



Genome-Wide CRISPR-Cas9 Screening for Identification of Cancer Essential Genes in Malignant Pleural Mesothelioma



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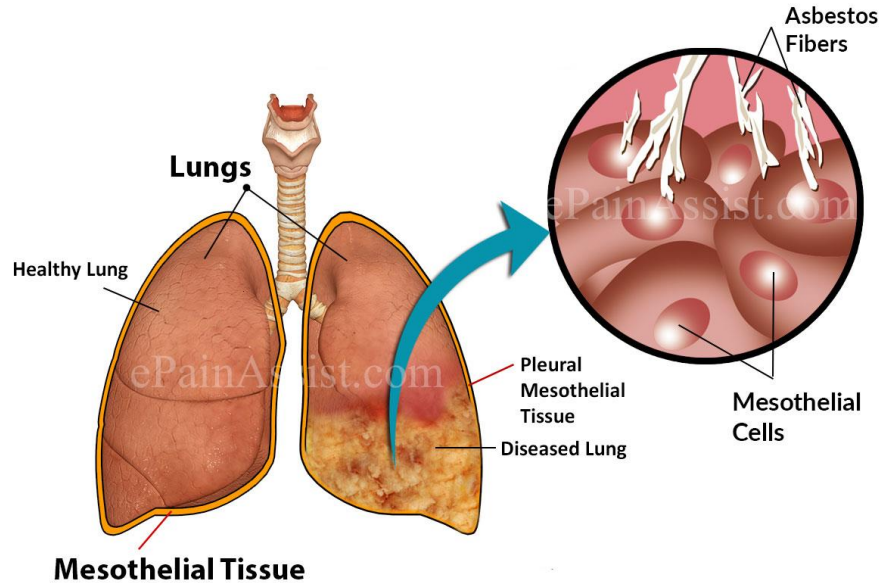
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Funded by TUBITAK, project no: 117Z227

Malignant Pleural Mesothelioma



➤ 80-90% of mesothelioma cases

➤ Asbestos exposure

- Irritation
- Oxygen free radicals
- Interfering mitosis
- Inflammatory cytokines
- Phosphorylation of protein kinases

