SYNBIO AUCTION

HANDBOOK

22.4.19

Made with ❤ for D.A.V. Public School, Velachery

iGEM 2019 SASTRA Team - Human Practices #1

Your Goal 💯

To develop and engineer an early diagnosis strategy for cervical cancer

A Tale of Cervical Cancer

Cervical cancer is one of the most common cancers affecting Indian women and has a strong association with Human Papillomavirus (HPV) infection, bearing an annual incidence of slightly over 100,000 cases with a 50% mortality rate[1]. The current gold standard for cervical cancer screening is the *Pap smear test*, which detects early cancerous changes in the cervical epithelium. Apart from the Pap smear test towards screening high-risk HPV types in cervical cancer, *liquid-based cytology* (LBC), *visual inspection with acetic acid* (VIA) and *HPV antibody detection* are the other traditional methods used. Notwithstanding their balance of pros and cons, they are limited either by the cost (in the case of LBC and molecular biology methods) or reliability issues for postmenopausal women due to changes in their endocervical junction (in the case of VIA) or high false-positive or false-negative rates (in the case of Pap smear)[2]. In addition, considering an Indian scenario, these tests are considered invasive by many women, rendering them as a ***social taboo***, potentially leading to lakhs of undetected but treatable cases of cervical cancer in the country. The scientific and societal barriers of these tests incentivize the development of simpler new diagnostic devices for cervical cancer.

Disclaimer: **If you lose, it's on the teacher.**

No microbes were harmed in the making of this game!

Rules and Regulations:

1. There are ***6 different phases*** for this auction where the teams ***choose a component*** from the given list in each of the phases to come up with a wholesome strategy towards early cervical cancer detection.
2. Each team will be given a fixed purse of **Rs. 500,000** and may expend only from that source.
    a. If you require any help from **iGEM SASTRA Members** during the course of the auction, you can pay and avail for our services using the ***Pay for Service*** 💰 option.
        i. Rs. 100 for a Yes/No Answer
        ii. Rs. 200 for Brief Answers
3. Each component in a phase **will be auctioned with their IDs** as mentioned in this document for **60s** 🕐. The teams are supposed to bid within this stipulated time from the base price.
    a. A member from the team should raise his/her hand 🖐 to initiate the bid for that team.
    b. A bid by a team will push the bidding time further and the auction for that component will proceed until the best bid arrives for it
    c. The best bid will be called out thrice before the bid closes for that component.👌
    d. If the teams do not wish to bid on a component further, the team that is calling first for that component will be given that component at the base price.
4. For an effective gameplay strategy, we suggest the following order:
    a. **Hosts** - will act as the "***vehicles***" 🚕🚕 that carries your desired components further from this stage.

    P.S: Make sure you choose appropriate Host (Vehicle) as it is likely to affect your fortunes further in the auction

b. **miRNAs** - The biomarker using which the disease is found stands essential for any diagnosis. In your case, there is a panel of biomarkers from which you need to choose the "**appropriate**" one considering their different properties.

c. **Plasmids** - This is basically an amplification phase where your objective is to choose the right plasmid component to create multiple copies of your desired components. These too act like *vehicles onto which your gene of interest is loaded and used further*.

d. **Restriction nucleases** - this phase allows you to choose the suitable "molecular scissor" ✂️ which could be used to cut a region of the plasmid chosen in the previous stage to load your gene and use it.

e. **Reporter genes** - this phase enables you to choose the proper "reporters" 🔊🔆 to indicate the presence or absence of the biomarker in the system.

f. **Detection systems** - The last phase of the auction wherein you are supposed to choose the apt system by which you can "detect" 🔎 the output of the "reporters"

5. All the above phases will have 7 components each with **a default component included in it** (**indicated by D**). Each team will have to choose a component from each phase and the team with no remaining money in their purse will end up with the default one.
6. Each team will be given 15 minutes to consolidate their strategies and a member from the team should present their solution in 5-10 minutes to the judges.
7. Final evaluation of the winners will be based on the amount left in their purse and "effectiveness" of their strategy towards the objective. The decision of the judges will be final.

