히 알려주는게, 좀 더 정확한 분석 계획을 세우는데 중요합니다.

“시료가 이미 준비되었는데, 질량분석 한번 해주세요”는 상당히 난감하지요.

시료정보			
Purpose			
Sample 수		Sample name	
Origin		Source	
Status		Sample buffer	
Concetration	ug/ul	Volume	ul
Supplement factor		Treated reagent	
additional information			

신청자	성명		전화	
			E-mail	
	기관명			
	플랫폼 활용방법	☐ 논문작성시 사사에 연구중심 병원플랫폼 활용 언급 ☐ 논문작성시 authorship 공유	☐ 상담관련 자문료 지급 ☐ 분석에 필요한 소모성 물품 제공	
	책임교수			