- Late diagnosis
- Invasive character
- Poor survival

➤ Rare

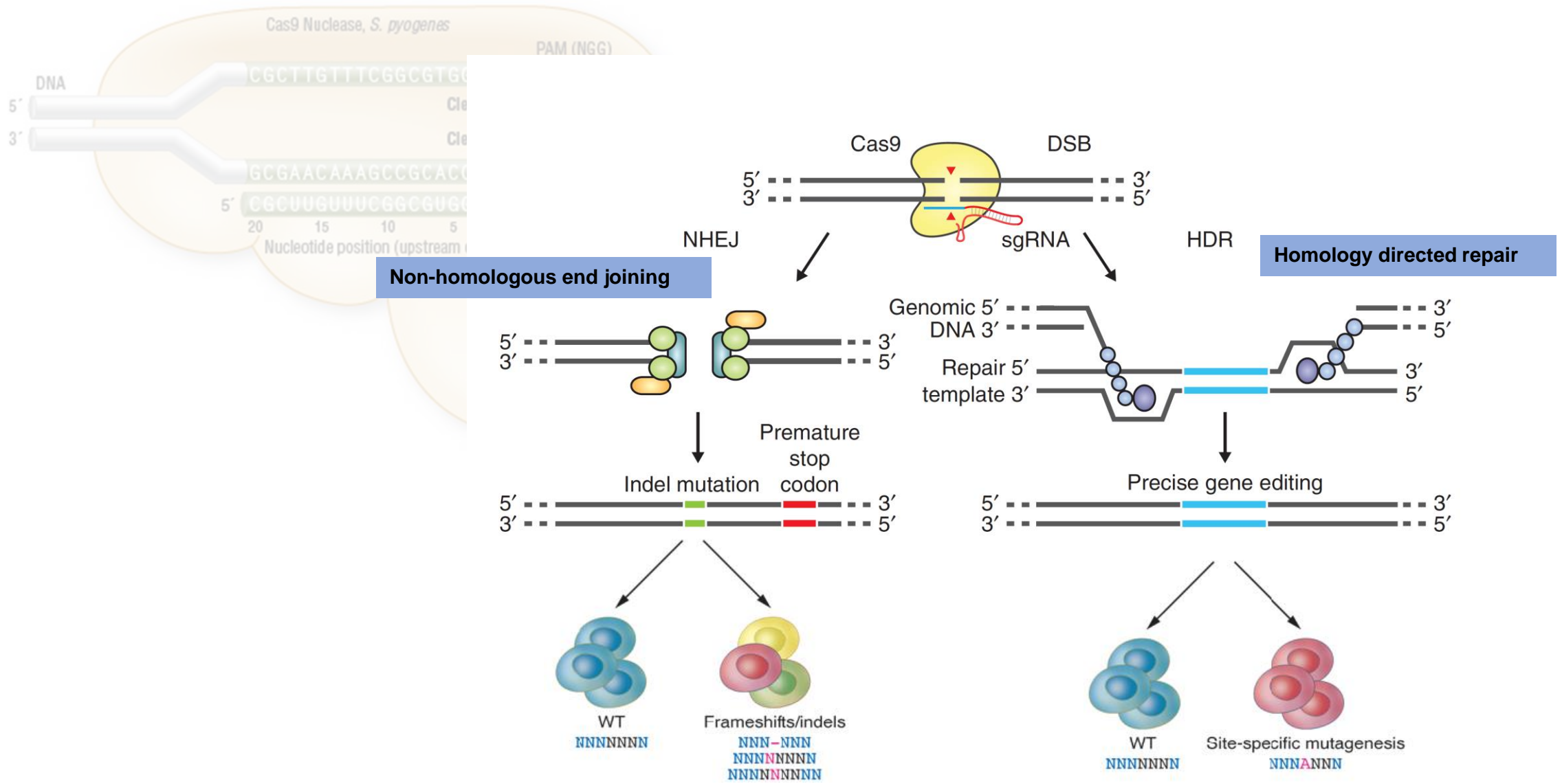
➤ Increasing incidence

- Multimodal/trimodal treatment → standard in the treatment of early-stage MPM
- Poor prognosis
- Low survival (~1-year mean survival; 9% 5-year survival)
- Pemetrexed - a folic acid-like chemotherapeutic agent- (FDA approval in 2004)