PHASE #1: HOST SELECTION

The ones who patiently hold together all your activities. Microscopic safe havens.

Host ID	Host Name	Merits	Demerits	Degree of Manipulation	Success rate (%)	Base Price (Rs.)
1001	*Escherichia coli (E. coli)*	Well studied; easy to manipulate; doubling time is less; easy to culture	Sometimes heavy engineering may be required	Very easy	93	5,000
1002	*Mycoplasma pneumoniae (M. pneumoniae)*	Minimal genome engineering required	Triggers Immune response	Cumbersome	67	3,000
1003	*Pseudomonas putida (P. putida)*	Metabolic diversity; ability to resist toxic compounds	Engineered cells are not that stable	Easy	80	3,800
1004	*Clostridium acetobutylicum (C. acetobutylicum)*	Broad range of substrates	Strict anaerobe	Cumbersome	64	2,800
1005 - **D**	*Saccharomyces cerevisiae (S. cerevisiae)*	Facultative anaerobic; well studied	Difficult to engineer; limited carbon source utilization	Difficult	40	-
1006	*Bacillus subtilis (B. subtilis)*	Well studied; easy to manipulate; easy to culture	Cell growth rate decreases with engineering	Very easy	89	4,700
1007	*Kluyveromyces marxianus (K. marxianus)*	Grows at high temperatures on a wide range of carbon sources	Major genetic engineering limitations	Cumbersome	55	2,500

PHASE #2: BIOMARKER SELECTION

The ones who are always there for you when something goes wrong. Or right :)

miRNA ID	**miRNA name**	**Prevalence**	**Comments**	**Success rate (%)**	**Base Price (Rs.)**
2001	miRNA-21	Highly prevalent	Displayed highest sensitivity and specificity for cervical cancer diagnosis	94	4,500
2002	miRNA-20a	Prevalent	Associated with aggressive progression of the disease	84	3,650
2003	miRNA-200a	Prevalent	Used for clinical monitoring	76	3,490
2004	miRNA-29a	Moderately prevalent	More prevalent in later stages of the disease	73	2,900
2005	miRNA-9	Not very prevalent	Plays a central role in the development of the disease	69	2,300
2006	miRNA-1275	Not very prevalent	Low specificity biomarker	46	1,650
2007 - **D**	miRNA-203	Very low prevalence	Not a credible biomarker	31	-

PHASE #3: PLASMID SELECTION

The ones who adopt others take them in as their own. The Odin to your Loki. The Wolf Pack to your Mowgli. The Uncle Ben and Aunt May to your Peter.

Plasmid ID	**Plasmid name**	**Copy Number**	**Insert Size (in bp)**	**Success rate (%)**	**Base Price (Rs.)**
3001	pET SUMO	Low	1455	60	1,000
3002 - **D**	pET15b	Low (~40)	964	57	-
3003	pRSET A	High (~250)	1953	96	2,500
3004	pDEST17	High	920	72	1,275
3005	pET-DEST42	High	1000	89	1,999
3006	pCOLA-2-DEST	High	990	80	1,498
3007	pET161-DEST	High	900	70	1,156

ROUGH WORK

PHASE #4: RESTRICTION ENDONUCLEASE SELECTION

They destroy old bonds and forge new ones. They need only a single weakness in the chain. Presenting to you, the Littlefingers of the molecular world.