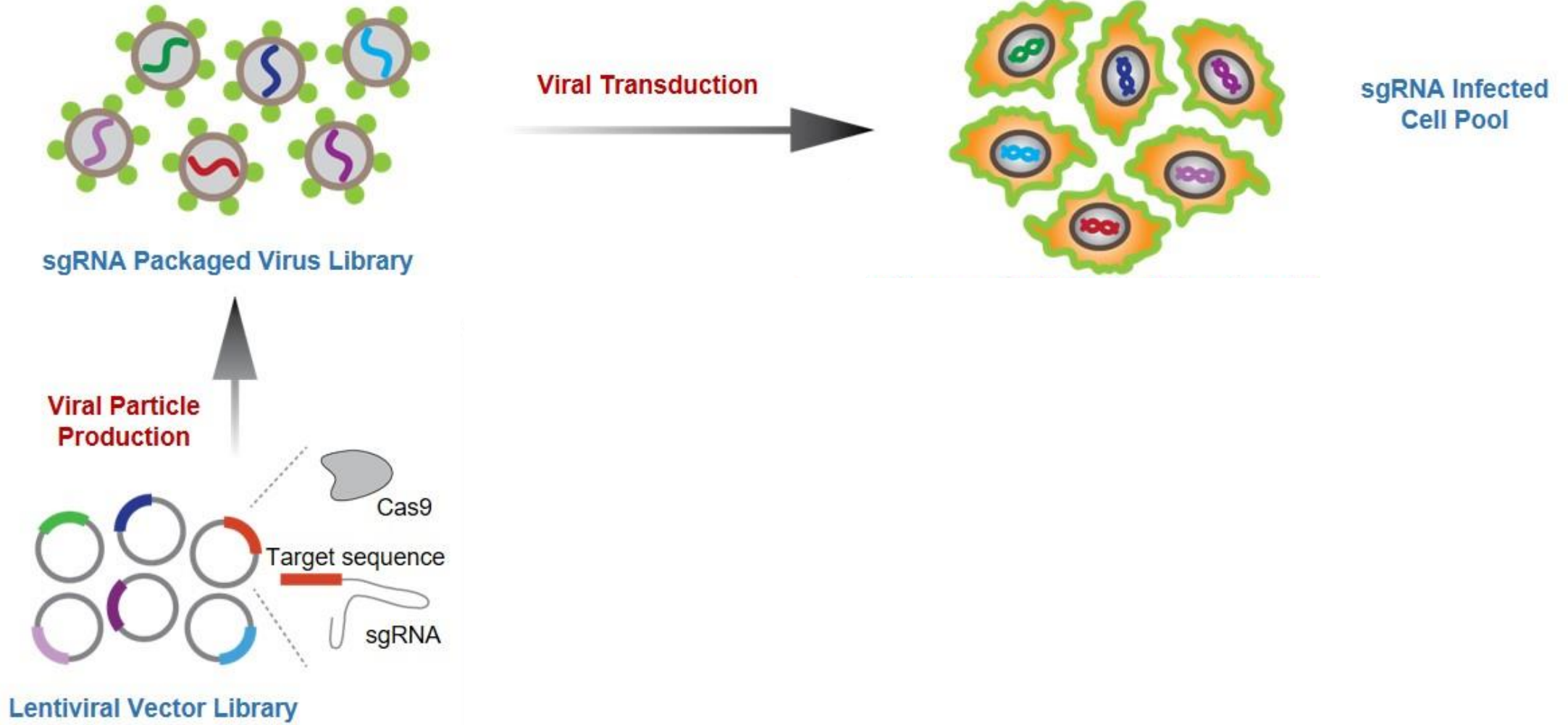
Our Aim

Identification and Functional Characterization of Essential Genes Involved in the Development of Malignant Pleural Mesothelioma





CRISPR Screens

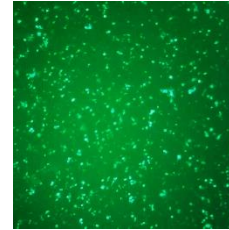


Clonal and Stable Cas9 Expression

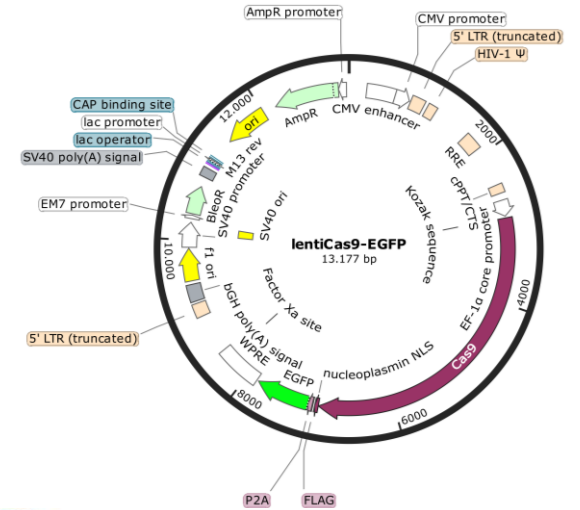


Infection

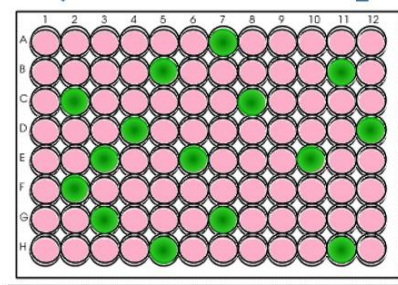
Transfection



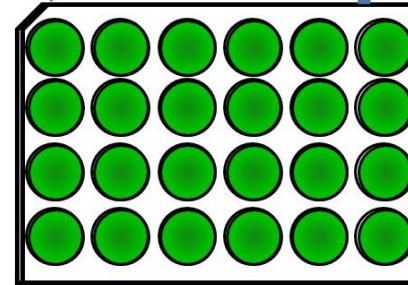
HEK293T



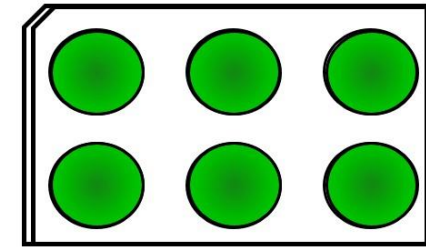
Sorting



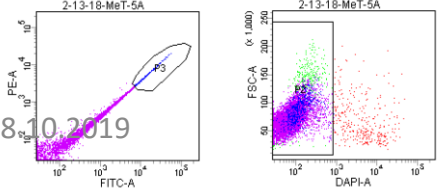
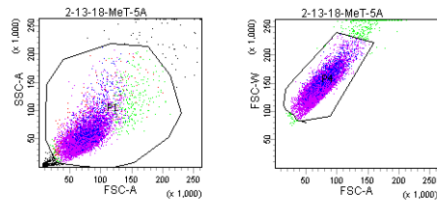
96 well plate



24 well plate



6 well plate



Cell Line	96-well plates seeded	Obtained Clones
MeT-5A	9	12
H2052	15	13
H2452	6	12
H28	10	11



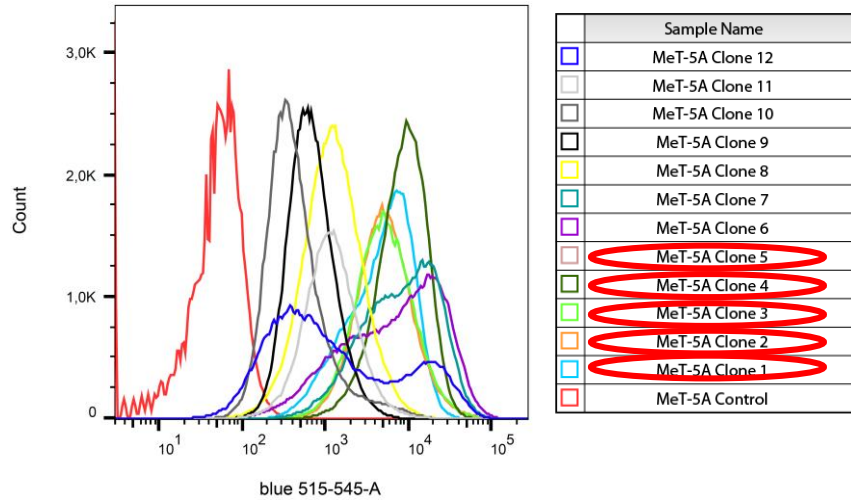
3 vials stock



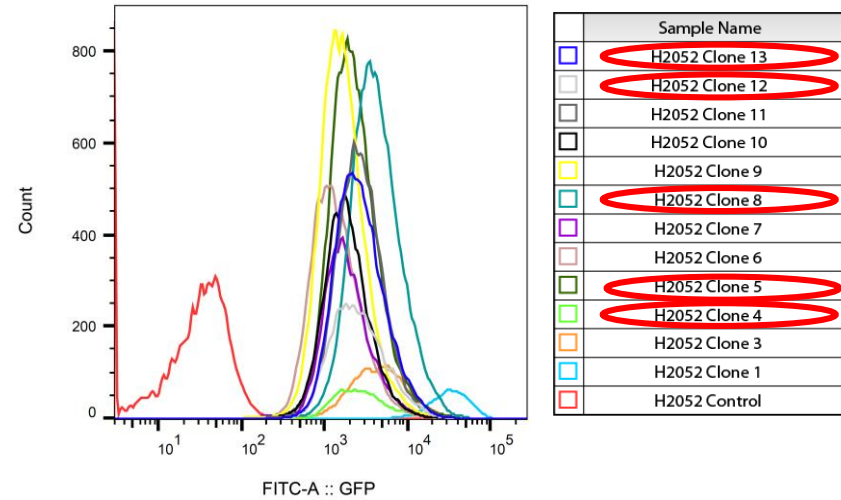
10 cm dish

Choosing The Right Clone

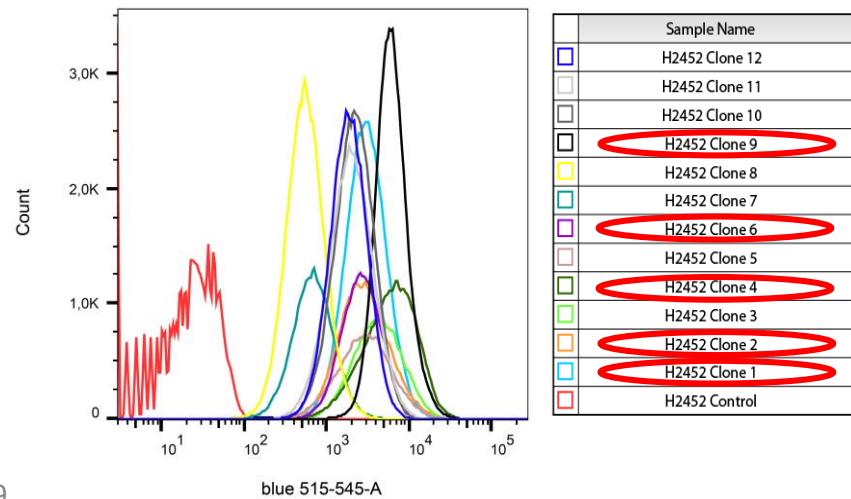
MeT-5A