RE ID	RE Name	Merits	Demerits	Errors	Success rate (%)	Base Price (Rs.)
4001	EcoR1	Isolated from *E.coli*; easily available; creates sticky ends; only require a low temperature of 37^{O}C	Needs magnesium ions as cofactors	Low error rate; errors increase at extreme temperatures	95	3,000 for the enzyme + 1,000 for the cofactor.
4002	Sma1	-	Produces blunt ends; harder for ligation of required DNA fragment; requires potassium as a cofactor	High error rate when the exact temperature isn't maintained; Activity drops to 50% even at 35^{O}C	80	2,000 for the enzyme + 900 for the cofactor.
4003	Type 1 RE	-	Cuts at a position far away from the recognition sequence	Cuts away from the sequence; high error rate	30	**D** 500 for the enzyme.
4004	HindIII	Can cut the DNA within 5-15 minutes; won't degrade the DNA; produces sticky ends	Needs magnesium ions as cofactors	High precision; low error rate	90	2,500 for the enzyme + 1,000 for the cofactor.
4005	Bcg1	Can cleave outside	Not very	Moderate error	75	1,500 for

		the recognition sequence; can help save space if the plasmid is very small; produces sticky ends	specific	rate		the enzyme.
4006	Xma1	Can cut the fragment within 5-15 minutes; good specificity	Produces blunt ends; harder for ligation of required DNA fragment	Prone to exhibit star activity when kept undisturbed for long periods of time	60	1,200 for the enzyme.

ROUGH WORK

PHASE #5: REPORTER GENE SELECTION

The sole outlet to see your carefully chosen components so far. This is the light at the end of the tunnel. Or maybe it's just fluorescence. :)

RG ID	RG Name	Merits	Demerits	Comments	Success rate (%)	Base Price (Rs.)
5001	Green Fluorescent Protein (GFP)	Substrate independent; more stable; brighter	Low levels cannot be detected; higher maturation time	Detected using fluorometer	92	15,990
5002	mCherry family	Substrate independent; low maturation time	Lesser brightness	Detected using fluorometer	86	14,350
5003	Red Fluorescent Protein (RFP)	Substrate independent	Lesser stability; higher maturation time	Detected using fluorometer	78	13,550
5004	LacZ	Rapid naked eye detection	Substrate dependent	Detected using enzymatic assay	67	20,500
5005	Luciferase	High sensitivity	Substrate dependent; time consuming	Detected using luminometer or optical microscope	56	19,450
5006	ChloramphenicolAcetyl Transferase (CAT)	Low levels can be detected easily	Radioactive labelling needed	Detected using Thin Layer Chromatography	44	17,800
5007 - **D**	β-glucuronidase (GUS)	Stable and good sensitivity	Substrate dependent; mostly used in plants	Detected using enzymatic assay	37	-

PHASE #6: DETECTION SYSTEMS FOR MEASUREMENT

Beauty lies in the eyes of the beholder. Choose your eyes carefully below.

DS ID	DS Name	Merits	Demerits	Success rate (%)	Base Price (Rs.)
6001	Optical	Preliminary qualitative analysis; differentiate positive and negative samples			
6001.1	Absorption	Low cost; moderate selectivity	Low Sensitivity; low reliability	84.3	3,250
6001.2	Fluorescence	High sensitivity; moderate selectivity	Saturation errors	87.9	25,110
6002	Electrochemical	Quantitative analysis; automated method; most utilized method for clinical diagnosis; high selectivity; high sensitivity; rapid detection	-	95	29,999
6003	Piezoelectric	Enhanced sensitivity and selectivity; fast response time	Temperature based changes in output	81.8	49,999
6004	Magnetic	Allows physical separation of components apart from detection; rapid detection	Low Sensitivity; frequent calibration required	75	5,990
6005	Calorimetric	Low cost; long life; portable	Frequent calibration required; susceptibility to damage	78	7,770

6006	Conductometric	Quantitative analysis; high selectivity; high sensitivity; rapid detection	-	92.2	23,200
6007 - **D**	Immunoassays	High specificity; both qualitative and quantitative analyses possible	Slower technique (need to wait for a day or so); reuse not possible; low sensitivity	62	-

ROUGH WORK

---------- END OF AUCTION HANDBOOK ----------