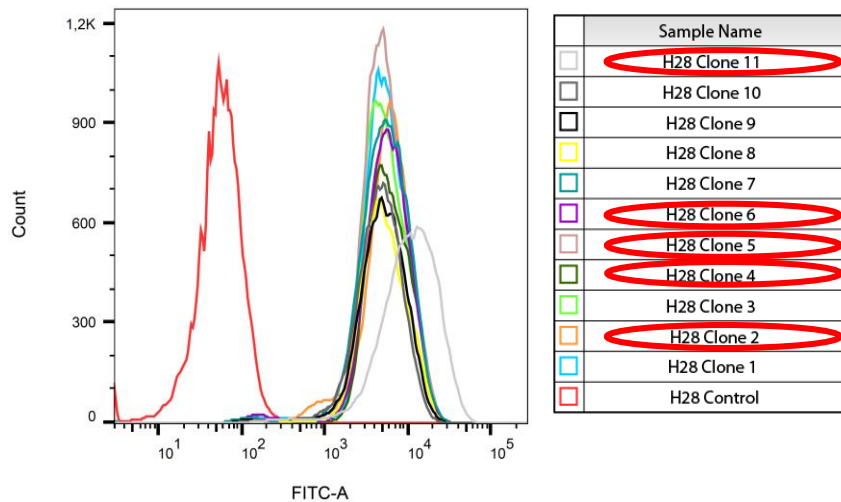
H2052



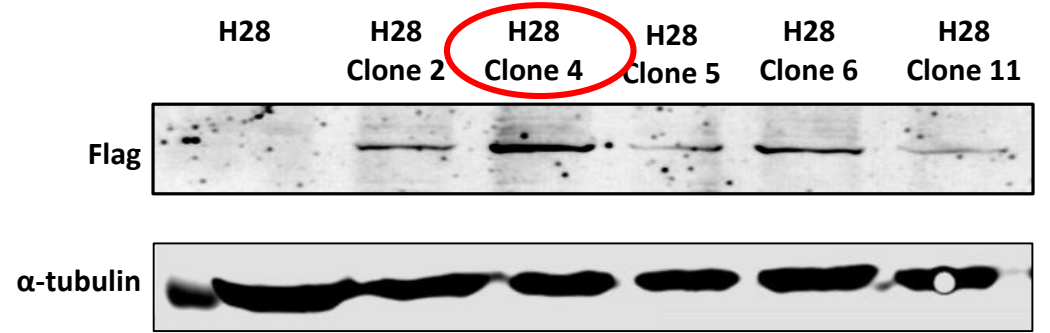
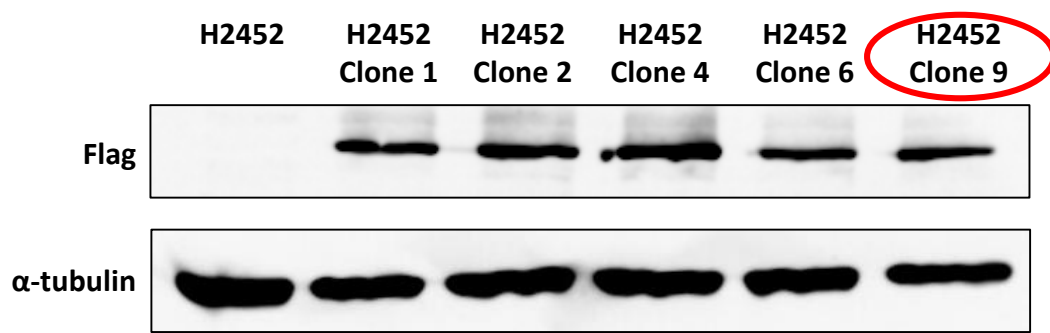
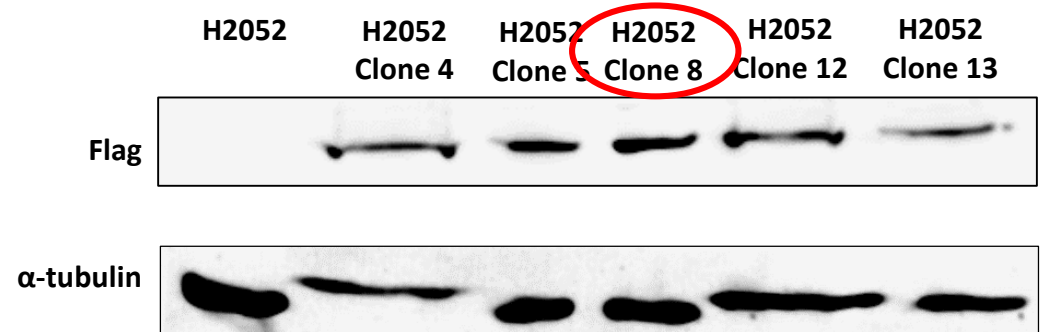
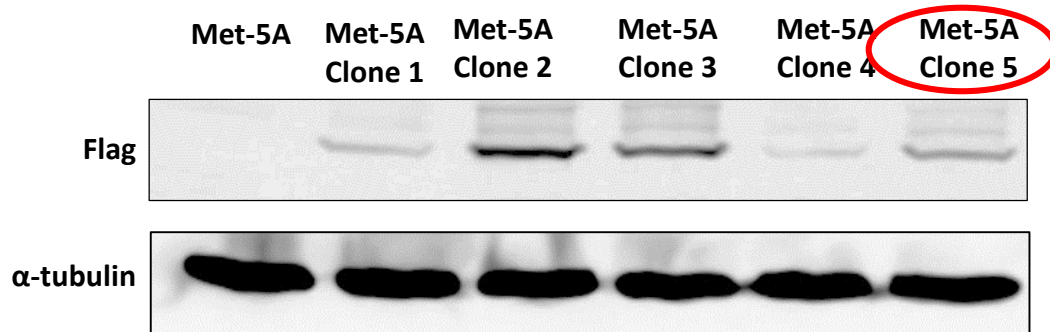
H2452



H28



Choosing The Right Clone

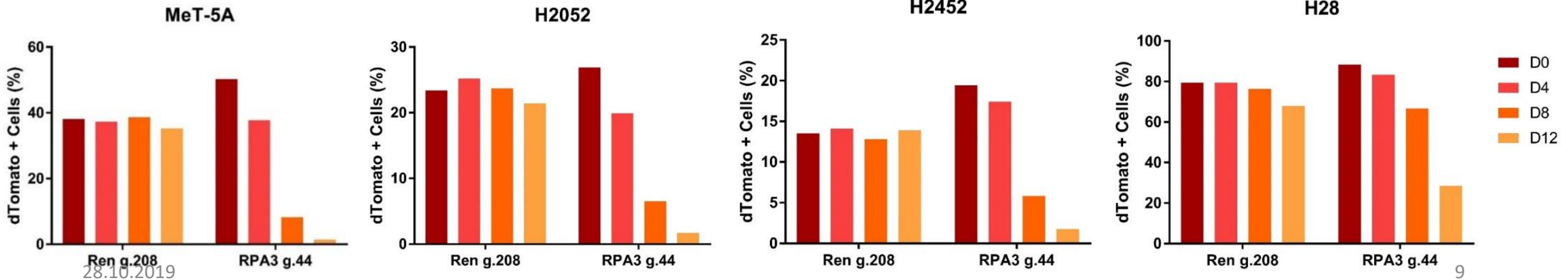
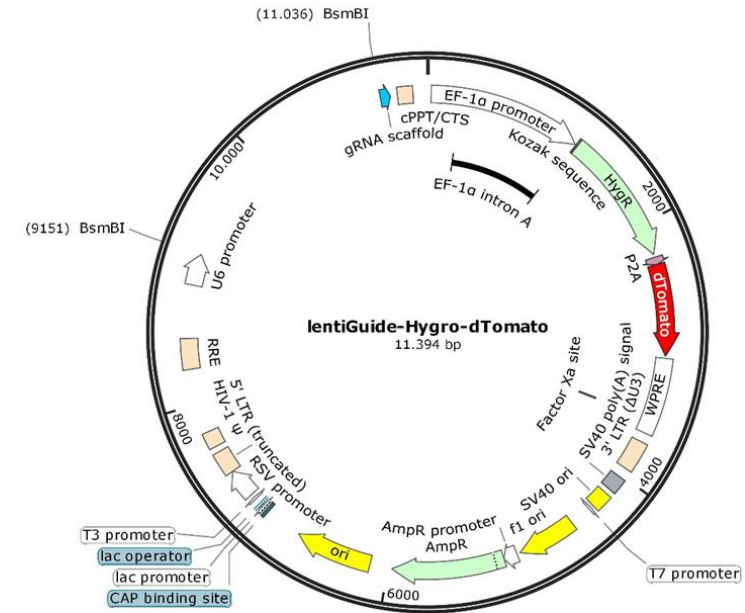


Testing Functionality of Cas9

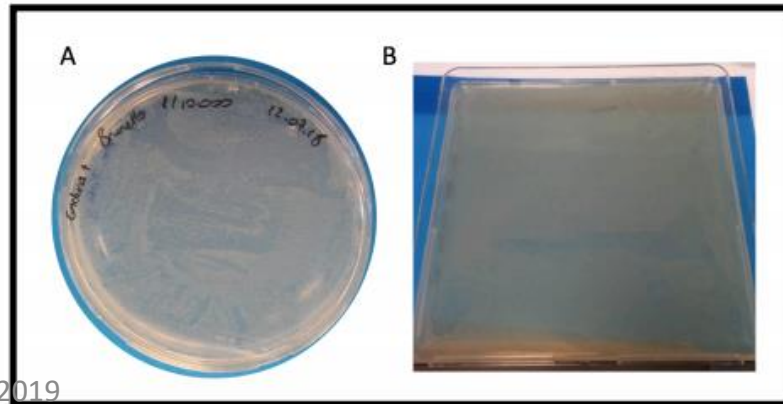
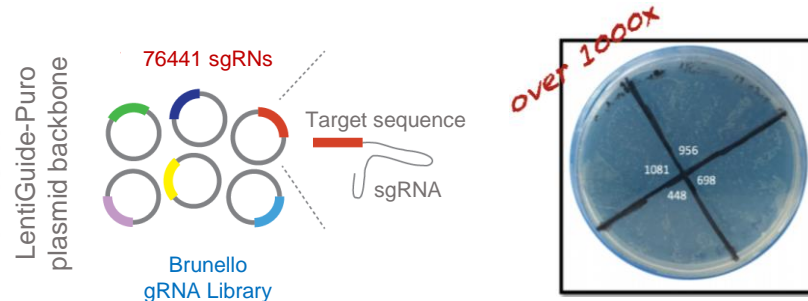
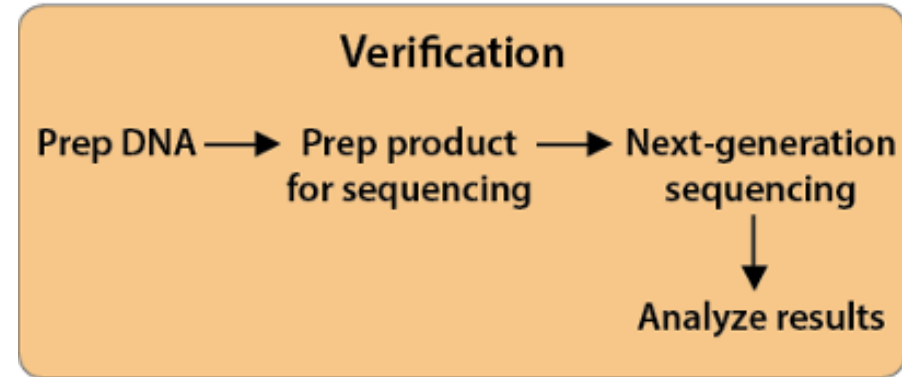
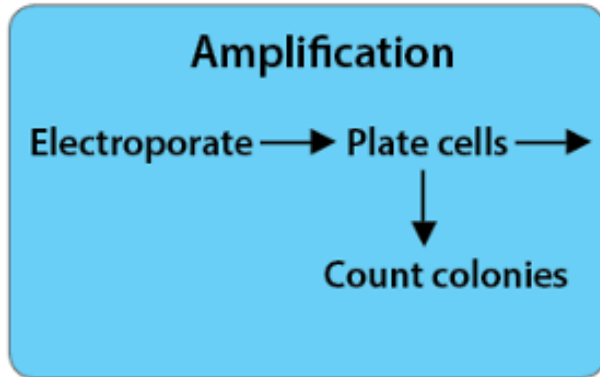
Competition Assay

MeT-5A-Cas9
 H2052-Cas9
 H2452-Cas9
 H28-Cas9

lentiGuide-Hygro-dTomato-Ren g.208
 lentiGuide-Hygro-dTomato-RPA3 g.44



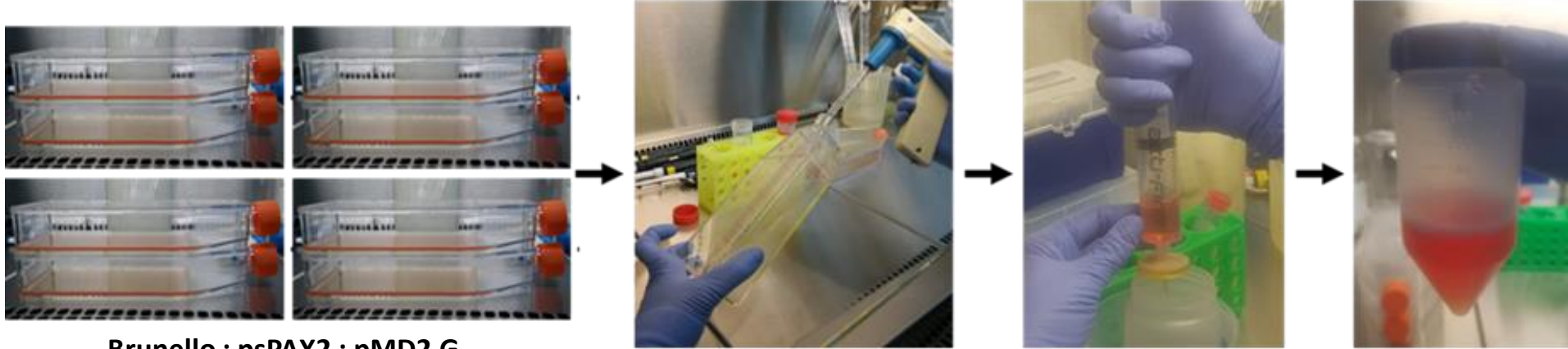
Lentiviral gRNA Library Amplification



NGS Result

	Addgene	Senturk
Perfectly Matching Guides (%)	93.5	93.6
Undetected Guides (%)	0.2	0.3
Skew Ratio	4.50	4.46

Large-Scale Virus Production



Brunello : psPAX2 : pMD2.G

4 : 2 : 1

DNA : PEI

1 : 5

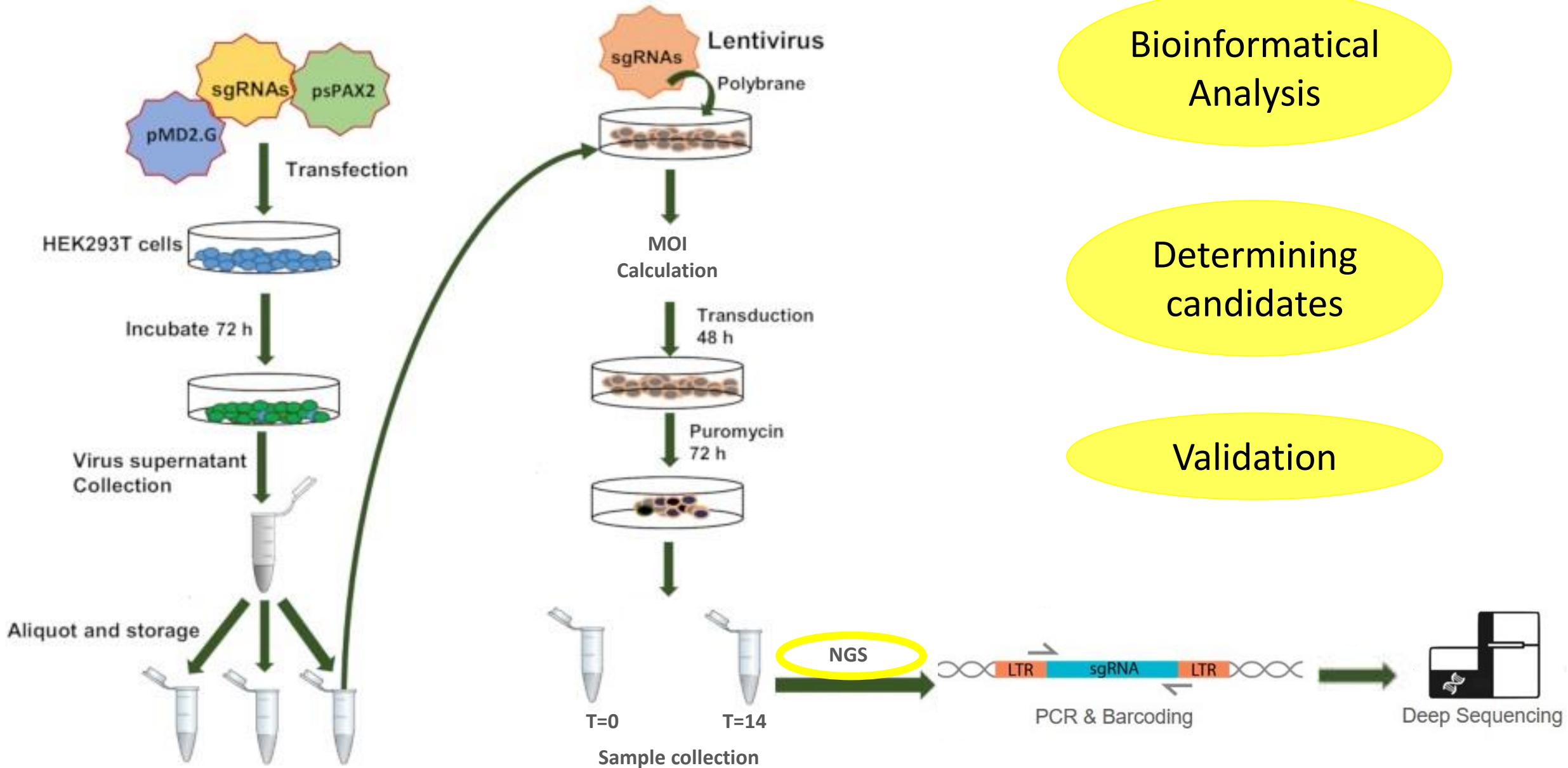
MOI
Calculation

MeT-5A → 1/128

H2052 → 1/48

H2452 → 1/24

H28 → 1/128



THANK YOU FOR YOUR ATTENTION

- **Advisor**
 - Doç Dr. Şerif Şentürk
- **Senturk Lab Members**
 - Özlem Şilan Coşkun
 - Aslı Kurden Pekmezci
 - Dilara Demirci
 - Bengisu Uluata Dayanç
 - F. Aybüke Mazi



Funded by TUBITAK, project no: 117Z227